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David M. Guidot
Ashish J. Mehta *Editors*

Alcohol Use Disorders and the Lung

A Clinical and Pathophysiological Approach

Humana Press

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Preface

Lung health is a precious commodity and is essential for human productivity as well as the quality of our individual lives. Unfortunately, there is a worldwide increase in mortality from lung diseases of various types that stands in sharp contradistinction to the declines in cardiovascular disease mortality. A progressively modernized world with ready access to tobacco products and constant exposure to air pollution in our larger and evermore industrialized cities is certainly a major cause. However, a major risk factor for acute and chronic lung diseases is rarely cited in textbooks and reviews of the global pandemic in lung diseases. Specifically, alcohol use and in particular excessive alcohol use and abuse contribute to millions of deaths per year worldwide from pneumonia and acute lung injury. However, its role in lung disease has frequently been overlooked and in the case of acute lung injury was unrecognized altogether until less than two decades ago. Although alcohol abuse was identified as a major risk factor for pneumonia more than two centuries ago, its diverse and devastating impact on overall lung health in a variety of forms and contexts is now being appreciated. There has been an explosion in laboratory research and clinical studies that are elucidating the remarkably diverse mechanisms by which this simple two-carbon compound impacts the delicate functions of the lung, from the upper airways to the resident immune cells in the alveoli and essentially every cell type and function in between. Therefore, we hope this textbook will be of interest to our colleagues but also a resource for them as the global pandemic of alcohol-related lung disease is recognized and, tragically, continues to grow. Although the great majority of this volume is focused on the epidemiology and pathophysiology of what we have termed "alcoholic lung," we hope that there will be an optimistic note as well in the discussions of evolving therapies. In this context, the exciting research discoveries made in the past two decades are already laying the foundation for the identification and testing of therapies designed to enhance lung health in those individuals who struggle with alcohol abuse and dependence and decrease the morbidity and mortality of alcohol-related lung diseases. Further, we are optimistic that such therapeutic approaches will have salutary effects on other organs such as the liver and brain. In fact, in many important instances the basic

research discoveries of the mechanisms by which excessive alcohol ingestion renders individuals susceptible to acute lung injury and pneumonia were guided by prior and/or parallel investigations of the effects of alcohol on other organs such as the liver, pancreas, and brain. Therefore, there is every reason to anticipate new treatment options to mitigate the pathological effects of alcohol in those who are at greatest risk. Although the ideal "solution" would be a society in which alcohol use is always moderate and in a safe context, the history of human societies and our current social challenges make it clear that no such "ideal solution" is imminent. Until such a lofty goal is achieved, it is imperative that we dissect the specific mechanisms by which alcohol perturbs health and identify biological interventions that can complement the important efforts in cognitive and behavioral therapy that are the focus of alcohol treatment programs.

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Part I
The Epidemiology of Alcohol Use and Lung Health

Chapter 1
A Brief History of Alcohol Use and Abuse in Human History

David M. Guidot and Ashish J. Mehta

Abstract There is clear archaeological evidence that dates the production and consumption of alcoholic beverages back ~20,000 years. Over the millennia the techniques of fermenting various organic materials have been gradually refined, and now there are thousands of different alcohol beverages that are produced, both commercially and noncommercially, throughout the world. Mankind's relationship with alcohol has been decidedly mixed, with its use associated both with celebration and with despair. The perceived salutary effects of alcohol in human culture have been celebrated in song and prose. In contrast, the negative effects of alcohol on behavior have led to its prohibition by various societies and religions since its use first became widespread in human culture many thousands of years ago. Independently of the arguments for and against the ingestion of alcoholic beverages, it is clear that its prohibition in free societies is not only impossible but in fact may also have unintended consequences such as the growth in organized crime and tragic side effects of consuming unsafe homemade products. Therefore, even with a growing public awareness of the adverse consequences of alcohol use and a justifiable tightening of laws that regulate its sale and distribution and punish dangerous alcohol-related activities such as driving while under the influence, it is clear that alcohol consumption will remain common throughout the world for the foreseeable future.

Keywords Alcohol • Alcoholism • Prohibition • Temperance

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Chapter Body

Alcoholic beverages of various kinds have been used, and abused, by humans for thousands of years. There is archaeological evidence that the fermentation of grains into beer dates back ~20,000 years and that similar fermentation of grape juice into wine is almost as ancient a custom. It is almost certain that the discovery of alcoholic beverages was accidental. For example, long before the domestication of grains and the development of farming there was evidence of the consumption of alcoholic beverages. Although we will never know for sure where the first alcoholic beverage was discovered, it appears that one of the first alcoholic drinks was fermented mare's milk in ancient Siberia, which likely was identified by trial and error when "spoiled" milk was found to have stimulant properties. In fact, a version of this alcoholic beverage, known as *kumis*, is still consumed in some parts of Russia.

In many cultures, both ancient and modern, the consumption of beer, wine, and spirits is a part of religious ceremonies, social events, and simple daily living. Although many religious and social groups have proscribed its use and temperance movements have arisen at various times in virtually every society, alcohol ingestion has proven to be an enduring human custom. Unfortunately, a significant proportion of individuals who consume alcohol on a regular basis develop patterns of alcohol abuse or even frank physical dependence, and the long-term health consequences of excessive alcohol use can be devastating.

Long before the negative consequences of alcohol abuse on physical health such as cirrhosis and dementia were recognized, its adverse effects on behavior and productivity were recognized. These social effects led to various forms of alcohol prohibition even in ancient cultures. Several major religions, including Islam and Mormonism, have clear bans on all alcohol ingestion, and in some countries such as Saudi Arabia the production, sale, and ingestion of alcohol are all prohibited by law because it violates religious doctrine. In the United States, a growing temperance movement in the nineteenth century that was driven largely by religious beliefs eventually led to the passage of the 18th Amendment to the Constitution in 1919, which imposed a complete prohibition on the production, sale, and consumption of alcoholic beverages within the United States. The "Prohibition Era" was marked by widespread disobedience and was in fact largely responsible for the rapid growth of organized crime in this country as the black market for alcoholic beverages was enormous. In parallel, the unregulated production of "homemade" liquors at times was associated with significant side effects from contaminants including methanol and lead. The 18th Amendment was repealed in 1933, and the unintended negative consequences of this attempt to eliminate alcohol from American society effectively ended the temperance movement as a meaningful political force. However, the prohibition of alcohol use by some religions and their influence on local laws remain evident in current times, as reflected best by the so-called dry counties in various states.

Whether or not one endorses or condemns the ingestion of alcohol on moral, religious, or social grounds, there can be no refuting that excessive alcohol use can have devastating health consequences and is directly or indirectly causative in millions of deaths worldwide each year.

Worldwide, alcohol is the most frequently abused drug [1]. In the United States, half of the general population regularly consumes alcohol, and 15–20 million individuals are alcoholics [2, 3]. According to the 2008 National Survey on Drug Use and Health, more than 50 % of the adult population in the United States consumes alcohol, which would roughly translate into more than 125 million people. In this same survey, almost 7 % reported heavy drinking [Substance Abuse and Mental Health Services Administration. (2009). *Results from the 2008 National Survey on Drug Use and Health*: *National Findings* (Office of Applied Studies, NSDUH Series H-36, HHS Publication No. SMA 09-4434). Rockville, MD]. Data from the 2001 National Epidemiologic Survey on Alcohol and Related Conditions reported that the lifetime prevalence of alcohol abuse was about 18 %, making alcohol the most widely used and abused among all drugs [4, 5]. While average alcohol intake has decreased over time, more recent data suggest that the incidence of alcohol use disorders has not changed [6]. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) has estimated that alcohol-related problems cost our society more than $185 billion per year [7]. Among persons admitted to general hospitals, 20–40 % have alcohol-related problems [8]. A more recent report published by the Centers for Disease Control and Prevention conservatively estimated that in the year 2001, there were ~76,000 alcohol-attributable deaths and more than 2.3 million years of potential life lost due to alcohol abuse in the United States alone [9]. The majority of these deaths were attributed to chronic conditions such as cirrhosis and to alcohol-related acute trauma, particularly automobile accidents. However, as we shall discuss in the context of acute lung injury, these estimates failed to include a large number of cases in which a causative role for alcohol abuse was unrecognized.

Perhaps most tragically, the prevalence of unsafe alcohol consumption in the so-called underage segment of our society (those under the age of 21) is rising dramatically. The NIAAA estimates that in 2009 ~10.4 million people in the United States between the ages of 12 and 20 had some degree of significant alcohol intake (http://www.niaaa.nih.gov/alcohol-health/special-populations-co-occurring-disorders/underage-drinking). Further, although people in this age group drink less often on average than their adult counterparts, they are far more likely to binge drink, defined as five or more drinks in one setting. Sadly, the NIAAA also estimates that ~5,000 people under the age of 21 die every year in the United States from alcohol-related injuries such as automobile accidents, burns, and drowning. Ironically, there is now anecdotal evidence that increasing the legal drinking age from 18 to 21 across the country by the National Minimal Age Drinking Act in 1984 may have actually increased the incidence of binge drinking, particularly among college students.

Summary

Alcohol is the most widely used and abused drug worldwide, and its production and ingestion have been woven into human cultures for thousands of years. Although the balance between the social benefits of alcohol and its negative consequences has been hotly debated for centuries and many societies have attempted to curb or even eliminate its use, the drinking of alcohol is inextricably connected to our modern society. Therefore, it is imperative to understand how excessive alcohol ingestion impairs human health and identify strategies to mitigate its impact as its complete prohibition is not feasible.

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Chapter 2
Overview of the Evolving Recognition of the Health Effects of Excessive Alcohol Use Over the Past Two Centuries Including the Classic Citations

David M. Guidot and Ashish J. Mehta

Abstract The negative behavioral and social consequences of alcohol ingestion, particularly when done so in excess, have been recognized for thousands of years and have led to various types of prohibition imposed on religious, moral, or social grounds in nearly every society at one time or another. However, the relatively modern era of medicine has only more recently documented and investigated the adverse health effects of excessive alcohol consumption. Although the pathophysiological effects of alcohol on the liver and the brain are more widely recognized and have attracted much of the attention by physicians and scientists, alcohol abuse has a myriad of systemic targets including the lung. In fact, it has been recognized for more than two centuries that alcohol abuse is a major risk factor for pneumonia. More recently, clinical observations have identified that alcoholics are at risk for much poorer outcomes if they develop certain pneumonias such as from *Streptococcus pneumoniae*. Further, even more recent epidemiological studies have identified that alcohol abuse significantly increases the risk of acute lung injury following acute insults such as sepsis or trauma. This chapter highlights some of the classic observations and discoveries of the relationship between alcohol use and lung disease over the past two centuries and sets the stage for the more detailed analyses and accounts of the current state of our knowledge of specific facets of this relationship in subsequent chapters.

Keywords Alcohol • Alcohol abuse • Pneumonia • Acute lung injury • ARDS

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Chapter Body

This chapter provides an overview of the relatively modern history of medical observations and investigations of the untoward effects of alcohol ingestion on respiratory health and highlights some of the seminal events in this history. Subsequent chapters in this book will focus on specific aspects of how excessive alcohol ingestion impairs airway and lung health in far greater depth and detail.

Unfortunately, a significant proportion of individuals who consume alcohol on a regular basis develop patterns of alcohol abuse or even frank physical dependence, and the long-term health consequences of excessive alcohol use can be devastating. Many of the medical complications of alcohol abuse, including hepatitis, cirrhosis, pancreatitis, cardiomyopathy, peripheral neuropathy, and dementia, are well known to both the general public and to the medical community [1]. By contrast, the ravages of alcohol abuse have been viewed as relatively sparing the lung. For example, an "alcoholic pneumopathy" or an "alcoholic pneumonitis" analogous to the aforementioned complications of chronic alcohol abuse has not been described. The notable exception is the long recognized link between alcohol abuse and pneumonia.

More than two centuries ago the first Surgeon General of the United States, Benjamin Rush, noted that pneumonia and tuberculosis were infectious complications more commonly encountered in people who drank alcohol, and, a century later, William Osler cited alcohol abuse as the major risk factor for pneumonia [2]. However, this risk has largely been attributed to alterations in immune function and/or structural/functional defects in the upper airway such as colonization of the oropharynx with gram-negative bacteria and the obvious risk of aspiration during inebriation. In fact, until relatively recently it had been generally assumed that chronic alcohol abuse had no effect on the lung parenchyma itself as there is no epidemiological evidence to implicate it as an independent risk factor for common pulmonary disorders such as bronchogenic carcinoma, asthma, emphysema, or interstitial lung disease.

Our understanding of the effects of alcohol abuse on the lung itself was changed when a novel epidemiological finding published in 1996 revealed for the first time that alcohol abuse independently increased the risk for developing a severe form of lung injury known as the acute respiratory distress syndrome (ARDS) in critically ill individuals [3]. Specifically, an otherwise healthy individual with an alcohol use disorder (i.e., "alcohol abuse" or "alcohol dependence") who suffers a critical illness such as pneumonia, sepsis, or trauma has a two- to fourfold increased risk of developing ARDS than nonalcoholics. Remarkably, this association and its impact had been missed even though independent risk factors for ARDS had been vigorously sought, and even two decades later they are not recognized routinely by the medical community.

This initial epidemiological observation inspired experimental and clinical studies that have led to an explosive growth in our understanding of the relationship between chronic alcohol abuse and pulmonary disease. The ensuing chapters in this

book will focus on the key aspects of research of the past decade on alcohol abuse and acute lung injury and synthesize the novel findings in this area with previous and ongoing studies of alcohol abuse and pulmonary host defense. Specifically, it is becoming increasingly clear that alcohol abuse, even in otherwise healthy individuals, causes significant oxidant stress within the alveolar space and impairs both alveolar epithelial and alveolar macrophage function via common pathophysiological mechanisms. Therefore, this textbook will integrate the parallel but often independent findings on immune dysfunction and susceptibility to acute lung injury in the "alcoholic lung" into a common pathophysiological scheme. In addition to in-depth analyses of the impact of underlying alcohol-use disorders on health outcomes in a variety of clinical contexts, the mechanisms by which alcohol impairs airway function and in particular lung immunity will be reviewed. Finally, we will discuss recent experimental findings that raise the possibility that novel therapies, targeted at the airway epithelial and macrophage dysfunction in alcoholic individuals, could limit the incidence and/or severity of lung infections as well as modify their dramatically increased risk of acute lung injury in the setting of serious lung infections and/or other critical illnesses. However, before we delve deeply into detailed analyses of the "alcoholic lung," it is worth remembering that alcohol abuse is in fact a systemic illness and that its devastating biological consequences do not spare any organ system.

Alcohol abuse causes a myriad of serious health consequences. Perhaps for obvious reasons, much of the medical attention has focused on alcohol-mediated pathophysiology within the gastrointestinal system. Following ingestion, alcohol is rapidly absorbed by the gastric and small intestinal mucosa and is metabolized primarily in the liver by alcohol dehydrogenase, a cytosolic enzyme with multiple isoforms that vary in their affinities for alcohol binding [4, 5]. Only the liver and the gastric mucosa have the high-affinity isoform, and therefore alcohol metabolism by alcohol dehydrogenase in tissues other than the liver and the stomach is limited [4, 5]. Alcohol can also be metabolized in microsomes via the cytochrome p450 component CYP2E1 [5]. This enzyme complex has a lower affinity for alcohol than the hepatic alcohol dehydrogenase enzyme and therefore may not contribute significantly to overall alcohol metabolism following occasional use. However, in the context of chronic use, the CYP2E1 enzyme metabolizes a significant percentage of ingested alcohol. Alcohol metabolism in the liver forms acetaldehyde and free radicals that have been implicated as direct causes of hepatocyte injury [4, 5]. As many as 35 % of heavy drinkers develop alcoholic hepatitis, and half of these individuals develop frank cirrhosis [4, 5]. Another prominent target of alcohol abuse within the gastrointestinal tract is the pancreas. An association between alcohol abuse and pancreatic injury was reported as early as 1878 [6], and alcoholic pancreatitis has become a well-recognized clinical entity since then that, although less common than alcoholic hepatitis, can cause significant morbidity and mortality in affected individuals. Alcohol consumption has diverse deleterious effects elsewhere throughout the gastrointestinal tract including gastroesophageal reflux, damage to the gastric mucosa, and malabsorption of nutrients in the small intestine [7].

Beyond the gastrointestinal system, alcohol abuse has diverse targets. For example, it impacts the endocrine system by disrupting the actions of hormones such as cortisol, testosterone, growth hormone, and prolactin, and it interferes with glucose and lipid metabolism [8]. Although much attention in recent years has been paid to the salutary effects of moderate alcohol consumption on the cardiovascular system, alcohol abuse can lead to significant morbidity and mortality from cardiomyopathy and vascular disease [9]. Further, alcohol abuse is clearly associated with certain cancers, such as esophageal and gastric carcinoma, and causes osteoporosis, myopathy, dementia, and peripheral neuropathy [1]. Therefore, one could argue that alcohol abuse is a truly systemic disorder in which the clinical manifestations may vary depending on the individual affected. As this textbook will focus on the effects of alcohol abuse on the lung, readers are directed to several excellent reviews of the medical complications of alcohol abuse that have been only briefly mentioned here [1, 10].

Summary

Alcohol abuse is common worldwide and has been a major cause of health problems for thousands of years. The effects of alcohol on the body are remarkably protean, with devastating consequences on the brain, liver, musculoskeletal system, and other organ systems. Although the association between alcohol abuse and pneumonia has been recognized for more than two centuries, it is only relatively recently that a link between alcohol and acute lung injury was identified. This textbook will detail the remarkable epidemiological and experimental findings that elucidate the mechanisms by which alcohol abuse renders the even otherwise healthy people susceptible to lung disease and will preview the novel therapies that have the promise of improving lung health in these vulnerable individuals.

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Chapter 3
Current Definitions of Alcohol Use Disorders and the Use of Validated Questionnaires in Clinical Practice and Research

Karen Drexler

Abstract Alcohol use among people varies widely from abstinence to high-risk alcohol use to addiction. Diagnostic criteria have varied somewhat over time and across the globe, but the essential features of severe alcohol use disorder, also known as alcohol dependence or alcohol addiction, share common elements. A variety of validated questionnaires have been developed to assist clinicians and researchers in screening for at-risk alcohol use and/or severe alcohol use disorders. This chapter provides a brief overview of the definitions and cardinal features of alcohol use disorders and the alcohol use questionnaires that have been developed and validated in clinical studies.

Keywords Alcohol abuse • Alcohol dependence • Alcoholism • Alcohol use disorders • Diagnosis • Screening

Introduction

Alcohol is one of the most widely used and is the most abused psychoactive substance worldwide. Initial mild intoxication causes euphoria, a feeling of relaxation and of warmth (as capillaries dilate), and increased energy. With higher amounts of alcohol ingestion, people experience a loss of inhibition, poor judgment, unsteady gait, lack of coordination, slurred speech, slowed reaction time, and drowsiness [1]. At higher blood alcohol levels, stupor, coma, and respiratory arrest can occur.

With prolonged intoxication on a daily basis over weeks to months, biological tolerance to the intoxicating effects of alcohol develops and one is able to maintain

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alertness and show diminished signs of intoxication at relatively high blood alcohol levels. When an individual has developed tolerance, signs of withdrawal often occur upon abrupt session or reduction of alcohol use. Withdrawal symptoms include anxious and irritable mood, insomnia, nausea, hand tremor, diaphoresis, tachycardia, and hypertension. More severe withdrawal can include vomiting, visual and auditory illusions or hallucinations, confusion, and withdrawal seizures. Severe alcohol withdrawal, classically termed "delirium tremens," can be life threatening due to cardiac arrhythmias and generalized tonic, clonic seizures [2].

Physiologic tolerance and withdrawal symptoms are together known as "physiologic dependence" [1, 3], which occurs when an individual has become addicted to alcohol. The biological factors that render an individual addicted to alcohol remain incompletely understood and some individuals with severe alcohol use disorders (particularly those with episodic alcohol dependence) do not manifest physiologic dependence.

Alcohol use disorders have been characterized along a spectrum from low-risk alcohol use to high-risk use to hazardous use (or mild alcohol use disorder) to alcohol dependence (or alcoholism, addiction, or severe alcohol use disorder). The majority of alcohol drinkers consume low to moderate amounts of alcohol without experiencing alcohol-related problems. These individuals are called "low-risk drinkers." According to the National Institute of Alcohol Abuse and Alcoholism (NIAAA), 72 % of adults in the USA never exceed the daily or weekly recommended limits. Other individuals (approximately 18 % of US adults) consume high amounts of alcohol without experiencing significant alcohol-related problems. These individuals are referred to as "high-risk" or "at-risk" drinkers. Unfortunately, a high percentage of heavy alcohol users experience significant alcohol-related problems. Those whose use is problematic but not compulsive are designated as "hazardous drinkers" by criteria in the 10th version of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) from the World Health Organization (WHO) [3, 4] or diagnosed with alcohol abuse or a mild alcohol use disorder by the criteria published in the 4th version of the Diagnostic and Statistical Manual of Mental Health Disorders (DSM-IV) [5]. Some heavy drinkers become addicted to alcohol when their use becomes compulsive such that they are not able to control their drinking and continue to use alcohol despite knowing that alcohol is causing significant medical, psychiatric, or other problems. By the WHO's ICD-10 guidelines and the American Psychiatric Association's DSM-IV criteria, these individuals are diagnosed with Alcohol Dependence and per DSM-V criteria the diagnosis would be further classified as a severe alcohol use disorder. The WHO diagnostic guidelines for Alcohol Dependence are illustrated in Table 3.1.

The WHO definition of psychoactive substance dependence is as follows:

> A cluster of physiological, behavioral, and cognitive phenomena in which the use of a substance or a class of substances takes on a much higher priority for a given individual than other behaviors that once had greater value. A central descriptive characteristic of the depen-

Table 3.1 WHO diagnostic guidelines for psychoactive substance dependence

A definite diagnosis of dependence should usually be made only if three or more of the following have been present together at some time during the previous year
(a) A strong desire or sense of compulsion to take the substance
(b) Difficulties in controlling substance-taking behavior in terms of its onset, termination, or levels of use
(c) A physiological withdrawal state when substance use has ceased or been reduced, as evidenced by: the characteristic withdrawal syndrome for the substance; or use of the same (or a closely related) substance with the intention of relieving or avoiding withdrawal symptoms
(d) Evidence of tolerance, such that increased doses of the psychoactive substances are required in order to achieve effects originally produced by lower doses (clear examples of this are found in alcohol- and opiate-dependent individuals who may take daily doses sufficient to incapacitate or kill nontolerant users)
(e) Progressive neglect of alternative pleasures or interests because of psychoactive substance use, increased amount of time necessary to obtain or take the substance or to recover from its effects
(f) Persisting with substance use despite clear evidence of overtly harmful consequences, such as harm to the liver through excessive drinking, depressive mood states consequent to periods of heavy substance use, or drug-related impairment of cognitive functioning; efforts should be made to determine that the user was actually, or could be expected to be, aware of the nature and extent of the harm

> dence syndrome is the desire (often strong, sometimes overpowering) to take psychoactive drugs (which may or may not have been medically prescribed), alcohol, or tobacco. There may be evidence that return to substance use after a period of abstinence leads to a more rapid reappearance of other features of the syndrome than occurs with nondependent individuals.

The terms "alcohol dependence" and "alcoholism" are synonymous with addiction to alcohol. The WHO has preferred the term "dependence"; however, the American Psychiatric Association (APA) prefers the term "substance use disorders." By comparison, the American Society of Addiction Medicine (ASAM) uses the term "addiction." ASAM issued a position statement in 2011 [6] updating their short definition of addiction as follows:

> Addiction is a primary, chronic disease of brain reward, motivation, memory, and related circuitry. Dysfunction in these circuits leads to characteristic biological, psychological, social and spiritual manifestations. This is reflected by an individual pathologically pursuing reward and/or relief by substance use and other behaviors.
>
> Addiction is characterized by inability to consistently abstain, impairment in behavioral control, craving, diminished ability to recognize problems with one's behaviors and interpersonal relationships, and a dysfunctional emotional response. Like other chronic diseases, addiction often involves cycles of relapse and remission. Without treatment or engagement in recovery related activities, addiction is progressive and can result in disability or premature death.

The ASAM definition of addiction is similar to that of the WHO and the APA, but adds information about the pathophysiology of the disorder, specifically dysfunction in brain circuits that mediate reward, motivation, memory, and inhibition. Discussion

of the neurobiology of reward and addiction is beyond the scope of this chapter, and interested readers are referred to an excellent review by Drs. Volkow and Li [7].

Low- and High-Risk Alcohol Use

The NIAAA has established guidelines for low-risk alcohol consumption [8], and these differ for men and women, and for men of different ages. For men between the ages of 21 and 64 years, the recommended amount is no more than 14 standard drinks per week and no more than 4 standard drinks on any occasion. For women of any age and for men 65 years and over, the recommendation is no more than 7 standard drinks per week and no more than 3 per occasion. What is a standard drink? A standard drink contains 14 g of pure ethanol and is equivalent to 1.5 oz of 80 proof spirits, 5 oz of table wine, 9 oz of malt liquor, or 12 oz of beer. Individuals whose alcohol consumption remains within these guidelines have less than a 1 % risk of developing an alcohol use disorder. However, those who exceed both the daily and weekly limits have about a 50 % chance of developing an alcohol use disorder at some time in their lives [8].

Many individuals suffering from a severe alcohol use disorder need medication and/or specialty care in an addiction treatment program in order to successfully abstain from alcohol use. However, selected high-risk drinkers respond well to brief interventions in primary care and other medical care settings [9, 10]. For this reason, the NIAAA and the Department of Veterans Affairs in the USA, along with the National Institute for Health and Clinical Excellence (NICE) in the UK, have recommended that general practitioners routinely screen for high-risk alcohol use.

Screening and Diagnostic Instruments

Even the most forthright individuals have difficulty quantifying their alcohol use based on the amount of alcohol (i.e., ethanol) they consume. Although the "standard drink" is defined above, in reality the preparation and consumption of alcoholic drinks, and particularly so-called "mixed drinks," makes it virtually impossible for someone to quantify their consumption based on recall alone. Therefore, a variety of questionnaires have been developed that can be used to routinely screen for high-risk alcohol use and alcohol use disorders. The NIAAA recommends a single-item screen, as follows [8]: The practitioner asks a prescreening question, "Do you sometimes enjoy beer, wine, or other alcoholic beverages?" Explicitly asking about beer and wine is important because some regard only spirits as "alcoholic beverages." If the patient answers affirmatively, then the follow-up screening question is asked: "How often in the last year did you have 5 or more drinks (for a man; 4 or more drinks for a woman) on one occasion?" If the answer to this follow-up screening question is anything other than "zero," then the screen is positive. Further questions

are needed to determine whether the person is an at-risk drinker or whether he or she may have an alcohol use disorder. If the person answers that they have not exceeded the daily limits within the past year, then the screen is negative and the practitioner is advised to remind the patient of recommended low-risk limits for alcohol use and commend the patient for healthy alcohol use.

The WHO and NICE recommend using the ten-question Alcohol Use Disorders Identification Test (AUDIT) when screening for alcohol use disorders [4, 11]. This instrument contains three questions about alcohol consumption and seven questions about symptoms of an alcohol use disorder, and each item is scored 0–4 points. The AUDIT can be self-administered on paper or on computer, and computerized versions can calculate the score and provide feedback on the individual's risk for alcohol use disorders and medical consequences. A score of 8 or more is considered positive on the ten-question AUDIT [12].

Although the ten-item AUDIT provides the information needed to screen for high-risk alcohol use and to make an alcohol use disorder diagnosis, it may be too lengthy for a busy medical practice. Therefore, the first three questions from the AUDIT about alcohol consumption have also been shown to provide an effective and valid screen for at-risk alcohol use and alcohol use disorders. The Department of Veterans Affairs Healthcare system screens enrolled veterans annually using this abbreviated version called the AUDIT-C where C' stands for "consumption." A score of 3 or more for a woman, or 4 or more for a man, is considered a positive screen on the AUDIT-C [13]. The clinician can then proceed with the full AUDIT or with a clinical interview to determine the presence of an alcohol use disorder.

Although there have been many different models for brief intervention for at-risk drinking, most have a few common elements. First, the screening and follow-up questions must be asked in a matter-of-fact and nonjudgmental manner as part of routine health screening. Because of the stigma associated with alcoholism, some patients may need reassurance that these questions are an important part of a general health screen. Secondly, if the screen is positive the clinician should determine whether an alcohol use disorder is present. If an alcohol use disorder is not present but the individual is at risk based on their consumption habits then the practitioner can recommend reducing drinking to within NIAAA guidelines or abstaining from alcohol completely, whichever is most appropriate. The NIAAA Clinician's Guide for Helping Patients Who Drink Too Much provides helpful patient education materials with strategies for cutting down or abstaining from alcohol and is a valuable tool for healthcare practitioners [8]. If an individual meets criteria for an alcohol use disorder then abstinence is recommended. Thirdly, if the patient is not willing to follow advice the clinician should reiterate their concern and encourage the patient to reflect on his or her reasons to continue drinking versus their reasons to quit, and express willingness to help whenever he or she is ready. If the individual's chief complaint or other pertinent medical conditions are exacerbated by alcohol use, the clinician should make the connection explicitly between their medical condition and their alcohol use and emphasize the importance of reducing or abstaining for health reasons. If the individual is willing to cut down or quit, the clinician should

assist them in setting a goal and provide support materials. Finally, the clinician should then follow up with questions about their alcohol use at subsequent visits. It is important to praise them for any approximations they have made toward achieving the drinking recommendations and offer to help with medication or referral to specialty addiction treatment as appropriate.

In addition to the AUDIT and AUDIT-C there are other clinician-administered and self-report questionnaires that have been used to screen for alcohol use disorders. The Michigan Alcoholism Screening Test (MAST) was the first such validated instrument [14]. There are several alternate versions of the MAST including the Short-MAST [15]. Both instruments have good reliability in men but may not be as reliable in women. They target severe alcohol use disorders and may not be sensitive to at-risk drinking.

The "CAGE" questionnaire has also been used successfully in inpatient hospital settings and trauma care settings to screen for alcohol dependence [16]. "CAGE" is an acronym for the four screening questions:

1. Have you ever felt the need to *Cut* down on your alcohol use?
2. Have you ever felt *Annoyed* by others concerns about your drinking?
3. Have you ever felt *Guilty* about your drinking?
4. Have you ever needed an *Eye-opener* first thing in the morning to treat the shakes or a hangover?

The CAGE questionnaire has the advantage of being easy to commit to memory and easy to score. A positive response to each question scores one point and even one positive answer should trigger more questions to elicit symptoms of an alcohol use disorder. Two positive responses is less sensitive, but highly specific for an alcohol use disorder. The disadvantage to the CAGE for screening is that it is unlikely to be positive for at-risk drinkers whose consumption is problematic and may be most responsive to brief interventions.

For research purposes, the Structure Clinical Interview for DSM (SCID) has been used to diagnose alcohol use disorders by DSM criteria [17–19]. It has good reliability and validity and is most often used in clinical trials to document the diagnosis of an alcohol use disorder [17, 20]. This clinician-administered questionnaire has modules for each of the major categories of mental disorders, as well as patient and non-patient versions. The entire SCID can be administered to determine substance use disorders and coexistent mental illness, or the alcohol use disorder module can be administered in isolation depending on the aims of the study. However, SCID administration requires 45–90 min for a complete evaluation, making it impractical for most clinical practices.

Summary

Alcohol use disorders span a spectrum from high-risk use to alcohol dependence or addiction. There are a variety of valid and reliable instruments available for screening for high-risk use or severe alcohol use disorders in clinical practice and research. Within clinical practice it is essential to screen everyone for underlying (and often

unrecognized) risky alcohol use and alcohol use disorders as there is overwhelming evidence that identifying such problems and providing supportive interventions can have positive effects. Within the research context and particularly relevant to the focus of this textbook, these instruments have proven to be invaluable in clarifying and quantifying the impact of alcohol use disorders on acute and chronic lung diseases.

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Chapter 4
The Epidemiology of Alcohol Abuse and Pneumonia

Kyle I. Happel

Abstract The association between alcohol abuse and pneumonia has been recognized for more than two centuries and represents an enormous health burden worldwide. The first published notation of alcohol as a clinical risk factor for the development of pneumonia is now over 200 years old, and since then there have been over a 1,000 references in the medical literature confirming these observations. Even in this modern era of medicine pneumonia remains a common infection that afflicts over 450 million persons worldwide annually and causes 7 % of all deaths. When one considers that alcohol is the most commonly abused substance in the world, the enormous excessive burden that alcohol contributes to the morbidity and mortality of pneumonia represents a major public health consideration. In this chapter we review the foundational literature that has chronicled the evolution of our understanding of the association between pneumonia and alcohol abuse over the past century. In addition, we discuss some of the specific pathogens that are particularly associated with serious lung infections in individuals with alcohol use disorders. Finally, we consider some of the specific guidelines for the treatment and prevention of pneumonia in the setting of alcohol abuse.

Keywords Alcohol abuse • Pneumonia • Risk factor • Pneumococcus • Anaerobic pathogens • *Haemophilus influenzae* • *Klebsiella pneumoniae* • *Pseudomonas aeruginosa*

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A Historical Perspective on Alcohol Abuse and Pneumonia

Most reviews on alcohol abuse and pneumonia credit Benjamin Rush, the first Surgeon General of the USA, as one of the earliest clinicians to recognize tuberculosis and pneumonia as infectious sequelae of excessive drinking, as was elaborated in his 1785 work entitled "An Inquiry Into the Effects of Ardent Spirits Upon the Human Body and Mind" [1]. This work also laid the foundation for the description of alcoholism as a disease rather than just a behavior as was the prevailing attitude at the time. Further credence to the association of alcohol abuse and pneumonia came in 1895 when Sir William Osler, often called the "Father of Modern Medicine," also advocated that excessive alcohol intake was a potent predisposing factor in the development of lobar pneumonia [2]. Interestingly, and quite ironically, at that time there were those who extolled the therapeutic use of alcohol as a *treatment* for pneumonia, particularly in cases with severe systemic manifestations. In his 1912 article, A.T. Jones of the London Royal College of Physicians describes his abandonment of therapeutic alcohol in cases of lobar pneumonia, after which his case mortality rate fell dramatically from 38 to 18 % after omitting alcohol as a component of his treatment regimen [3]. This experience led him to conclude that alcohol was expensive and of very little help, if any, in treating pneumonia and therefore that a patient's money would be likely better spent on nutrients to secure their recovery. Shortly thereafter in 1923 came a somewhat more robust description of case mortality rates observed in 3,422 patients admitted to Cook County Hospital for pneumonia from 1911 to 1922. In this work, Capps and Coleman showed both a stepwise increase in the incidence of lobar pneumonia, as well as greater case mortality rates (22, 34, or 50 %) in patients who were essentially abstainers, moderate, or heavy drinkers, respectively [4]. Even when their subjects were stratified for age, substantial differences in fatality results remained with a nearly 70 % mortality rate in heavy drinkers over the age of 60. These observations were supported by subsequent studies that associated alcohol abuse with twofold to threefold increased mortality rates from pneumonia [5, 6]. These dramatic clinical observations of an association between alcohol abuse and pneumonia prompted Branch and Stillman to perform landmark experimental studies, published in a series of papers beginning in 1924, in which they demonstrated that acute alcohol administration in mice caused septicemia and death following pneumococcal aerosolization [7]. These provocative studies in the so-called "pre-antibiotic era" identified a clear and independent mechanistic association between alcohol abuse and pneumonia and laid the foundation for intensive investigations on the part of clinical and basic science researchers that continue to this day. Unfortunately, although these research efforts have elucidated the clinical features and underlying pathophysiologic mechanisms, the devastating impact of alcohol abuse and pneumonia on our society remains as problematic as when the association was first chronicled.

Alcohol Abuse: A Persistent Risk Factor for Pneumonia in the Age of Antibiotics

While modern medicine is characterized by several notable milestone achievements, in the context of the study of infectious diseases perhaps none is greater than the serendipitous discovery of penicillin by Alexander Fleming in 1928 [8]. Subsequent large-scale synthesis of the drug for the war effort saved the lives of many allied soldiers in World War II, and soon thereafter this wonder drug found widespread civilian application as an effective antimicrobial treatment against most pathogens causing bacterial pneumonia. As a result, pre-antibiotic pneumonia mortality rates within the US population, previously estimated at 200 deaths per 100,000, plummeted to 31 per 100,000 by 1949 [9]. In parallel, rates of serious complications such as empyema also declined precipitously [10–12]. Despite these initial and subsequent advances in antibiotic therapies, alcohol abuse as a significant risk factor for pneumonia continued. For example, in a large series of alcoholics in Canada who sought treatment for their addiction, investigators identified a threefold and sevenfold increase in pneumonia death rates in female and male drinkers, respectively, when compared to nonalcoholics with pneumonia [13]. A US study performed at that same time showed that alcoholism was an underlying condition in 35 % of hospitalized pneumonia cases [14]. Further, in a prospective series of 900 admissions to Yale's Grace-New Haven Hospital, Nolan prospectively identified alcoholics as having three times the rate of hospitalization for pneumonia as nondrinkers, and nearly three quarters of alcoholics studied were admitted to the hospital for reasons considered directly attributable to their drinking [15]. In another well-performed study of enlisted US Navy personnel, Kolb demonstrated a doubling of the hospital admission rate for pneumonia in a cohort of 2,191 sailors who met criteria for alcohol abuse [16]. Further complicating matters, alcohol abusers are also at increased risk for developing *recurrent* pneumonia, as reflected by the observation that 40 % of patients with recurrent infection in one series had no other predisposing condition besides alcoholism [17]. Examining middle-aged adults in a case–control fashion, Fernandez-Sola found excessive alcohol intake as the only independent risk factor for community acquired pneumonia (CAP), and alcoholics had more severe symptoms, more multi-lobar involvement and parapneumonic effusions, and greater mortality than their nonalcoholic counterparts [18]. Even now as we are well into the twenty-first century, excessive alcohol consumption remains as an independent risk factor for the development of CAP and other serious lung infections [19–23], and the specific microbial pathogens that are overrepresented in these vulnerable individuals will be discussed next.

The Pneumococcus

When Osler characterized pneumonia as "the captain of the men of death", it was undoubtedly due in large part to the intrinsic respiratory tract virulence of *Streptococcus pneumoniae* [24]. Since then, the majority of CAP series in which a specific etiologic agent could be identified have demonstrated that *S. pneumoniae* continues to be the most common pathogen, and this remains true as well in the subset of individuals with alcohol abuse [20, 22, 23, 25–31]. Even in the modern era of antibiotics and an effective vaccine, the overall case mortality rate for pneumococcal CAP in most recent series ranges from 10 to 30 % [32–36]. However, the independent effects of alcohol abuse on mortality in individuals hospitalized for pneumococcal CAP is difficult to quantify. As previously discussed, many historical studies have shown that alcohol abuse increases the risk of CAP mortality [4–6]. Consistent with this, a more recent Spanish study identified high alcohol intake as a risk factor for CAP mortality; however, pneumococcus was not a frequently isolated pathogen in that population [18]. Further, although the study of CAP in 2004 concluded that alcohol abuse was associated with increased mortality in a univariate analysis, this association was lost in the multivariate analysis [37]. Similarly, De Roux and colleagues studied a large cohort of individuals hospitalized for CAP (the majority of whom had *S. pneumoniae* infection) and found that despite a strong association between current or previous alcohol abuse and pneumococcal pneumonia, mortality in current alcoholics was not different than in nondrinkers [38]. This finding of equivalent in-hospital pneumonia mortality in alcoholics and nonalcoholics has also been reported in other recent CAP cohorts [25, 39, 40]. These apparently conflicting results on the effect of alcohol abuse on CAP mortality are at face value confounding. However, a possible explanation for these discrepancies can be found in the results of a recent study by Goss and colleagues. Specifically, they determined that in a cohort of hospitalized low-risk CAP patients who could have otherwise been treated as outpatients, 49 % of them were alcoholics, 44 % of them were homeless, and 20 % of them were acutely intoxicated on presentation [41]. Therefore, individuals with underlying active alcohol use disorders may be admitted to the hospital for treatment for reasons unrelated to the severity of their pneumonia. If so, this could decrease their overall observed hospital mortality by including many individuals with a lower pneumonia-specific severity of illness as compared to the nonalcoholic subjects in the studies.

What appears less controversial is the increased risk for developing invasive (i.e., bacteremic) pneumococcal pneumonia in alcoholics. Using 1999 and 2000 data, the Centers for Disease Control (CDC) estimated that pneumococcal bacteremia occurs ten times more often in alcoholics compared to otherwise healthy adults [42]. Approximately 20–25 % of pneumococcal pneumonia cases are associated with bacteremia [21, 43], and mortality rates for bacteremic pneumonia are generally somewhat higher than in non-bacteremic disease [44, 45]. In a prospective series of 100

cases of proven pneumococcal pneumonia, alcohol abuse was the only demographic risk factor predictive of bacteremia [33]. Indeed, results from multiple studies in separate cohorts have consistently demonstrated clear and convincing evidence of a heightened risk for invasive pneumococcal disease, including bacteremic pneumococcal pneumonia, in alcohol abusers [25, 46–52], and alcoholics are four times more likely to die from bacteremic pneumococcal pneumonia than nonalcoholics [53]. A particularly poor outcome is seen in alcoholics with bacteremic pneumococcal pneumonia, sepsis, and leukopenia in whom mortality exceeded 80 % [54]. This series led to the coining of the term "*A*lcoholic *L*eukopenic *P*neumococcal *S*epsis Syndrome" or ALPS Syndrome. Many of these patients developed a severe form of lung injury known as the acute respiratory distress syndrome (ARDS), and a subsequent chapter in this book discusses in detail the more recently identified association between alcohol abuse and ARDS in critically ill individuals [55].

An unavoidable consequence of the widespread use of antibiotics is the eventual selection of resistant pathogens, and drug-resistant *S. pneumoniae* (DRSP) is now prevalent in several countries [56]. In the joint American Thoracic Society and Infectious Diseases Society of America CAP guidelines published in 2007, alcoholism is cited as a risk factor for infection with *S. pneumoniae* resistant to beta-lactam antibiotics [22]. A study published subsequent to these guidelines found a fivefold increased risk for DRSP infection in alcohol abusers [57], although other studies have not found such a significant association [38, 58].

Anaerobic Pathogens

Commensal anaerobes make up the dominant microbial population of the oropharynx [59] and although not particularly virulent, these pathogens are quite capable of causing pneumonia if they are aspirated into the lower airways. As a direct result of impaired consciousness and depressed cough reflex, alcohol intoxication facilitates the aspiration of substantial amounts of oropharyngeal secretions in which colonizing bacteria (commensal or otherwise) gain access to the usually sterile lower airways where they can cause pneumonia [60, 61]. Dominant pathogens in anaerobic pneumonia include *Peptostreptococcus*, *Bacteroides*, *Prevotella*, and *Fusobacterium* species, although mixed flora, including gram negative rods, are increasingly recognized participants [62–64]. Due to significant exotoxin production by anaerobic bacteria, many cases evolve into a necrotizing pneumonia or lung abscess, with or without frank empyema formation [29]. Case series of anaerobic pleuropulmonary disease have suggested that up to 70 % of cases are associated with alcohol abuse [65–70]. Fortunately, the mortality rates from these infections have dropped considerably (from 34 to 5 %) since the introduction of antibiotics, and, equally fortunately, at least to date the pathogens causing anaerobic pleuropulmonary disease generally remain antibiotic-susceptible [71].

Haemophilus influenzae

A moderate amount of evidence supports the conclusion that alcohol abuse also increases the risk of pneumonia caused by *H. influenzae*. Several studies in the mid- to late twentieth century described a high prevalence (up to 50 %) of alcohol abuse in cases of *H. influenzae* pneumonia [72–75], although there is some suggestion that alcoholic liver disease must also be present for there to be a significantly increased susceptibility to this pathogen [76]. It is not clear which capsular subtypes and/or or untypeable strains are responsible for these pneumonias in alcohol abusers, and therefore at least at this time the current CDC recommendations do not include alcoholism per se as an indication for routine *H. influenzae* B vaccination in adults [77].

Klebsiella pneumoniae

Initially described in the nineteenth century as by Friedlander and therefore known as "Friedlander's bacillus", this highly virulent gram negative rod is often found in the pharyngeal flora of alcoholics [78] and is a deadly respiratory pathogen that is often identified as the etiology of pneumonia in these susceptible individuals [14, 79–83]. Early studies noted the majority of patients with *Klebsiella* infection were alcoholic, many of whom produced a characteristically thick and bloody sputum that was often referred to as "currant jelly sputum" that was believed to consist of necrotic lung, hemorrhage, and the bacteria's mucoid capsule. Highlighting the prognostic value of the blood leukocyte count in such cases, in 1956 Limson described a series of 22 individuals with *K. pneumoniae* lung infection in whom all of those who were alcoholic and had a low or even normal circulating leukocyte count succumbed to their acute disease [84]. More recent studies continue to show an association between *Klebsiella* pneumonia and alcohol abuse including excessive rates of bacteremia and death despite aggressive antibiotic therapy and supportive management in the intensive care unit [18, 85–89]. Many of these recent (and some older) studies attest to the antibiotic resistance of this pathogen, and this challenge of antibiotic resistance has worsened in recent years [90]. Some experts now consider *K. pneumoniae* as the prototypical organisms to express extended spectrum beta lactamase (ESBL) activity [91], and more recently some *K. pneumoniae* isolates from several countries have even been found to express carbapenemase, thereby rendering them resistant to one of the most effective gram-negative antibiotic classes [92]. Therefore, clinicians caring for individuals with underlying alcohol use disorders presenting with pneumonia, particularly those who are severely ill, need to consider these factors carefully in selecting appropriate therapy.

Pseudomonas aeruginosa and Acinetobacter Species

Although not commonly considered community-acquired lung infections, pneumonias caused by virulent gram negative pathogens such as *P. aeruginosa* and *Acinetobacter* species nevertheless do occur and carry excess morbidity and mortality [22]. In a study of individuals with severe CAP, Marik found that infection with these pathogens imposed a very high mortality (82 %), and the only clinical factor that appeared to increase the risk of infection with these microbes was a history of alcohol abuse [93]. Subsequent work in Taiwan found that alcoholism frequently accompanied severe CAP caused by *Acinetobacter baumanii* [94], and it is concerning that this association was seen in relatively younger individuals. A recent and comprehensive summary of community-acquired *Acinetobacter* infections consistently identified alcohol abuse as a risk factor in the majority of case series [95]. In developed countries, pneumonia caused by these pathogens is more commonly associated with a nosocomial infection (i.e., acquired in the hospital) and usually in the context of a critically ill individual receiving mechanical ventilation. In this setting, individuals with underlying alcohol abuse experience increased rates of pneumonia with these and other drug-resistant pathogens, and they are more likely to succumb to infection than are their nonalcoholic counterparts [96–101]. As those who abuse alcohol are ostensibly forced to suspend their drinking while they are hospitalized, many develop an acute alcohol withdrawal syndrome. To complicate matters further, the occurrence of this acute withdrawal syndrome increases the risk of nosocomial pneumonia [97, 100], and the development of pneumonia in this setting is a strong predictor of increased hospital mortality [101]. Additional discussion of the care for hospitalized alcoholics can be found in a subsequent chapter (Chap. 14).

Mycobacterium tuberculosis

Just as long-recognized as the association between alcohol abuse and bacterial pneumonia is the increased incidence of pulmonary tuberculosis in the alcoholic patient [1]. Although also a bacterial pathogen, the acquisition of pulmonary tuberculosis is quite different than that of other "typical" bacterial pneumonias. While most cases of bacterial pneumonia are caused by the pulmonary aspiration of pathogen-colonized oropharyngeal secretions, *M. tuberculosis* inoculates the lung by direct inhalation of aerosolized organisms (as is true of Legionella, influenza, and other respiratory viruses) [102]. As a result, person-to-person spread is a major feature of tuberculosis not seen in most bacterial pneumonias, making it a major public health scourge over many centuries in which epidemics rise and fall over many decades within a discrete population. In this regard, an individual's environment and community strongly influence the chance of being exposed to and contracting tuberculosis. Unfortunately, individuals with chronic alcohol use disorders are more likely to be homeless and to subsequently reside in dense cohorts (e.g., shelters,

group homes, jails, and prisons), environments that are conducive to effective tuberculosis transmission [103–105]. This confounding effect has led some investigators to caution against a definitive conclusion regarding a causative effect of excessive alcohol intake on tuberculosis incidence [106–108], while others conclude that alcohol abuse does indeed confer increased susceptibility to this pathogen [109–111]. In support of a causal effect of alcohol abuse on pulmonary tuberculosis is a collective body of experimental evidence from animal studies showing that a multitude of pulmonary host defenses mechanisms against tuberculosis are adversely impacted by alcohol [112–115]. For example, live infection with tuberculosis in a mouse model has shown that chronic alcohol feeding is associated with impaired pulmonary clearance of pathogen, a defect likely caused by reduced T cell responses and granuloma formation in the lung [115]. More recently, statistically robust international analyses of the association between alcohol use and tuberculosis conclude that alcohol abuse confers a nearly threefold increase in the relative risk for active tuberculosis infection [116, 117]. Equally concerning from a clinical perspective are the findings that active alcohol users exhibit altered pharmacokinetics of anti-tuberculous medicines, and, not surprisingly, are less compliant with their treatment regimen [108, 118]. The latter facet is likely a key determinant in the alarming findings that alcoholics are more likely to become reinfected with tuberculosis and are more likely to harbor a drug-resistant tuberculosis strain [119–121]. While the emergence of infection with the human immunodeficiency virus (HIV) is the overwhelming factor underlying the global resurgence of tuberculosis rates, it is nevertheless still currently estimated that 10 % of tuberculosis cases worldwide can be attributed to alcohol abuse [117]. A thorough discussion of the pulmonary sequelae of alcohol abuse and HIV infection is provided in a subsequent chapter (Chap. 15).

Alcohol and Pneumonia: The Current Socioeconomic Burden

According to the 2010 National Survey on Drug Use and Health, 16.9 million Americans reported heavy alcohol use, representing 6.7 % of the US population over the age of 12 [122]. Globally, the World Health Organization estimates that 2.5 million persons die each year as a result of alcohol use [123], which represents 4 % of global deaths and reflects a greater impact than the number of deaths caused by HIV, violence, or tuberculosis. In fact, worldwide alcohol abuse is the third greatest risk factor for the development of disease and disability, and in upper middle income countries it is now the greatest risk factor, with Russia and the former Soviet Union countries exhibiting some of the highest alcohol-attributable death rates. Although the exact contribution of alcohol to current pneumonia incidence and death is difficult to determine, a recent and large meta-analysis by Samokhvalov demonstrated a dose–response curve to drinking and CAP incidence, with individuals clinically classified as having an alcohol use disorder exhibiting eight times the risk for CAP [124]. Given these findings and recent data indicating that pneumonia and influenza were the 8th leading cause of death in America in 2011 [125], it is

clear that alcohol abuse remains a pertinent, pervasive, and potentially modifiable adverse health factor from a public health policy perspective.

The estimated total cost of excessive alcohol consumption in the USA alone in 2006 was over 200 billion dollars [126]. While only a part of this cost is direct healthcare utilization, this figure nevertheless reflects the considerable economic burden of alcohol abuse to our society. A study of pneumonia hospitalizations in Massachusetts found that cases associated with alcohol-related diagnoses had a longer length of stay, a 50 % greater intensive care unit utilization, and a significant increase in hospital charges—although mortality rates were similar (10 %) [127]. A more recent large cross-sectional study of Danish persons aged 50–64 revealed a nearly doubling of risk for pneumonia hospitalization in heavy drinkers, as defined by greater than 50 drinks per week [128]. Similar studies from European nations have also demonstrated a greater length of stay, increased ICU use, and increased need for mechanical ventilation in alcohol abusers with CAP [38, 39, 129–131]. A further contributor to the economic burden of pneumonia in alcoholics is the previously discussed need to hospitalize a greater proportion of these patients than would otherwise be warranted on the basis of their infection severity [41]. A similar picture has emerged in the context of hospital-acquired pneumonia; specifically, alcoholics are more likely to develop nosocomial pneumonia despite a lower overall illness acuity, and the cost to care for such events is substantial [98, 132]. Even after discharge from the hospital following a pneumonia admission, alcohol abusers are more likely to return to a primary care center or emergency department within 30 days of discharge [133].

Preventative measures against bacterial pneumonia in the individuals suffering from alcohol use disorders at present are essentially limited to addressing the alcohol consumption behavior itself and vaccination against infection, and more data are needed to assess the cost effectiveness of these expenditures in preventing pneumonia. Currently, the CDC Advisory Committee on Immunization Practices recommends pneumococcal and yearly influenza vaccination for all alcohol abusing persons [134, 135]. Unfortunately, in practice there appears to be less frequent vaccination against these pathogens in alcohol abusers, even in those with additional risk factors for pneumonia such as COPD or advanced age [136–139]. This finding is quite disappointing, particularly as the available clinical data support the efficacy of the 23-valent polysaccharide pneumococcal vaccine in heavy drinkers, including those who are elderly [140, 141]. Whether the newer peptide-conjugate pneumococcal vaccine is of lesser, equal, or greater benefit in heavy alcohol users is yet to be determined, and therefore at the time this chapter was prepared its administration to this population is not currently recommended.

Summary

Alcohol abuse remains common throughout the world, and the medical literature is incontrovertible in its depiction of alcohol abuse as a risk factor for both an increased incidence and severity of bacterial pneumonia, whether acquired in the community

or the hospital setting. The spectrum of pathogens causing pneumonia in the alcohol abuser is somewhat wider and more virulent than in non-abusers, such that the clinical history of alcohol abuse has a direct impact on the clinical care and outcome of such patients. In an era of spiraling global health care costs and regionally scarce health care resources, it would seem prudent to continue or even expand efforts to determine optimal prevention and treatment strategies for bacterial pneumonia and other sequelae of alcohol abuse in these persons. Such measures could have a significant effect not only on the outcome of disease for an individual, but also on the incidence of disease and alcohol's overall economic burden to society. Although not discussed in this chapter, the association between alcohol and other health concerns such as HIV infection, tobacco use, and illicit substance use further supports comprehensive and robust research programs to identify and intervene in those alcohol abusing populations most amenable to response.

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Chapter 5
The Epidemiology of Alcohol and Acute Respiratory Distress Syndrome

Brendan J. Clark and Ellen L. Burnham

Abstract The acute respiratory distress syndrome (ARDS) is defined by the acute onset of hypoxemic respiratory failure and alveolar infiltrates in the absence of an elevated left atrial pressure. ARDS is a common cause of admission to an intensive care unit with nearly 200,000 reported cases in the USA each year. Likewise, alcohol misuse, or consumption of alcohol in excess of recommended limits is common in the setting of critical illness and present in up to 40 % of patients admitted to an ICU. The epidemiologic evidence reviewed in this chapter demonstrates a clear association between alcohol misuse and *common risk factors for ARDS*, as well as an independent association between alcohol misuse and the *development of ARDS*. Furthermore, the presence of severe alcohol misuse is independently associated with poor outcomes in patients with established ARDS. The consistency of these findings and the translational studies that we review provide a compelling case that the association between alcohol misuse and poor outcomes in ARDS is causative.

Keywords Lung injury • Pneumonia • Oxidative stress • Critical care

Introduction

The acute respiratory distress syndrome (ARDS) is heralded by the acute onset of hypoxemic respiratory failure and bilateral alveolar infiltrates in the absence of evidence of an elevated left atrial pressure [1]. ARDS is commonly encountered in the intensive care unit, with nearly 200,000 reported cases in the USA each year, with

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an associated mortality in observational studies of 40 % [2]. Likewise, alcohol misuse is common in patients admitted to the ICU with up to 40 % of ICU patients consuming alcohol in excess [3]. The epidemiologic evidence reviewed in this chapter has driven an understanding that the intersection between alcohol misuse and ARDS is more than just a coincidental meeting of two commonly encountered conditions. We first review the definitions of alcohol misuse, and the effect of alcohol misuse on the development of common risk ARDS factors. We also review the epidemiologic evidence linking alcohol misuse with the development of ARDS, and with poor outcomes once ARDS is established. Finally, we briefly summarize the current understanding of the pathophysiology that suggests a causal inference between alcohol misuse and the development of pneumonia, ARDS, and poor ARDS outcomes.

Defining Alcohol Misuse, Alcohol Abuse, and Alcohol Dependence

As highlighted in previous chapters, *alcohol misuse* refers a spectrum of excess alcohol consumption [4]. On the mild end of the spectrum, *at-risk drinking* is present when excess alcohol consumption is not associated with adverse consequences. When drinking is associated with significant impairment or distress, by the criteria established in the Diagnostic and Statistical Manual, fourth edition (DSM-IV), *alcohol abuse* is present. The DSM-IV also establishes criteria for *alcohol dependence* that include evidence of tolerance, withdrawal in addition to unsuccessful efforts to cut down. Alcohol abuse and dependence are often collectively referred to as an *alcohol use disorder (AUD)* [5].

The epidemiologic studies discussed in this chapter use a variety of methods to establish the presence of alcohol misuse. Validated questionnaires such as the Short Michigan Alcohol Screening Test (SMAST) and the Alcohol Use Disorders Identification Test (AUDIT) were developed as sensitive instruments to detect alcohol misuse [6, 7]. Higher scores are associated with more severe alcohol misuse and an increased probability of having alcohol abuse or dependence. However, these screening tests by themselves do not establish a diagnosis of alcohol abuse or dependence. Alternatively, physician diagnosis can be relied upon to diagnose alcohol abuse or dependence. Physicians in the inpatient medical and surgical setting are more likely to identify the severe end of the spectrum of alcohol misuse (i.e., patients with severe alcohol abuse or alcohol dependence), thus classifying some patients with moderate alcohol misuse as low-risk drinkers [8]. Because of the heterogeneity used to identify and describe alcohol misuse in these studies, *we will highlight the method of defining alcohol misuse in each of the studies discussed in this chapter to most accurately characterize the severity of alcohol misuse present in the study population, and to facilitate between studies comparisons.*

Association of Alcohol Misuse with ARDS Risk Factors

Patients who have certain pulmonary conditions, including pneumonia or the aspiration of gastric contents, as well as specific non-pulmonary conditions, such as sepsis, trauma, or receiving multiple blood product transfusions, place patients "at risk" for ARDS. Published epidemiologic data support a strong relationship between alcohol misuse and pneumonia and sepsis that has ramifications on the association between alcohol use and ARDS.

Pneumonia. Individuals with alcohol misuse worldwide are disproportionately affected by *S. pneumoniae* pulmonary infections. One Spanish study of 1,347 patients hospitalized with community-acquired pneumonia determined that 10 % had a history of alcohol dependence, defined as daily alcohol consumption of >80 g for men and >60 g for women during the last 2 years before hospitalization. Patients with alcohol dependence were more likely to be infected with pneumococcus when compared to patients without alcohol dependence [9]. In a subsequent Spanish series of over 1,041 consecutive patients with pneumococcal pneumonia, approximately 20 % of patients were characterized as having heavy alcohol consumption (>80 g of alcohol per day for at least the past year) [10]. In another series of 1,039 adults from Toronto, Ontario, CA, who were assessed between 2002 and 2006, 18 % of patients with invasive pneumococcal disease had a history of alcohol abuse [11]. A 2-year prospective study (2000–2002) of 129 patients with laboratory-proven bacteremic pneumococcal pneumonia in Edmonton, Alberta, CA determined that while 12.7 % of inpatients hospitalized for bacteremic pneumococcal pneumonia had a history of alcohol abuse, an even higher 34.5 % of critically ill ICU patients hospitalized for this reason carried an alcohol abuse comorbidity [12].

These reports echo earlier observations from Argentina where among 101 patients with bacteremic pneumococcal pneumonia alcoholism, cigarette smoking, COPD, and CHF were the most common comorbid associated conditions [13]. More recently, in a study of 222 French patients admitted to the ICU for severe community-acquired pneumococcal pneumonia between 2001 and 2008, the prevalence of alcohol abuse among patients was 29 % (65/222); along with tobacco use, alcohol misuse was one of the two most common comorbidities present in this cohort [14]. A systematic review and meta-analysis examining the dose–response relationship between alcohol consumption or alcohol use disorders and the incidence of community-acquired pneumonia (CAP) determined that the relative risk for CAP increased monotonically as the daily amount of alcohol increased from 24 to 60 g and finally to 120 g of pure alcohol daily. Patients with clinically defined alcohol use disorders had an associated risk ratio of 8.22 (95 % CI, 4.85–13.95) for developing CAP [15].

Along with an increased risk for pneumonia, an increased *severity of illness* in the setting of pneumonia among patients with alcohol misuse has also been reported. For example, in DeRoux et al.'s Spanish series of patients hospitalized with CAP,

patients with alcoholism were more likely to develop bilateral or multilobar pneumonia, were more likely to require ICU admission, and were more likely to require mechanical ventilation [9]. In a 13 year series of 4,715 cases of CAP, 261 (6 %) developed an empyema or complicated parapneumonic effusion. In patients with CAP and a pleural effusion, the presence of alcoholism was associated with an odds ratio of 2.09 (95 % CI 1.3, 3.36) for developing an empyema or complicated parapneumonic effusion [16]. This finding validated earlier investigations performed in the UK where alcohol misuse was independently associated with empyema or complicated parapneumonic effusion [17].

In summary, alcohol misuse is commonly found in individuals with CAP, particularly pneumococcal pneumonia. Moreover, when pneumonia develops in individuals with alcohol misuse, its course tends to be more severe, requiring ICU-level care, and additional therapy to address extra-pulmonary extension of disease.

Sepsis. Sepsis, or the systemic spread of infection, can be a further complication of pneumonia but can also occur in the setting of other primary infections (e.g., intra-abdominal infections). The incidence of sepsis and septic shock (sepsis with cardiovascular dysfunction and need for blood pressure supporting agents) in association with poorer outcomes has been reported among patients with alcohol misuse, as illustrated in a large administrative cohort from a single urban medical center in Denver, CO. Using these data, investigators examined whether the presence of alcohol dependence was independently associated with the development of sepsis. Of the 9,981 unique patient admissions over a 6 year period, 1,222 (12 %) had alcohol dependence. The presence of alcohol dependence was associated with higher rates of sepsis (12.9 % vs. 7.6 %, $p<0.001$), organ failure (67.3 % vs. 45.8 %, $p<0.001$), septic shock (3.6 % vs. 2.1 %, $p=0.001$), and hospital mortality (9.4 % vs. 7.5 %, $p=0.022$) [18]. These associations between alcohol dependence and the development of sepsis, septic shock, and mortality remained after adjustment for known confounding variables, and, interestingly, were mostly confined to the population of patients who did not receive a blood transfusion. Further supporting the association between alcohol misuse and the development of sepsis, in the Garcia-Vidal series of over 1,000 Spanish patients with pneumococcal pneumonia, approximately 10 % of the entire cohort presented with shock (114/1,041), and again, patients with heavy alcohol consumption comprised 20 % (23/114) of those patients [10].

Alcohol Use and ARDS Risk

The independent association between alcohol misuse and the development of ARDS was first described by Moss and colleagues in 1996 [19]. In a single center, prospective cohort study conducted in 351 patients with one of seven at-risk diagnoses for the development of ARDS, physician diagnosis was used to identify alcohol abuse. Using this method, alcohol abuse was found to be present in 34 % of the at-risk patients, supporting previously described studies of the association between alcohol abuse with pneumonia and sepsis. In a univariate analysis, alcohol abuse was

associated with a near doubling of the risk of developing ARDS, from 22 % in patients without alcohol abuse to 43 % in patients with alcohol abuse ($p<0.001$). After adjusting for gender, at-risk diagnosis, and severity of illness measured by APACHE II scores, patients with an ARDS risk factor and alcohol abuse had 2.79 (95 % CI 1.68, 4.83) times the odds of developing ARDS when compared to patients without alcohol abuse. When stratified by ARDS risk factor, the effect of alcohol abuse on the development of ARDS was most pronounced in patients with septic shock.

Building on this single center study, Moss and colleagues conducted a multicenter cohort study in patients with septic shock in order to prospectively confirm the independent association between alcohol misuse and the development of ARDS [20]. The authors defined chronic alcohol abuse as a SMAST score ≥3. Of the 220 patients enrolled between 1995 and 1999, 30 % had chronic alcohol abuse. In a univariate analysis, the incidence of ARDS in patients with chronic alcohol abuse was 70 % compared to 31 % in patients without a history of chronic alcohol abuse ($p<0.001$). This association persisted after adjustment for potential confounding variables (adjusted OR 3.70; 95 % CI 1.83, 7.71; $p<0.001$).

Several other epidemiologic studies have confirmed an association between alcohol misuse and ARDS. Licker and colleagues conducted a retrospective cohort study in 879 patients undergoing thoracic surgery for non-small cell lung cancer at a single institution [21]. Chronic alcohol abuse was defined as daily consumption of five or more drinks for months or years and was not further stratified based on severity of alcohol misuse. Chronic alcohol abuse was associated with nearly twice the odds of developing ARDS (OR 1.87; 95 % CI 1.09, 4.56; $p=0.012$) in the postoperative setting, and was one of four identified independent risk factors in this cohort. In a case–control study examining risk factors for the development of transfusion related acute lung injury, chronic alcohol abuse was more common in patients who developed TRALI [22]. The association between alcohol abuse and ARDS has also been confirmed in a national survey conducted in the Netherlands [23].

Alcohol Misuse and ARDS Outcomes

Understanding the results of studies examining the effect of alcohol misuse on ARDS outcomes first requires knowledge of how alcohol use is stratified in epidemiologic studies. A u-shaped association between alcohol consumption and poor outcomes has consistently been reported in the literature, such that individuals with *low-risk alcohol use* have a lower risk of adverse outcomes when compared to individuals who are *abstinent* [24]. Several potential explanations for these associations are possible. First, abstinence may be associated with unmeasured comorbidity which, in turn, could disproportionately influence outcomes [25–28]. Second, individuals who are abstinent are more likely to have lower socioeconomic status which is known to be linked to poor health outcomes in the USA [29]. Finally, individuals with alcohol dependence who have stopped drinking may be

misclassified as being abstinent, regardless of the duration of their sobriety [30]. Therefore, ideally, patients with low-risk alcohol use (and not those with abstinence) should be used as the control group when examining the influence of alcohol consumption on clinical outcome variables. With these caveats in mind, one can compare investigations that have specifically examined outcomes in ARDS in patients with alcohol misuse.

In the previously discussed prospective cohort study [19], the effect of alcohol abuse on mortality was examined in the 29 % who developed ARDS. In the subgroup of patients with ARDS, unadjusted in-hospital mortality rate for patients with alcohol abuse was 65 % compared to 36 % in patients without alcohol abuse ($p=0.003$), an association that remained significant after adjustment for severity of illness as measured by APACHE II scores (adjusted OR 6.26; 95 % CI 2.22, 20.38; $p=0.001$). Notably, in this observational study, alcohol abuse was identified using physician diagnosis that possibly biased investigators towards including patients with more severe alcohol abuse or dependence, but excluding those with less severe alcohol misuse.

In the follow-up prospective cohort study that employed a more rigorous definition of alcohol abuse (e.g., SMAST score ≥ 3) to examine its association with ARDS in patients with septic shock, the influence of alcohol abuse on mortality among severely septic patients who subsequently developed ARDS was examined [20]. In these 93 patients, alcohol abuse was not associated with higher mortality (59 % vs. 55 %, $p=0.74$). Unlike the previous study, use of the SMAST may identify and therefore promote inclusion of patients with less severe forms of alcohol misuse at this cutoff and, thus, potentially explain the discrepancy in these two investigations.

The most recent comprehensive study examining the association between alcohol misuse and outcomes in patients with established ARDS was a secondary analysis of patients enrolled in three treatment trials conducted by the National Heart Lung and Blood Institute ARDS network [31]. AUDIT scores were used to classify the 1,133 patients into four groups: abstinent, low-risk alcohol use, mild–moderate alcohol misuse, and severe alcohol misuse. Unadjusted rates of death or rehospitalization were 34 %, 26 %, 27 %, and 36 % for patients with abstinence, low-risk alcohol use, mild–moderate alcohol misuse, and severe alcohol misuse, respectively, with significant differences observed between low-risk alcohol users and patients who were either abstinent ($p<0.05$) or who had severe alcohol misuse ($p<0.05$) (Fig. 5.1). After adjusting for comorbidities, severity of illness, and other baseline differences between groups, the higher rate of death or rehospitalization in patients with abstinence when compared to low-risk alcohol use was no longer significant. However, after adjusting for the same confounding variables, the higher rate of death or persistent hospitalization in patients with severe alcohol misuse when compared to those with low-risk alcohol use persisted (adjusted OR 1.70; 95 % CI 1.00, 2.87; $p=0.048$), suggesting that the u-shaped association between alcohol use and poor outcomes reported in other medical contexts extends to the setting of ARDS. The low overall mortality observed in this more recent investigation in comparison to the two previously published observational studies may be

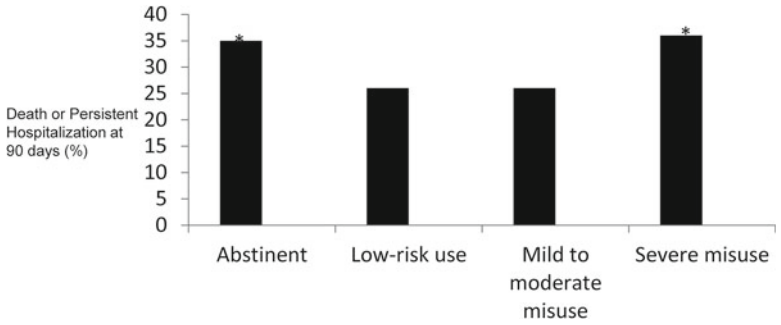

Fig. 5.1 Unadjusted rates of death or persistent hospitalization at 90 days in patients with abstinence, low-risk alcohol use, mild to moderate misuse, and severe alcohol misuse. *$p<0.05$ for comparison with zone 2 (modified from [32])

explained by differences in inclusion and exclusion criteria across these studies. For example, in the ARDS Network studies, patients with class C cirrhosis were excluded, while no such criteria were present in earlier investigations. Additionally, improvement in the care of ARDS patients, most notably the widespread use of low tidal volume ventilation, has likely extended a beneficial effect to patients with ARDS more generally [32].

Pathophysiology of Alcohol Misuse and ARDS Development

Alcohol's Effects on Innate Immunity

Given the predisposition for the development of pneumonia among those with alcohol misuse highlighted by epidemiologic investigations, understanding the pathophysiologic mechanisms of alcohol on lung immunity could inform new strategies to diminish the risk of pneumonia, or to decrease the likelihood of ARDS development that may occur due to pneumonia. We will focus on clinical investigations that highlight some potential reasons why alcohol misuse may contribute to pneumonia development.

Alveolar macrophages represent a first line of defense against small inocula of invading pathogens, where they function in maintaining lung sterility. They also secrete specific cytokines, chemokines, and growth factors necessary to initiate an appropriate immune response. Tumor necrosis factor (TNF)-α is one such pro-inflammatory cytokine integral to the early innate immune response. Ex vivo stimulation of alveolar macrophages by lipopolysaccharide (LPS), a component of the gram negative cell wall, was demonstrated to result in less tumor necrosis factor (TNF)-α release by cells from chronic alcohol misusers, regardless of smoking history [33]. Given the key early role in the immune response, decreased

production of TNFα could have important downstream effects and subsequently impair the overall immune response to pathogens. Chronic alcohol misuse has also been associated with enhanced NADPH oxidase (Nox) expression by alveolar macrophages in the absence of overt lung disease. Noxes are primary sources of reactive oxygen species (ROS) generation in the lungs under physiologic conditions. Since ROS are known to have an important influence on cell signaling (e.g., via transcription factors such as NFkB) and apoptosis [34], it follows that excessive ROS production by alveolar macrophages could have a prominent role in modulating immunity to microbial pathogens in lung. Additional studies have examined gene expression differences by alveolar macrophages in subjects with alcohol misuse compared to healthy controls, in the absence of infectious stimuli. In this setting, an increase in gene expression for proteins relevant to programmed cell death, including upregulation of vascular endothelial growth factor (VEGF), perforin-1, granzyme, superoxide dismutase-2, and CXCR4 were reported [35]. The interaction of perforin and granzyme, when released by their manufacturing cells, is to induce apoptosis in target cells. While such activity could be beneficial in clearing the lung of dead and dying cells to limit excessive release of pro-inflammatory cytokines, excessive apoptosis may contribute to the dissemination of infection via unnecessarily eliminating host defense cells still needed to appropriately clear pathogens [36].

Finally, the presence of adequate quantities of antimicrobial substances within lung to combat invading pathogens may also be important in averting pulmonary infections. These "natural antibiotics" may be produced by myeloid cells within the lung, including alveolar macrophages as well as alveolar epithelial cells. Our group observed that epithelial lining fluid from subjects with alcohol misuse had less potency in killing live pneumococcus than that of healthy subjects that could be partially explained by differences in the quantity of lactoferrin and activity of lysozyme present in the airspace [37], the quantitatively most abundant antimicrobials in lung. Moreover, alveolar macrophages of patients with alcohol abuse cultured ex vivo secreted a decreased quantity of these antimicrobial proteins when compared to alveolar macrophages of patients without alcohol abuse.

Alcohol's Effects on Oxidative Stress and Alveolar-Epithelial Permeability

The association between ARDS and intrapulmonary oxidative stress mediated via abnormal glutathione homeostasis was reported two decades ago [38, 39]. In these studies, ARDS patients were found to have significantly decreased total glutathione in their alveolar-epithelial lining fluid with an increased percentage in its oxidized form, when compared to normal subjects or patients with cardiogenic pulmonary edema. Glutathione is manufactured in the liver and transported to the lung where its concentration is manifold higher than what is

measured in plasma. Given its manufacture by the liver, the effect of alcohol misuse on pulmonary glutathione homeostasis is probable, and in fact, both alcohol use disorders, and alcohol-associated cirrhosis have been demonstrated to be associated with decreased reduced glutathione in epithelial lining fluid, along with an increase in the percentage of its oxidized form, GSSG, analogous to what has been reported in ARDS [40, 41]. Pulmonary glutathione homeostasis remains virtually unchanged in individuals with alcohol misuse despite 7 days' abstinence from alcohol [42, 43], even when oral nutraceuticals are administered specifically to modulate oxidative stress [43]. Importantly, increased oxidative stress indices, as indicated by abnormal glutathione homeostasis, may be detected in exhaled breath condensate collected from subjects with alcohol misuse, suggesting the possibility of noninvasively identifying patients with the most abnormal glutathione homeostasis for therapeutic interventions [44]. Nevertheless, although glutathione repletion may represent a modality to normalize oxidative stress in these individuals, optimal therapies that would serve in this capacity and their appropriate route for administration remain to be established.

Another cardinal feature of ARDS is abnormal alveolar-epithelial permeability and the development of non-cardiogenic pulmonary edema with attendant respiratory failure. Alcohol has been associated with increased permeability involving a variety of organ systems including the gut and the blood–brain barrier [45, 46]. Based on these data, our group has previously examined the influence of alcohol misuse on alveolar-epithelial barrier function in human subjects. We first established that protein concentrations in epithelial lining fluid among individuals with alcohol misuse are increased, and do not decrease significantly after a week of abstinence from alcohol [42]. In a separate study, subjects with alcohol misuse and healthy controls group matched for cigarette smoking were administered an inhaled radiolabelled isotope, and the washout of the isotope was measured over a 2 h period. The half-life of the isotope in lung was found to be significantly shorter among the group with alcohol use disorders, indicating differences in alveolar-epithelial permeability associated with alcohol consumption [47]. Additional investigations have been performed using a semi-invasive PiCCO® catheter system to assess extravascular lung water (EVLW) as a means to estimate alveolar-epithelial permeability in the context of alcohol misuse and critical illness. When severely septic patients who had a history of alcohol misuse were followed prospectively, those who ultimately developed ARDS had a mean increase in extravascular lung water almost twofold greater than septic patients who went on to develop ARDS, but who did not misuse alcohol [48]. Subsequent investigations by this group examining PiCCO catheter assessments of EVLW for a period of 7 days after the development of ARDS confirmed that EVLW was indeed significantly higher among ARDS patients with alcohol misuse, and the resolution of EVLW was significantly delayed in the group who misused alcohol [49]. Collectively, these data suggest abnormalities in alveolar-epithelial permeability could enhance the development of non-cardiogenic pulmonary edema in

the setting of ARDS, and also hamper its resolution, thereby contributing to the poorer outcomes among this population.

Conclusion

Alcohol misuse remains one of the most common comorbidities in ICUs in the USA and worldwide. The intensity and duration of alcohol use has not been characterized consistently throughout the literature, and subsequently, direct comparisons and accurate quantification of the magnitude and effects of alcohol in the setting of critical illness have been challenging. However, the consistent observations across numerous studies of alcohol misuse in association with common ARDS risk factors, its frequent association with ARDS development, and with poorer ARDS outcomes suggest that alcohol use is a causal factor in the pathophysiology of ARDS. Translational investigations support specific mechanisms underlying these associations. Refining our understanding of the epidemiology underlying alcohol's association with ARDS will drive future research efforts aimed at attenuating the incidence and morbidity of ARDS among those with alcohol misuse.

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Part II
The Pathophysiology of the "Alcoholic Lung"

Chapter 6
Alcohol, the Upper Airway, and Mucociliary Dysfunction in the Conducting Airways

Todd A. Wyatt and Joseph H. Sisson

Abstract Innate mucosal defense in the airways of the lung involves mucociliary clearance of inhaled particles, microbes, and toxins. Alcohol consumption results in both rapid and transient stimulation of the frequency of cilia beat as well as a prolonged exposure-mediated desensitization of cilia stimulation leading to diminished clearance. This alcohol-induced ciliary dysfunction (AICD) is the result of the direct exposure of the airways to condensed alcohol from the bronchial circulation during exhalation. In addition, alcohol can alter mucus production, pro-inflammatory cytokine release, barrier function, cell migration during wound repair, and smooth muscle airway hyperresponsiveness in exposed airways. Such alterations impact normative airway functions in response to other inhaled injurious agents such as viruses, cigarette smoke, and organic dusts.

Keywords Cilia • Airway epithelial • Mucociliary clearance • Nitric oxide • cAMP • cGMP • PKC

Abbreviations

PKG	cGMP-dependent protein kinase
PKA	cAMP-dependent protein kinase
PKC	Protein kinase C
PKCε	PKC epsilon

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AICD	Alcohol-induced ciliary dysfunction
HSP90	Heat shock protein 90
eNOS	Endothelial isoform of NO synthase
CBF	Ciliary beat frequency
AUDs	Alcohol use disorders
RSV	Respiratory syncytial virus
MAA	Malondialdehyde and acetaldehyde
SPD	Surfactant protein D
CAFO	Confined animal feeding operation

Introduction

The first line of cellular defense in the innate protection of the lung against disease is the mucociliary apparatus. This consists of mucus secretions lining the airways to trap inhaled particles and pathogens, which are then propelled up and out of the lungs via the whip-like action of the cilia. Because the action of the cilia is regulable, the speed at which the cilia move can govern this clearance. Therefore, environmental exposures can modify ciliary beating speed and thus impact the effectiveness of mucociliary defenses. Agents that slow cilia enhance the persistence of a toxin or microbe in the airway, leading to changes in lung injury and repair. Thus, normal protection against environmental inhalation injury is compromised by co-exposure to those factors that impair effective lung innate mucociliary protection.

Alcohol interferes with mucociliary clearance in a wide variety of animal models including cat [1], rabbit [2], and mouse [3], as well as in the human [4]. Studies addressing the mechanisms of cilia dysfunction in response to alcohol consumption in the context of innate lung defense against both viral and bacterial pathogen infection are limited. Likewise, only a few infection models of human lung disease with regard to chronic lung disease exacerbations have been conducted. Acknowledging the impact of alcohol on other upper airway defenses against airway infection including antimicrobial saliva production [5], the cough reflex [6], and macrophage function [7], this chapter focuses exclusively on the mechanisms by which alcohol compromises lung mucociliary defense against inhalation injury.

Cilia Action

Trapped particles are cleared from the lungs very effectively by what we commonly refer to as the “ciliary flight response.” This term describes the significant (>2 Hz) increase in the beat frequency of the cilia after response to a stimulus. Waveform and frequency of cilia motion are regulated differentially. The cilia are always in a baseline state of motion regulated by the utilization of calcium and adenosine triphosphate (ATP). Changes in temperature, pH, mechanostimulation, and

chemostimulation all lead to changes in cilia beating. However, enhancements to the speed of cilia beat are produced by cellular elevations in cyclic nucleotides. Agents that stimulate the production of nitric oxide lead to the guanylyl cyclase-mediated production of cGMP and protein kinase G (PKG)-mediated stimulation of increased cilia beat frequency [8]. In a parallel pathway, beta agonist-stimulated increases in adenylyl cyclase-mediated production of cAMP and protein kinase A (PKA) stimulation also result in increased cilia beating. Either of these cyclic nucleotide kinases, when activated, is capable of phosphorylating a ciliary dynein-associated ATPase. This ATPase provides the necessary energy for the increased motion of the cilia beat.

Conversely, little is known about the process by which cilia are slowed. In response to many toxicants, significant decreases in cilia beating have been observed. Cilia-slowing toxicants are generated by viruses, bacteria, and fungi. Oxidants and particles contained in air pollution or cigarette smoke are also capable of slowing cilia. Mechanisms of cilia slowing have been proposed to consist of cyclase inhibition, phosphatase activation, and protein kinase C (PKC) activation. Interestingly, a host of agents (phorbol esters, sodium metabisulfite, calcitonin gene-related peptide, aldehyde adducted proteins, and neuropeptide Y) that have known cilia-slowing characteristics all share the characteristic of being PKC epsilon (PKCε) activators. Some agents, such as alcohol (ethanol), can both stimulate and depress cilia action.

Alcohol in the Airways

Upon oral ingestion, the majority of alcohol consumed is delivered by first pass to the liver via the hepatic portal vein where phase one metabolizing enzymes (principally alcohol dehydrogenase) effectively metabolize it into acetaldehyde. Subsequently, much of this acetaldehyde is then metabolized into acetate via the action of aldehyde dehydrogenase and excreted. However, a significant amount of alcohol enters the bloodstream through direct tissue permeability throughout the oral–esophageal–intestinal pathway. This portion of alcohol is subject to lung exhalation as the principal excretion mechanism. Indeed, such an excretory pathway is the basis of the Breathalyzer™ test [9]. Less appreciated is the nature of such concentration of alcohol into the airways of the lung. Through simple diffusion, alcohol moves from the bronchial circulation directly through the ciliated epithelium and vaporizes into the conducting airways where it can either be exhaled or condensed as a liquid back onto the airways [10]. Importantly, such condensation or "rain-effect" has the potential to concentrate alcohol in the conducting airways for direct and extended exposure to the airway epithelium in a manner unique to the conducting airways of the lung. Thus, the innate defense function of the airway epithelium is readily susceptible to modification by alcohol whether it is mucus production, ciliary clearance, or barrier protection under physiologic conditions, and cytokine production or wound repair under pathologic conditions.

Alcohol-Induced Ciliary Dysfunction (AICD)

Our group was the first to show that nitric oxide (NO) could stimulate increases in cilia beating using cellular models in vitro [11]. Similarly, direct exposure of cultured ciliated cells to alcohol results in rapid (1 h) and transient NO-mediated increases in cilia beating [12]. This observation was recapitulated in an organelle model system. Very low concentrations of alcohol (10 mM) rapidly (by 1 min) stimulated isolated demembranated cilia to beat faster in a cell-free system indicating that the alcohol cilia activation machinery is present and functional in the organelle [13]. Recent studies have determined that alcohol triggers this rapid and transient stimulation of ciliary beating by activating the chaperone function of heat shock protein 90 (HSP90) via threonine phosphorylation of HSP90. This activation increases HSP90's association with the endothelial isoform of NO synthase (eNOS) in cilia [14]. Once activated, HSP90 promotes the translocation of eNOS and likely other cilia activating enzymes from the cilia basal body up into the cilia axoneme, where the cilia motor molecules called dyneins are located, causing ciliary beat frequency (CBF) to speed up. Importantly and in contrast to the stimulatory effect of brief and modest concentrations of alcohol on airway cilia, a very different effect on cilia motility occurs when the airways are exposed to alcohol for sustained periods of time.

Longer exposures (>6 h) to alcohol not only fails to stimulate cilia beating, but this more "chronic" exposure to alcohol actually desensitizes the ciliated cell to the actions of any other ciliostimulatory agents (such as beta agonists) [15]. Subsequently, it was identified that alcohol uniquely requires an NO-stimulated pathway of PKA action and that chronic alcohol exposure prevents the activation of PKA via the uncoupling of the NO pathway [16]. Further, these studies of AICD were reproduced in several alcohol-exposed animal models in vivo including rat, sheep, and mouse [17–19]. Not only was cilia beating frequency and subsequent PKA activity desensitized to subsequent beta agonist stimulation in animals fed an alcohol-containing diet, but the functional consequences of such desensitization was demonstrated by showing defective clearance of *Streptococcus pneumoniae* from the lungs of infected rodents [20]. This work defined that one mechanism of lung injury to innate defense is the alcohol-mediated suppression of ciliary PKA action.

Alcohol and Respiratory Syncytial Virus

In addition to AICD as functionally applied to the effective clearance of bacteria from the lungs, the role alcohol plays in lung viral infections is a topic of interest. Individuals with alcohol use disorders (AUDs) are well known to be at increased risk for a wide variety of lung infections and are susceptible to these infections being more severe and longer in duration. One of the most common viral respiratory infections is respiratory syncytial virus (RSV). This is a particularly burdensome

clinical problem in the Veterans population where AUDs occur at three times the general population. In addition, RSV infection is now viewed as a serious problem in older adults, as the initial RSV infection commonly predisposes to a secondary bacterial infection or chronic lung disease exacerbation. Using a mouse model of RSV infection, it was observed that alcohol-fed mice fail to effectively clear RSV from the lungs in a timely manner [21]. In addition, the magnitude of the intensity and duration of cilia slowing is significantly enhanced in mice consuming alcohol. This enhanced cilia slowing is regulated by the activation of PKCε. Alcohol feeding also enhances RSV-induced ciliated cell detachment in mice. This occurs sequentially after the PKCε-induced cilia slowing and is regulated by the auto-downregulation of PKCε [22]. Using the PKCε knockout mouse, the importance of this kinase in the control of RSV-induced cilia slowing was confirmed [23].

The functional significance of these findings is that innate defense against viral-mediated lung injury is compromised by co-exposure to alcohol. Humans have little to no resistance to RSV infection, but we typically recover from this virus due to innate lung antimicrobial action and airway re-epithelialization leading to a rapid restoration of the mucociliary escalator. In doing so, secondary bacterial infections are minimized. In stark contrast, drinking alcohol decreases the repair process to the RSV-injured mucociliary transport apparatus. Future studies will no doubt also examine the impact of alcohol exposure on lung antimicrobial defensins and when integrated into our evolving understanding of ciliary dysfunction will provide a more complete understanding of how alcohol impairs airway defenses. For example, it is known that chronic alcohol decreases surfactant expression in an ovine model [18] and modifications to normal surfactant expression in response to alcohol-derived aldehyde adduction produces significant changes in innate defense [23]. However, to date the role of alcohol in the regulation of other lung antimicrobial peptides has not been determined in the context of innate defense against viral and bacterial injury to the airway epithelium.

The Combination of Smoke and Alcohol

Estimates are that greater than 95 % of adults with an alcohol use disorder (AUD) smoke cigarettes. Conversely, 30–50 % of all cigarette smokers consume alcohol. Given these observations, it seems highly relevant to study the combined effects of both cigarette smoke and alcohol on lung innate defense, and rodent models of smoke and alcohol co-exposure have been established [17, 19, 24]. These models utilize small animal whole-body cigarette smoke exposure systems for the generation of environmental cigarette smoke in an alcohol-fed mouse model. Using such a co-exposure system, it has been shown that functional clearance of control particles, bacteria, or viruses is significantly delayed under conditions of both smoke and alcohol as compared to the individual exposure to smoke or alcohol alone [20]. This observed decrease in clearance corresponds to a significant decrease in baseline cilia beating. Of importance, only under the smoke plus alcohol co-exposure

condition is PKCε activated in the airway epithelium [23]. This was an important new observation that differed from that of AICD in which alcohol blocks any stimulated increases in cilia beating. Under conditions of smoke plus alcohol, cilia actually beat *slower* than at baseline.

It remains to be determined exactly how the unique condition of cigarette smoke combined with alcohol leads to the activation of PKCε and the subsequent slowing of cilia. Burning tobacco generates large amounts of acetaldehyde [25], and alcohol metabolizes into both acetaldehyde and malondialdehyde. Together, these reactive aldehydes can form protein adducts. One stable hybrid adduct of malondialdehyde and acetaldehyde (MAA) adducts to lung protein under conditions of combined smoke and alcohol exposure [23]. The amount of aldehydes required for the formation of MAA-adducted proteins in the lung is only produced under co-exposure conditions [26]. Smoke plus alcohol co-exposure leads to the formation of MAA-adducted surfactant protein D (SPD-MAA) in a mouse model of smoke and alcohol co-exposure [23]. Further, when mice are nasally instilled with purified SPD-MAA in the absence of smoke or alcohol, airway epithelial PKCε is activated and cilia beating is slowed.

A possible mechanism(s) for PKCε stimulation by SPD-MAA may involve scavenger receptor A (SRA), which is found on lung macrophages and airway epithelial cells. SRA appears to rapidly bind to MAA-adducted proteins and internalize them within an hour and this precedes PKCε activation in both cell and animal models. In studies using both PKCε KO mice as well as SRA KO mice, neither with SPD-MAA nor with the combination of smoke and alcohol is cilia slowing observed. Thus, scavenger receptor–ligand interactions may govern inside-out signaling resulting in the modulation of innate lung defense.

Bidirectional Control Hypothesis

Combination exposures, such as cigarette smoke and alcohol, could potentially play an even more significant role in lung innate defense and environmental injury when viewed within the context of a bidirectional control hypothesis. Agents that activate PKA enhance ciliary beating and should enhance mucociliary clearance. Chronic alcohol use would lead to the desensitization of a PKA-activating cilia stimulatory event. On the other hand, PKCε activating events trigger the cilia slowing. Because the combination of smoke and alcohol causes PKCε activation, the combination exposure could result in blocking the resolving pathway while activating the injury pathway with regard to lung mucociliary clearance. This PKA-mediated anti-inflammatory pathway versus PKC-mediated pro-inflammatory pathway axis may have broader applications to lung innate defense. Indeed, similar observations have been reported with regard to PKA/PKC dual opposing regulation of airway epithelial migration into wound repair [27–29] and bronchial epithelial release of pro-inflammatory cytokines [30]. Thus, bidirectional modulation of pathways would only be observed and likely be of most importance when investigating co-exposure conditions.

Alcohol and Mucus

While mucus-related symptoms from alcohol abuse such as nonallergic rhinitis and mucus dehydration have long been noted, there are few reports concerning the direct effect of alcohol on mucins or mucus production. Early studies utilizing a frog palate model suggest that both the amount and viscosity of mucus are increased in response to alcohol treatment, although mucociliary clearance rates were dependent upon the "ciliotoxic" action of alcohol [31]. In the frog palate study, 50 % alcohol treatment doubled the secreted glycoprotein concentration while 20 % alcohol resulted in nearly a twofold increase in the apparent viscosity of mucus. Consistent with the observation that alcohol increases mucus production, Verma and Davidson found that 100 mM alcohol elevated tracheobronchial mucin mRNA levels up to eightfold in human bronchial epithelial cells [32]. However, it is generally understood that increased symptoms of mucus production in the upper airways is primarily the result of nonallergic rhinitis or an allergy response to components in the alcoholic beverage consumed (such as sulfites or histamines) and not to a direct reaction to alcohol itself.

Lung Function and Airway Hyperresponsiveness

At toxic doses, alcohol can have a dramatic effect on lung function as regulated by the central nervous system. However, the maintenance of smooth muscle tone in the airways can also be directly impacted by alcohol. For centuries, alcohol has been utilized as a treatment for asthma symptoms (reviewed in [33]). Underlying this practice, it has been demonstrated that alcohol is capable of producing a dose-dependent bronchodilation in asthmatics resulting in increased airways conductance [34, 35]. Similar to mucus production, bronchospasm in response to drinking alcoholic beverages is likely the result of the allergy-inducing components contained in certain alcoholic beverages as pure alcohol does not induce bronchospasm [36]. In contrast, the primary metabolite of alcohol, acetaldehyde, enhances airway bronchospasm and is associated with the exacerbation of asthma symptoms due to mast cell degranulation and histamine release [37]. In contrast, studies in canine models [38, 39] demonstrate that alcohol directly produces a concentration-dependent airway smooth muscle relaxation response. The mechanism underlying this response may involve alcohol-induced nitric oxide generation from airway epithelium [40] leading to cGMP elevation in target smooth muscle cells, or possibly alcohol-stimulated increases in cAMP directly in the lung smooth muscle through activation of adenylyl cyclase 7 [41]. Indeed, alcohol reversibly blocked airway responsiveness to a methacholine challenge in mice fed 20 % alcohol in drinking water or given an intraperitoneal injection of alcohol [42]. This bronchodilation response was regulated at the level of the airway smooth muscle cell as 100 mM alcohol blocked methacholine-induced rat smooth muscle cell contraction in vitro [43]. In these cells, alcohol activates PKG through the NO-cGMP pathway. Antagonist analogs to cGMP block alcohol-induced PKG activation and prevent

ethanol from blocking methacholine-induced cell contraction, suggesting that alcohol can directly relax airway smooth muscle. Alcohol administration also blunts airway hyperresponsiveness in an ovalbumin-sensitized mouse model of allergic asthma [44]. However, the effects of alcohol on airways with normal responsiveness have not been identified, and this must be considered if alcohol is used as a drug solubility vehicle in airway function studies.

Barrier Function

In comparison to the establishment of alveolar cell resistance to permeability, the maintenance of airway barrier protection against leak is the subject of less investigation, although of significant functional importance. The integrity of functional tight junctions between airway epithelial cells is required for proper resistance to permeability in the maintenance of barrier protection. Previously, it was established that both alcohol and acetaldehyde decrease intestinal epithelial barrier function and promote leak via the downregulation of tight junction proteins [45]. Indeed, the impact of alcohol on cell–cell junctional proteins in the alveolar epithelium is established as a mechanism for alcohol-mediated acute lung injury [46, 47], and this is the central topic of a subsequent chapter. Similar to alveolar leak, alcohol also decreases bronchial epithelial cell resistance in primary human bronchial cells [14]. This alcohol-induced bronchial permeability is associated with the decreased expression of ZO-1, claudin-1, claudin-5, and claudin-7 at the cell membrane. Interestingly, alcohol requires the presence of active PKCα in the bronchial cell to stimulate the downregulation of tight junction protein and decrease cell monolayer resistance [14]. This mechanism is consistent with previous observations that activated PKCα leads to tight junction permeability in kidney epithelium [48] and that alcohol has the potential to directly activate PKCα in certain cells [49]. However, the functional significance of airway leak remains less obvious than that of lower airway edema. Targeted injury to the airway epithelium, such as that observed in response to viral infection, has been shown to involve the disruption of tight junctions and a loss of epithelial barrier function [50]. Indeed, the reestablishment of cellular tight junctions is the final step in the restoration of the epithelial monolayer after wound injury and prior to epithelial re-differentiation into a polarized cell phenotype [51]. In this context, the impact of alcohol on barrier function in the conducting airways can be viewed as an important regulator of injury repair.

Wound Repair

Injury to the airway epithelium in response to pathologic challenges such as viral respiratory infections or cigarette smoke exposure results in the loss of cilia, the loss of ciliated cells, and the sloughing of significant areas of the epithelium

lining the conducting airways [52]. Repair of the wounded area requires a multistep process involving migration of the remaining epithelial cells into the wound, proliferation of cells, and re-differentiation of epithelial cells into a specialized phenotype [53]. The rate at which epithelial cells migrate into a wound area and facilitate restitution of the epithelial monolayer is a rate-limiting step toward wound repair and the return of homeostatic function. Enhanced cell migration into a wound is stimulated, in part, through the activation of PKA [28] and the inhibition of Rho-kinase [54]. Consistent with the observation that sustained alcohol treatment of bronchial epithelial cells desensitizes PKA from activation [15], chronic alcohol exposure decreases airway epithelial migration into a wound via the downregulation of PKA [27]. In addition, adenosine-mediated PKA enhancement of bronchial epithelial wound repair [55] is prevented by alcohol blocking the nucleoside transporter [56]. Alternatively, agents that activate PKC have been shown to decrease epithelial cell migration. While the alcohol metabolite acetaldehyde only activates PKC in very high concentrations in vitro [25], stable protein adducts can form from pathophysiologic concentrations of acetaldehyde and malondialdehyde [57]. These MAA adducts decrease airway epithelial wound repair through a PKC-dependent pathway [58]. Such concentrations of MAA and formation of MAA-adducted protein have been shown to occur in vivo in a mouse model of alcohol and cigarette smoke co-exposure [26]. In this scenario, MAA-adducted protein requires a scavenger receptor-mediated activation of PKC that results in the significant release of IL-8 from the bronchial epithelial cell [59]. The impact on epithelial wound repair by autocrine IL-8 signaling is not established, but it has been shown that IL-8 can uncouple beta-agonist stimulated PKA action [60]. These findings underscore two interesting points concerning the impact of alcohol on wound repair: First, alcohol exposure can suppress epithelial migration in response to wound healing through a bidirectional approach of direct PKA inhibition and indirect PKC activation, and second, alterations in inflammatory cytokines and mediators subsequent to alcohol exposure may negatively impact airway repair and remodeling.

Pro-inflammatory Cytokine Release

Alcohol generally plays an immunosuppressive role in the lungs as evidenced by increased host susceptibility to pneumonia from alcohol abuse. Such immunosuppression has been the focus of numerous studies at the level of the alveolar macrophage and alveolar epithelium [61]. However, the airway epithelium also functions to produce and release pro-inflammatory cytokines in response to pathogen stimulation. Studies suggest that alcohol can modulate these pathways as bronchitis is an established health problem in alcohol abuse [62]. As important pattern recognition receptors for innate immunity, Toll-like receptors bind bacterial cell wall components. Alcohol exposure increases both TLR2 message and protein expression in bronchial epithelial cells [63] through a

NO-cGMP-PKG mediated pathway [64]. This suggests that alcohol could enhance a bacterial inflammatory response that might otherwise be relatively tolerated in non-alcohol exposed airway epithelium. These findings demonstrate a potentially important aspect of alcohol exposure as it relates to environmental co-exposures in inflammatory lung disease. Pro-inflammatory chemokine release is relatively unaffected by alcohol exposure alone to the bronchial epithelium. However, a significant enhancement in chemokine release is observed in response to the combination of alcohol and cigarette smoke exposure [19]. As mentioned, alcohol-exposure-derived MAA adducted protein stimulates the production of IL-8 in bronchial epithelial cells [59]. Surfactant protein A is a likely major target for such adduction in the lung that also stimulates chemokine release when it is modified with this hybrid aldehyde adduct [65]. Consistent with these observations, the combination of alcohol and RSV leads to an enhanced production of epithelial-derived TNFα and MCP-1 as opposed to RSV infection alone in the absence of alcohol [21]. In contrast, co-exposure studies in a mouse model in which they are exposed to the agricultural product known as "combined swine confined animal feeding operation" (CAFO) dust in combination with alcohol show a significant suppression of inflammation [66]. In this model, dust-induced inflammation pathways are suppressed, thereby leading to significant animal weight loss and mortality. This counterintuitive observation of seemingly less inflammation but increased mortality has yet to be understood, particularly as localized alcohol-induced decreases in lung TNFα but elevated systemic TNFα levels result were observed. This suggests that alcohol does not ameliorate the injury induced by CAFO dust, but rather facilitates greater harm to the host than merely that of dust-mediated lung inflammation alone.

Summary

Owing to the fact that the lungs are an organ that actually excretes consumed alcohol, the conducting airways represent a key target for the alcohol-induced modulation of important innate defense mechanisms. Principally, mucociliary clearance becomes dysfunctional after sustained alcohol exposure, resulting in a desensitization of the intracellular signaling pathways upregulating increased cilia beating. This inability to effectively clear inhaled particles, whether pollutants, viruses, or bacteria, can result in sustained exposure leading to enhanced injury. To compound such enhanced injury, studies suggest that alcohol exposure also decreases airway epithelial barrier function, repair responses to wound healing, and both innate and adaptive airway immunity. Collectively, this alcohol-mediated dysfunction may have a significant deleterious effect on the ability of the lung to withstand environmental and occupational exposures and result in a variety of acute and chronic inflammatory diseases of the airways.

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Chapter 7
Alcohol and the Alveolar Macrophage

Samantha M. Yeligar, Yan Liang, and Lou Ann S. Brown

Abstract Compared to nonalcoholics, patients with a history of alcohol-use disorders have increased susceptibility to lung infections, leading to sepsis and in a disproportionately high percentage of cases the development of the acute respiratory distress syndrome. A primary cause for increased risk of respiratory infections in alcoholics is impaired immune function of the alveolar macrophage. Macrophages are key components of innate immunity in various tissues and serve as a first line of defense against invading pathogens by generating pro-inflammatory responses to kill microbes and facilitate the clearance of foreign debris from tissues. Macrophages can be characterized into three phenotypes based on their mechanism of activation and functional characteristics: classical activation (pro-inflammatory), alternative activation, and deactivation (the latter two are anti-inflammatory). Alcohol induces an alternatively activated phenotype in alveolar macrophages, which is characterized by increased oxidative stress, via up-regulation of transforming growth factor beta and NADPH oxidases and phagocytic dysfunction. A range of treatments that increase glutathione and zinc bioavailability, granulocyte–macrophage colony-stimulating factor signaling, or activation of nuclear factor erythroid 2-related factor 2 for the attenuation of alcohol-induced oxidative stress have been identified as strategies that can restore alveolar macrophage immune function in the alcoholic lung.

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Keywords Alcohol • Alveolar macrophage • Macrophage activation • ARDS • Respiratory infections

Abbreviations

AUD	Alcohol-use disorder
ARDS	Acute respiratory distress syndrome
TGFβ	Transforming growth factor beta
Noxes	NADPH oxidases
GM-CSF	Granulocyte/macrophage colony-stimulating factor
Nrf2	Nuclear factor erythroid 2-related factor 2
ARDI	Alcohol-Related Disease Impact
SA	Small aggregate
LA	Large aggregate
GBS	Group B *Streptococcus pneumoniae*
IFNγ	Interferon gamma
IL	Interleukin
LPS	Lipopolysaccharide
SOCS	Suppressor of cytokine signaling
JAK	Janus-associated kinase
TLR	Toll-like receptor
ATF	Activating transcription factor
TNFα	Tumor necrosis factor alpha
PPARγ	Peroxisome proliferator-activated receptor gamma
TZD	Thiazolidinediones
iNOS	Inducible nitric oxide synthase
COPD	Chronic obstructive pulmonary disease
MRC1	Mannose receptor 1
MHC	Major histocompatibility complex
MT	Metallothionine
Arg1	Arginase 1
ARE	Antioxidant response element

Epidemiology and Statistics

Alcohol consumption has been an aspect of popular culture for thousands of years, where the prevalence to imbibe alcohol legally is greater than abstinence. According to the 2011 National Survey on Drug Use and Health, over 50 % of the adult population in the United States consumes alcohol. Although there is ample epidemiological evidence that moderate alcohol ingestion (≤1 drink/day for women and ≤2 drinks/day for men) can have positive health benefits, chronic alcohol abuse has become a

worldwide heath problem. According to the Centers for Disease Control (CDC), excessive alcohol use includes heavy drinking (≥1 drink/day for women and ≥2 drinks/day for men, on average), binge drinking (≥4 drink/day for women and ≥5 drinks/day for men, during a single occasion), and any drinking by pregnant women or underage youth. The CDC's Alcohol-Related Disease Impact (ARDI) reports that there are about 80,000 deaths annually in the United States that are attributable to excessive drinking, making excessive alcohol use the third leading lifestyle-related cause of death [1]. In fact, according to ARDI, alcohol abuse is responsible for 2.3 million years of potential life lost or an average of about 30 years of potential life lost for each death. In 2006, excessive alcohol consumption was responsible for ~$223.5 billion in economic costs, including over $1.2 million in emergency room visits and $2.7 million in physician office visits [2]. Patients with a history of alcohol abuse are twice as likely to develop acute respiratory distress syndrome (ARDS) compared to matched nonalcoholic subjects [3], and their in-hospital mortality rates are double that of nonalcohol abusers [4]. Further, alcoholic patients who develop pneumonia are twice as likely to develop sepsis [5], and those who develop sepsis are twice as likely to develop ARDS [4, 6]. ARDS pathogenesis is characterized by the development of pulmonary edema and inflammation in response to aspiration, trauma, or sepsis that results in the activation of systemic pro-inflammatory cascades [7]. Current experimental models of lung infection and injury in the context of chronic alcohol exposure are discussed in the following section.

Models of Chronic Alcohol Consumption and Pulmonary Injury

Currently, there are several experimental models of lung infection and injury that include chronic alcohol use as a confounding factor. In each model, the combined effects of alcohol abuse and pulmonary infection and/or injury lead to exacerbation of either effect alone. Here, we will explore models of chronic alcohol use and ventilator-induced lung injury, cecal ligation-mediated sepsis, and respiratory infections with *group B Streptococcus pneumoniae* and *Klebsiella pneumoniae*.

Mechanical ventilation may induce or exacerbate inflammatory lung injury [8–10] during critical illnesses such as sepsis, trauma, or infection. Ventilator-induced lung injury is characterized as lung tissue damage caused by shear stresses during mechanical ventilation. Mechanical ventilation with relatively smaller tidal volumes, known as "lung-protective ventilation," was associated with a lower mortality rate in patients with ARDS [11]. Using rats fed with or without alcohol for 6 weeks and ventilated *ex vivo* with low-volume "protective" or high-volume "injurious" strategies, combined alcohol- and ventilator-mediated challenges on lung tissue and alveolar macrophages were investigated [12]. Chronic alcohol consumption attenuated injurious mechanical ventilation-mediated pro-inflammatory responses but did not promote ventilator-induced lung injury. However, the innate immune response of alveolar macrophages to phagocytose bacteria during injurious mechanical

ventilation was blunted with alcohol ingestion. These data demonstrate that alcohol ingestion suppresses ventilator-induced inflammation but exacerbates alveolar macrophage immune dysfunction [12] and may in part explain the increased risk of ventilator-associated pneumonia seen in patients with a history of alcohol-use disorders (AUDs).

Sepsis is the most common risk factor associated with ARDS [13]. It has been well established that chronic alcohol ingestion impairs surfactant production in the lung [14–16] and that patients with ARDS have increased ratios of inactive small aggregate (SA) surfactant phospholipids to bioactive large aggregate (LA) surfactant phospholipids. In that same model in which rats were fed with or without alcohol (36 % of total calories) for 6 weeks, rats were made septic via cecal ligation and perforation [13]. Although prior chronic alcohol ingestion had no significant effect on physiological indices of sepsis severity such as respiratory rate, arterial blood pressure, or plasma lactate level, it significantly increased lung lavage protein levels (reflecting transepithelial leak), worsened hypoxemia, and altered the pool of functional surfactant phospholipids. These experimental findings that alcohol ingestion in the absence of malnutrition or other lifestyle factors can exacerbate sepsis-mediated lung dysfunction provided additional mechanistic evidence of why there is an increased risk of ARDS in alcoholic patients.

The alveolar macrophage is the first line of defense against pulmonary infections in both the adult and the fetal lung. Importantly, fetal alcohol exposure increases the risks of neonatal injury and infection. Using a guinea pig model of fetal alcohol exposure, timed-pregnant guinea pigs were fed with or without alcohol and after delivery their term pups were given *group B Streptococcus* (GBS) pneumonia by intratracheal administration [17]. Pups exposed to alcohol in utero exhibited increased lung GBS infection and sepsis, and alveolar macrophages isolated from these pups demonstrated impaired GBS phagocytosis; taken together, these findings implicate alcohol exposure in utero as a cause of neonatal lung defense derangements against bacterial infection [17]. In adults, alcoholics also have an increased incidence of GBS infections [18]. In a study of young adult rats fed with or without alcohol for 6 weeks and given GBS intratracheally, alcohol decreased GBS clearance from the lung, increased colony formation in the liver and spleen, and exacerbated acute lung injury induced by GBS [19]. Further, studies of alcohol-fed rats that were inoculated with intratracheal *K. pneumoniae* showed decreased lung bacterial *Klebsiella* clearance compared to control-fed rats [20, 21]. Collectively, these studies suggest that chronic alcohol consumption increases the susceptibility of the lung to bacterial infections.

The above models of pulmonary injury and infection in the context of chronic alcohol abuse demonstrate the ability of alcohol to exacerbate ventilator-induced lung injury, cecal ligation-mediated sepsis, and respiratory bacterial infections. Since alveolar macrophages are the first line of immune defense in the lung, the following sections discuss alveolar macrophage development and their role in immunity.

Alveolar Macrophage Lineage and Differentiation

Alveolar macrophages are derived from peripheral blood monocytes that travel through the pulmonary circulation to become sequestered in pulmonary capillaries, where they then migrate into the interstitial and alveolar spaces [22]. As these monocytes migrate to the alveolus through the pulmonary interstitium as "interstitial macrophages," they represent an intermediate differentiation stage between monocytes and alveolar macrophages (Fig. 7.1) [23]. Monocytes highly express adhesion molecules such as those in the CD11 family. Compared to mature alveolar macrophages, interstitial macrophages more closely resemble monocytes in that they are smaller in size and have blunted pseudopodia. As macrophages mature in the alveolar space, they grow larger in size and their density decreases [22]. Alveolar epithelial type II cells secrete granulocyte/macrophage colony-stimulating factor (GM-CSF) into the alveolar space to prime the terminal differentiation of circulating monocytes into mature, functional alveolar macrophages [24]. Studies of GM-CSF knockout mice showed that these animals exhibited normal bone marrow maturation but had impaired alveolar macrophage maturation and development of an abnormal lung phenotype that resembled pulmonary alveolar proteinosis in humans [25]. Mature alveolar macrophages express minimal levels of adhesion molecules, have significantly greater capacity for phagocytosis [26], and play a key role in innate and acquired immunity [27].

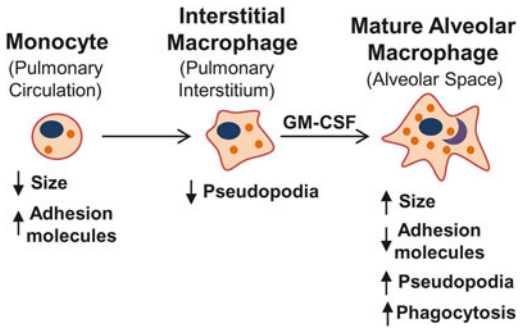

Fig. 7.1 Terminal differentiation of alveolar macrophages. Circulating pulmonary monocytes are small in size and have high expression of adhesion molecules. These monocytes migrate through the pulmonary interstitium as interstitial macrophages that exhibit blunted pseudopodia. Alveolar type II cells in the alveolar space secrete granulocyte/macrophage colony-stimulating factor (GM-CSF), which primes the terminal differentiation of circulating monocytes into mature alveolar macrophages. The mature macrophages are larger in size, express low levels of adhesion molecules, and exhibit increased pseudopodia and increased phagocytic capacity

Macrophage Function in Immunity

Macrophages are specialized cells in host innate and acquired immunity that play key roles in the pathogenesis of many inflammatory disorders, such as atherogenesis and liver and lung injury. Macrophages reside in nearly every tissue and display marked heterogeneity in their expression of cell surface markers, location, and physiologic function [28]: in the spleen, red pulp macrophages are a subset of functionally distinct macrophages [29]; in bone, macrophages form multinucleated osteoclasts at the periosteum; in the central nervous system, macrophages comprise the microglia and are interspersed among neurons; in the kidney, macrophages are mesangial cells that form a network around glomeruli [30]; in the vasculature, macrophages occupy the subendothelial space of large arteries [31]; in the liver, macrophages are Kupffer cells that line the sinusoids [30]; and in the lung, alveolar macrophages are found within the alveolar space [32]. These resident tissue macrophages are long-lived and perform common as well as tissue-specific functions. They provide the first line of defense against invading pathogens by generating inflammation, respiratory bursts, and initiating antigen presentation to stimulate adaptive immunity [33].

Macrophages are recruited to sites of injury to both initiate and resolve inflammation [34, 35]. For example, chemokine secretion by endothelial cells, parenchymal cells, or lymphocytes recruits monocytes to sites of tissue injury where they differentiate into resident macrophages. Pro-inflammatory molecules initially released by macrophages are protective in that they scavenge, degrade, and clear cellular and foreign debris from tissue. However, if excessive and persistent, the inflammatory response becomes harmful and can contribute to disease progression as has been identified in obesity, insulin resistance, and atherosclerosis [34, 36]. Therefore, the molecular mechanisms involved in the dynamic regulation of macrophage inflammatory responses play a critical role in both the initiation and resolution of inflammation. For example, one of these regulating mechanisms involves the induction of negative feedback loops that transcriptionally or post-transcriptionally hinder pro-inflammatory signaling pathways. Macrophage stimulation with IFNγ, interleukin (IL)-4, or LPS rapidly induces proteins known as "suppressor of cytokine signaling" or SOCS, in turn causing inhibition of Janus-associated kinase (JAK)-STAT and LPS signaling [37, 38]. Further, the binding of LPS to toll-like receptor-4 (TLR4) induces activating transcription factor (ATF)3 expression, resulting in suppression of inflammation [39].

In the lung, alveolar macrophages make up approximately 93 % of the macrophage population, with the remainder comprising pulmonary tissue macrophages [40]. They act as early effectors of the innate immune response against bacterial pathogens if they reach the distal airways [41]. Alveolar macrophages employ various microbicidal molecules to kill pulmonary pathogens [42]. While the role of nitric oxide in pathogen killing by human macrophages has been debated [43], activated human alveolar macrophages increase nitric oxide production following challenge with pathogens such as *K. pneumoniae* and *S. pneumonia* [44, 45]. Reactive oxygen

species additionally contribute to the killing of important pathogens including fungi, a variety of gram-negative bacteria, and *Staphylococcus aureus* [46]. Upon activation, alveolar macrophages are also key sources of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), which are involved in neutrophil recruitment [42]. During inflammation resolution, alveolar macrophages may induce the apoptosis of inflammatory cells including monocytes, lymphocytes, and neutrophils, which they can then phagocytize [47]. However, activation of peroxisome proliferator-activated receptor gamma (PPARγ) with thiazolidinediones (TZDs) down-regulates IFNγ-induced Th1-cytokines, blunts the inflammatory response, and attenuates the activity of the inducible nitric oxide synthase (iNOS) in alveolar macrophages [48]. Smoking and chronic alcohol consumption can impair alveolar macrophage phagocytic function, which is also a characteristic of chronic obstructive pulmonary disease (COPD) and of the ARDS [49–51]. Collectively, these studies show the importance of alveolar macrophages in protecting the lung through phagocytosis of invading pathogens and inflammatory mediators.

Phenotypic States of Macrophage Activation

Macrophages respond to external stimuli with activation programs that allow functional plasticity and specificity in tissue and recruited macrophages [52]. Although there are multiple ways to classify macrophages into subtypes [34, 35, 53–55], Gordon and colleagues first proposed a simple schematic to describe the functional importance of mature macrophages in health and disease [35]. In their schema, terminally differentiated macrophages are divided into the following three states: classical activation (M1), alternative activation (M2), or deactivation [35] (Fig. 7.2). However, in vivo, macrophage phenotypes demonstrate plasticity along the continuum of these three states [55, 56]. Macrophages in these states are classified by their ability to secrete inflammatory mediators and kill pathogens, promote tissue repair, inhibit immune responses to antigen, or any combination of these [57].

Macrophages become classically activated in response to the Th1-cytokines, IFNγ, IL-1β, and microbial stimuli, including LPS, which activate signaling of TLR4 [35, 58, 59]. Classically activated macrophages utilize a microbicidal program to confer immunity against intracellular pathogens [34, 35, 53–55]. The macrophage respiratory burst and pro-inflammatory pathways associated with classical activation are necessary for the clearance of invading pathogens. M1 classically activated macrophages produce pro-inflammatory cytokines, including TNFα, IL-6, and IL-12 [35]. However, chronic and aberrant expression of this microbicidal program contributes to the pathogenesis of several diseases, such as atherosclerosis and insulin resistance induced by obesity [60]. As a result, genetic ablation of genes involved in classically activating macrophages, including IFNγ and its receptor, iNOS, TNFα, or IL-6, results in protection from atherosclerosis and obesity-induced insulin resistance [60, 61], illustrating the importance of the classically activated macrophage microbicidal program in tissue-specific immunity.

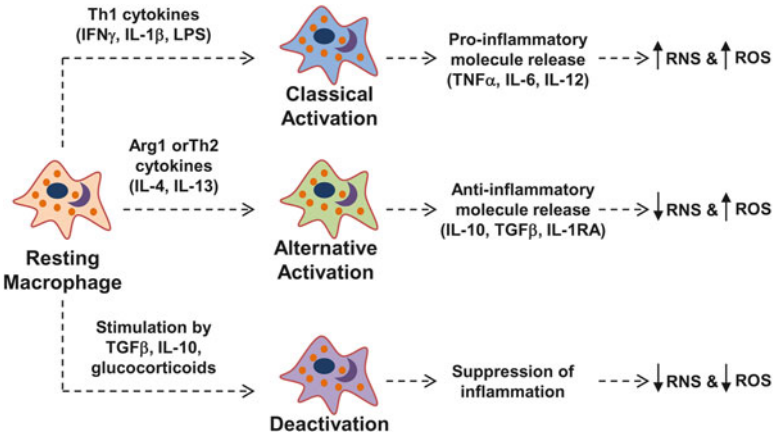

Fig. 7.2 Macrophage functional heterogeneity is achieved through different states of macrophage activation. Recruited and resident macrophages can undergo distinct activation programs in response to cytokine signaling [35]. Stimulation with the Th1-cytokines, interferon (IFN)γ, interleukin (IL)-1β, or lipopolysaccharide (LPS) leads to classically activated macrophages that release pro-inflammatory molecules such as tumor necrosis factor (TNF)α, IL-6, and IL-12. Arginase-1 (Arg1) induction or stimulation with the Th2 cytokines, IL-4 and IL-13, leads to alternatively activated macrophages that release anti-inflammatory molecules such as IL-10, transforming growth factor (TGF)β, and IL-1 receptor antagonist (IL-1RA). Stimulation by TGFβ, IL-10, and glucocorticoids leads to macrophage deactivation and suppression of inflammation. *RNS* reactive nitrogen species, *ROS* reactive oxygen species

Macrophages exhibit plasticity and can switch from the M1 to M2 phenotype and vice versa in response to specific signals [35]. Alternative macrophage activation is mediated by the Th2-cytokines IL-4 and IL-13 [62]. It was initially believed that IL-4 prevented respiratory and inflammatory burst in human monocytes and macrophages by antagonizing classical activation pathways regulated by IFNγ in phagocytes [63, 64]. Later studies identified dramatic expression of the mannose receptor (MRC1) in IL-4-activated macrophages [65] and revealed that IL-4 induced the expression of major histocompatibility complex (MHC) class II [66]. These observations led to the concept of alternative activation generating an anti-inflammatory phenotype, additionally characterized by a decrease in both the respiratory burst and phagocytosis.

Several studies have identified a transcriptional signature of alternatively activated macrophages [67–73]. With alternative activation, macrophages clear immune complexes, attenuate inflammatory responses, and promote wound healing by up-regulating growth factors [55, 74]. Arginase 1 (*Arg1*), the best studied marker of alternative activation [35], plays a role in ornithine and polyamine synthesis, both of which are essential for the reparative functions of macrophages [75]. This synthesis program reduces cellular arginine levels, and the decreased substrate availability of arginine attenuates nitric oxide synthesis, which some pathogens exploit for intracellular growth [76, 77]. During alternative activation of macrophages, various phagocytic receptors, cytokines, chemokines, and secreted products are induced in

addition to *Arg1* [35, 78]. The development and trafficking of alternatively activated macrophages in various tissues can be tracked due to the specificity and availability of these markers [79]. In addition, M2 alternatively activated macrophages can be recognized by their production of anti-inflammatory factors such as IL-10, TGFβ, and IL-1 receptor antagonist, which appear to promote angiogenesis and tissue repair following injury [35, 80].

Macrophage deactivation, the third phenotypic state, is an active process that switches off classically and alternatively activated macrophages, thereby attenuating antigen presentation and subsequently increasing immunosuppression [35]. Potent stimulators of macrophage deactivation include TGFβ, IL-10, and glucocorticoids, all of which reduce MHC class II molecule expression, down-regulate antigen presentation, and attenuate inflammation [35]. Similarly, macrophage phagocytosis of apoptotic cells renders macrophages resistant to classical activation by LPS through increasing the release of TGFβ and the deactivating cytokine IL-10 [81–83]. Ingestion of apoptotic cells by macrophages also activates nuclear receptor signaling, resulting in inflammation suppression [35]. Although significant strides have been made in elucidating the molecular mechanisms by which IL-10 and glucocorticoids deactivate macrophages [84–86], the scarcity of cell surface markers that characterize this deactivation state has made understanding the physiological functions of deactivated macrophages in vivo difficult. To complicate matters, the role of TGFβ both as a factor released by the alternatively activated macrophage and as a factor that stimulates the deactivated macrophage demonstrates plasticity between these phenotypic states as well. Collectively, studies investigating the phenotypic states of the macrophage support the notion of plasticity along the continuum of classical activation, alternative activation, and deactivation.

Chronic Alcohol and Alveolar Macrophage Activation

Depending on varying stimuli within the microenvironment of the alveolar space, “resting” alveolar macrophages can become classically activated, alternatively activated, or even deactivated. The plasticity of these cells to move between these activation states is important for proper immune response and lung tissue defense. Invading pathogens must be phagocytized by classically activated alveolar macrophages and killed using the respiratory burst. Alternatively activated and deactivated alveolar macrophages perform “protective” functions in that while these cells do not have proper immune function, they instead decrease inflammation and promote tissue repair. However, impairment of the immune response by chronic alcohol consumption results in a susceptibility to lung injury. In a study of chronic alcohol exposure using primary rat alveolar macrophages and the rat alveolar macrophage cell line NR8383, alcohol induced a phenotype characteristic of alternatively M2 activated alveolar macrophages [87]. In parallel, chronic alcohol exposure impaired macrophage phagocytic function and increased the expression of TGFβ and other markers of alternative activation, such as arginase-1, IL-13, and galectin-3 [87].

Alternative activation of alveolar macrophages has also been shown to induce lung fibrosis in the context of chronic alcohol use through production of pro-fibrotic factors such as fibronectin. Alveolar macrophages were isolated from the bronchoalveolar lavage fluid collected from patients with a history of AUDs and otherwise healthy control subjects. Compared to controls, patients with a history of AUDs demonstrated increases in fibronectin expression in alveolar macrophages and in lung fibroblasts collected from epithelial lining fluid [88]. AUD patients also exhibited decreases in alveolar macrophage matrix metalloproteinase expression. These studies show that chronic alcohol consumption, leading to an alternatively activated alveolar macrophage phenotype, can activate tissue remodeling which might contribute to increased susceptibility to ARDS [88]. While alternative activation may decrease the contribution of alveolar macrophages to a pro-inflammatory state in the alveolar space, chronic suppression of phagocytosis and respiratory burst results in decreased killing of pathogenic microbes and an increased risk of respiratory infections.

It is important to stress that alveolar macrophages within the alcoholic human lung not only exhibit plasticity between the phenotypic states of terminally differentiated macrophage activation described above but also represent a mixed population of differentiated cells. As pulmonary macrophages mature in a healthy lung, they increase in size and decrease in density. Alveolar macrophages containing a high nuclear-to-cytoplasm ratio represent the most immature cells, while macrophages containing a low nuclear-to-cytoplasm ratio represent the most mature cells. In a guinea pig model of chronic alcohol consumption, alveolar macrophages were fractionated into subpopulations of various maturational stages based on their cell densities using a Percoll gradient. Over 75 % of alveolar macrophages isolated from control guinea pigs separated within the two most mature fractions, whereas less than 30 % of the macrophages isolated from alcohol-fed guinea pigs separated in these fractions. Compared with controls, chronic alcohol ingestion resulted in a 60 % decrease in the percentage of mature alveolar macrophages [22]. These studies indicate the presence of mixed populations of alveolar macrophages in varying stages of maturity after chronic alcohol ingestion.

Alcohol-Induced Alveolar Macrophage Oxidative Stress Leads to Phagocytic Dysfunction

Increased respiratory infections and higher in-hospital mortality rates of patients with a history of AUDs are very likely due at least in part to impaired immune response of the alveolar macrophage. These macrophages are deficient in their capacity to phagocytose and clear invading pathogens. Impaired phagocytic function results from decreased availability of the critical antioxidant glutathione (GSH), reduced intracellular zinc levels, impaired GM-CSF signaling, decreased nuclear factor erythroid 2-related factor 2 (Nrf2) levels, and increased expression and activity of $TGF\beta_1$ and NADPH oxidases (Noxes) (Fig. 7.3).

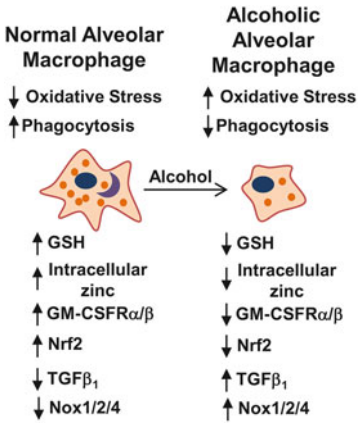

Fig. 7.3 Chronic alcohol induces alveolar macrophage oxidative stress, leading to phagocytic dysfunction. There are several pathways involved in alcohol-mediated alveolar macrophage derangements, including decreased glutathione (GSH) availability, low levels of intracellular zinc, reduced levels of granulocyte/macrophage colony-stimulating factor receptors alpha and beta (GM-CSFRα/β), decreased nuclear factor erythroid 2-related factor 2 (Nrf2) levels, increased transforming growth factor-beta1 ($TGF\beta_1$) signaling, and enhanced expression and activity of NADPH oxidases (Noxes)

Alcohol-induced GSH depletion in the lung occurs through poor GSH homeostasis which leads to chronic oxidative stress. Alcohol is metabolized by alcohol dehydrogenase in the liver and gastric mucosa, and, if concentrations remain elevated, alcohol can also be metabolized by cytochrome p450 in the lung. Both of these pathways result in the metabolism of alcohol into acetaldehyde, which generates oxidative stress through oxygen radical production and lipid peroxidation. Acetaldehyde generation results in antioxidant utilization and subsequent depletion. GSH, predominantly synthesized in the liver, is critical for cellular protection due to its involvement in detoxifying reactive oxygen species, conjugating and excreting toxic molecules, and controlling inflammatory cascades [89]. Chronic alcohol abuse alters GSH homeostasis within the lung by decreasing GSH levels in the epithelial lining fluid by 80 %, compared to GSH levels found in healthy controls (typically >400 μM), even when controlled for smoking histories [90]. Studies of alveolar macrophages from chronic alcohol-fed rats [32] and of alveolar macrophages isolated from mice and exposed to alcohol *ex vivo* [91] demonstrate increased levels of oxidative stress and impaired phagocytosis. Oxidative stress was evidenced by a 30 mV oxidation of the GSH/GSSG redox potential in alveolar macrophages isolated from alcohol-fed rats. Regarding immune function, alveolar macrophages from control-fed rats phagocytized ~80 % of fluorescent *S. aureus*, whereas only 20 % of macrophages from alcohol-fed rats were able to internalize the bacteria. When the GSH precursors procysteine and *N*-acetyl cysteine were added to the alcohol diet, GSH and oxidative stress were normalized to control levels in both the epithelial lining fluid and alveolar macrophages, and this was associated with

subsequent normalization of macrophage phagocytic function [32]. These studies suggest that attenuated GSH availability in the alcoholic lung leads to increased oxidative stress and alveolar macrophage dysfunction.

In addition to GSH homeostasis, zinc bioavailability also plays a key role in alveolar macrophage phagocytic function. Zinc deficiency is strongly associated with chronic alcohol abuse and impacts both epithelial and immune cell functions. Adequate zinc levels are important for innate defenses involving the alveolar macrophage and adaptive defenses provided by T and B lymphocytes [20]. For example, zinc deficiency interferes with mounting a proper immune response to pneumococcal antigen, leading to increased susceptibility to severe pneumococcal pneumonia [92]. It had generally been assumed that zinc deficiency in alcoholics resulted from poor nutrition. However, chronic alcohol ingestion decreases the expression of the primary zinc transporter in the intestinal epithelium such that systemic zinc deficiency can develop even with adequate dietary zinc levels [93]. These effects on zinc transporters were widespread, as alcohol ingestion altered the expression of critical zinc transporters and storage proteins in the small intestines (ZIP1, ZIP4, and ZNT4) and lungs (ZNT4, metallothionine-1 (MT1), and MT2 in alveolar epithelial cells and alveolar macrophages) [93] and increased oxidation of the thiol redox pair cysteine and cystine [21]. Further, alcohol-fed rats inoculated with intratracheal *K. pneumoniae* exhibited a fivefold decrease in lung bacterial clearance when compared to control rats [21]. In contrast, dietary zinc supplementation in these alcohol-fed rats resulted in increased alveolar macrophage intracellular zinc levels, enhanced GM-CSF receptor expression [93], attenuated oxidative stress [21], and restored bacterial phagocytic capacity and lung bacterial clearance [21, 93]. A more thorough review of the role of zinc deficiency in the alcoholic lung is provided in a later chapter.

GM-CSF and its receptors also play a role in alveolar macrophage dysfunction in response to chronic alcohol use. As mentioned earlier, GM-CSF regulates the terminal differentiation of peripheral blood monocytes into alveolar macrophages [24]. GM-CSF knockout mice exhibit attenuated surfactant expression, bacterial clearance, phagocytosis, and altered receptor expression in their alveolar macrophages [27, 94]. Studies using a rat model of chronic alcohol ingestion showed that although alcohol had no effect on GM-CSF levels in the alveolar space, the expression of the GM-CSF receptor subunits, GM-CSFRα and GM-CSFRβ, was significantly decreased in the membranes of alveolar macrophages [95]. Further, chronic alcohol ingestion decreased alveolar macrophage cellular and nuclear binding of PU.1, a transcription factor primarily responsible for activation of GM-CSF-dependent macrophage functions. Interestingly, treatment of alcohol-fed rats with recombinant rat GM-CSF intranasally (which aerosolized it into the lower airways) restored alveolar macrophage GM-CSF receptor expression, PU.1 protein levels and nuclear binding, and alveolar macrophage function [95].

In addition to alveolar macrophage immaturity, alcohol-induced oxidative stress is also crucial to resultant alveolar macrophage dysfunction. As described earlier, oxidation of the redox thiol pair GSH and glutathione disulfide as well as of cysteine and cystine following chronic alcohol exposure leads to increased oxidative stress

in the alveolar macrophage. In the healthy lung, acute stresses lead to activation of the antioxidant response element (ARE), which is a programmatic induction of a wide variety of antioxidants and cellular defense molecules. ARE activation is mediated by its major transcription factor Nrf2 and thereby serves as a cytoprotective program in response to inflammatory and oxidative stresses. However, this fundamental defense pathway is inhibited by chronic alcohol abuse ingestion [21], a novel and previously unidentified effect of alcohol that appears to explain how chronic alcohol ingestion leads to such profound oxidative stress in the lower airways of experimental animals and patients with a history of AUDs [14, 90]. In the aforementioned study, dietary zinc supplementation restored the ability of the lung to activate the ARE/Nrf2 pathway and replenished lung GSH levels in addition to preserving bacterial clearance by alveolar macrophages [21].

$TGF\beta_1$ signaling and activation of Noxes are two additional mechanisms by which chronic alcohol may induce oxidative stress in the alveolar macrophage. Chronic alcohol treatment increases $TGF\beta_1$ signaling and downstream IL-13 expression in a rat alveolar macrophage cell line, NR8383, producing a phenotype characteristic of alternative activation in which alveolar macrophages have increased oxidative stress and reduced capacity for phagocytosis. Treatment with $TGF\beta_1$-neutralizing antibody abrogates these effects and restores phagocytosis in NR8383 cells [87]. TGF β_1 consistently up-regulates and activates Nox4 [96], an NADPH oxidase isoform that along with Nox1 and Nox2 produces ROS in the alcohol-exposed alveolar macrophage. In fact, either Nox1 or Nox2 is sufficient to upregulate Nox4 [91]. These studies implicate activation of $TGF\beta_1$ and Noxes in alveolar macrophage oxidative stress and dysfunction in response to chronic alcohol consumption.

Collectively, the above studies suggest that patients with a history of AUDs have increased risk of respiratory infections and higher in-hospital mortality rates due to impaired immune function of the alveolar macrophage. Alcohol-exposed alveolar macrophages have impaired phagocytic capacities and cannot clear invading pathogens. Impaired phagocytic function results from oxidative stress via decreased availability of GSH, reduced intracellular zinc levels, low levels of GM-CSF receptors, decreased Nrf2 levels, and increased expression and activity of $TGF\beta_1$ and Noxes. Treatment with GSH precursors, zinc, and GM-CSF attenuate chronic alcohol-induced alveolar macrophage oxidative stress and dysfunction.

Summary

Compared to nonalcoholics, individuals suffering from AUDs are more susceptible to lung infections, sepsis, and ARDS. Alcoholics are twice as likely to develop ARDS [3], and their in-hospital mortality rates are double that of nonalcohol abusers [4]. In fact, alcoholic patients who develop pneumonia are twice as likely to develop sepsis [5], and those who develop sepsis are twice as likely to develop ARDS [4, 6]. A primary cause for increased risk of respiratory infections in

alcoholics is impaired immune function of the alveolar macrophage. Resident macrophages, differentiated from circulating monocytes, are critical for innate and acquired immunity in various tissues, including neurons, kidneys, bone, adipose tissue, liver, and lungs. These cells are the first line of defense against invading pathogens by generating a pro-inflammatory response to kill pathogens and clear foreign debris from tissues. Macrophages exhibit three distinct phenotypes, in which plasticity allows for functional specificity to be achieved. Classically activated macrophages respond to stimulation by Th1 cytokines, such as IFNγ and IL-1β, as well as to LPS to produce pro-inflammatory molecules including TNFα, IL-6, and IL-12. In contrast, alternatively activated macrophages respond to stimulation by Th2 cytokines such as IL-4 and IL-13 to generate anti-inflammatory molecules including IL-10, TGFβ, and IL-1 receptor antagonist. A third state of macrophage deactivation is mediated through stimulation by TGFβ, IL-10, and glucocorticoids to suppress inflammation. Chronic alcohol ingestion induces an alternatively activated phenotype in alveolar macrophages, which is characterized by increased oxidative stress, via up-regulation of TGFβ and Noxes, and leads to decreased phagocytic function. In contradistinction, attenuating alcohol-induced oxidative stress with treatments that preserve GSH and zinc bioavailability, enhance GM-CSF signaling, and reactivate the Nrf2-ARE pathway restore alveolar macrophage immune function. The studies reviewed in this chapter emphasize the importance of alveolar macrophage phagocytic function in clearing respiratory infections and how alcohol abuse impairs this critical component of lung host immunity. Understanding the cellular and molecular mechanisms that underlie alveolar macrophage immune dysfunction in the alcoholic lung is imperative to develop novel therapeutic strategies to decrease the risk of infection and lung injury in this vulnerable population.

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Chapter 8
Alcohol and the Alveolar Epithelium

Samuel A. Molina and Michael Koval

Abstract The distal airways are covered with a heterogeneous layer of cells known as the alveolar epithelium. Alveolar epithelial cells provide the major barrier between the airspace and fluid filled tissue compartments. As such, regulation of the alveolar epithelium is critical to maintain a healthy lung and for optimal gas exchange. In this chapter, we discuss functional roles for alveolar epithelial cells with particular emphasis on intercellular junctions and communication. As a thin layer of cells directly exposed to atmospheric oxygen, alveoli are particularly sensitive to oxidant insults. Alcohol significantly diminishes the normal antioxidant reserves of the alveolar epithelium, thereby rendering it sensitized for an exaggerated damage response to acute and chronic injuries. The effects of alcohol on alveolar epithelia are discussed along with open questions and potential therapeutic targets to prevent the pathophysiology of alcoholic lung disease.

Keywords Pneumocyte • Alveolus • Claudin • Connexin • Tight junction • Gap junction • Ethanol • Barrier function • Pulmonary edema • Fluid clearance

Introduction

Gas exchange occurs in the terminal airspaces of the lung, known as alveoli. The alveolar epithelium consists of two distinct cell populations where >90 % consists of Type I cells and the remainder are Type II cells (Fig. 8.1). Type I cells are exceptionally thin squamous cells that allow efficient gas permeability between the

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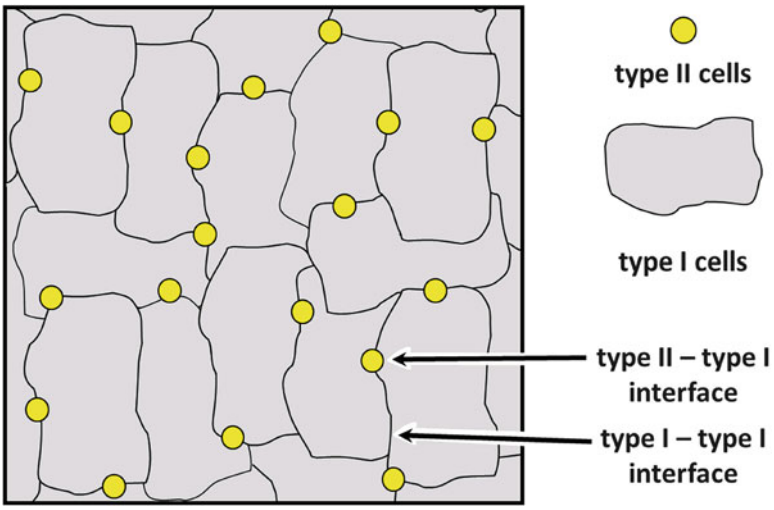

Fig. 8.1 Morphology and distribution of alveolar epithelial cells. The en face representation of alveolar epithelium shows the relative size and number of type I and type II cells [115]. The monolayer is heterogeneous and predominantly contains two classes of cell–cell interfaces: type I–type I cell junctions and type I–type II cell junctions. Adapted from [26]

airspace and bloodstream. Type II cells are cuboidal epithelia that serve several functions including the production and secretion of pulmonary surfactant that maintains alveolar surface tension and promotes innate immunity. Type II cells produce cytokines that are critical for regulation of the alveolar barrier and in the regulation of alveolar immune cells. Type II cells also respond to injury and have the ability to proliferate and differentiate into Type I cells to repair damaged alveoli.

Critically, Type I and Type II cells function in a cohesive manner in order to regulate alveolar fluid balance. This depends on functional interactions between these cells, which are regulated in large part by intercellular junctions at sites where cells are in direct contact. Paracrine signaling by cytokines and extracellular ATP also integrate alveolar epithelial cell function.

The alcoholic lung presents a unique pre-disease state that renders the tissue susceptible to damage when presented with a significant inflammatory insult. This is in large part because the ability of the lung to maintain a proper air/liquid interface is compromised due to a deficit in alveolar epithelial barrier function. Here we focus on how alcohol impairs alveolar epithelial physiology, with particular emphasis on that critical barrier function.

Effects of Alcohol on Alveolar Glutathione

Lung airspaces are directly exposed to environmental oxygen, which makes them vulnerable to oxidant stress. In patients requiring oxygen supplementation, there is even greater oxidant stress. As a major component of antioxidant defense, lungs use

the tri-peptide thiol antioxidant glutathione [1]. Levels of glutathione in the alveoli are among the highest in the human body and are present at concentrations of 400 μM or more in the alveolar fluid. Type II cells also contain high levels of intracellular mitochondrial and cytosolic glutathione pools that enable them to resist oxidant stress.

In contrast to the unstressed lung, chronic alcohol ingestion increases oxidative damage by depleting glutathione [2, 3]. Type II cells from alcohol-fed rats contain 20 times less total glutathione than controls and airspace glutathione is depleted [2, 4]. Glutathione levels are further decreased during endotoxemia where whole lung glutathione was reduced by 25 % in alcohol-fed septic rats as compared to control-fed septic rats, consistent with an additive effect of sepsis as a second hit on oxidative stress when it occurs in alcoholics [5, 6].

Metabolsim of dietary alcohol (ethanol) to acetaldehyde directly depletes glutathione, causing oxidant stress [6, 7]. Alcohol also induces cell-signaling pathways that contribute to oxidant stress, including increasing the production of Transforming Growth Factor β (TGF-β), which appears to play a critical role in mediating the alcoholic lung phenotype [5, 8]. TGF-β decreases lung glutathione by inhibiting γ-glutamylcysteine synthetase expression [9, 10] and further increases oxidant stress by increasing Nox expression [11, 12] and H_2O_2 production [13]. Consistent with this effect on oxidant stress, TGF-β accelerates the progression of acute lung injury [14, 15].

The effects of alcohol on the total cell glutathione pool suggest the potential of antioxidant therapies as a means to reduce the severity of acute lung injury. Consistent with this, supplementing the diets of alcohol-fed rats with the glutathione precursor procysteine reduces oxidant stress and reverses many of the pathophysiological effects of alcohol on the lung [16–18]. Antioxidant therapies have not yet been proven to be effective in decreasing mortality in critically ill patients since they have never been directly tested in individuals with alcohol use disorders. However, antioxidants can prevent the severity and duration of acute lung injury [19, 20]. Therefore, bolstering the antioxidant reserves of the lung are likely to be best used as an adjunct to other therapeutic approaches.

Alveolar Epithelial Barrier Function and Fluid Balance

The alveolar epithelium controls fluid balance through a combination of processes. First, the alveolus provides a permeability barrier which prevents fluid efflux from the bloodstream, lymphatic system, and tissue into the airspaces. Second, the alveolus actively promotes fluid efflux from the airspaces through a system of ion channels where water osmotically accompanies ion transport. To some extent, barrier function and ion transport can compensate for each other; however, the extent of compensation is not unlimited.

Most progress on defining the mechanistic basis for the effects of alcohol on alveolar barrier function have been through studies of rodents fed the isocaloric Lieber DeCarli alcohol diet that carefully controls for nutrition and thereby can

"isolate" the independent effects of alcohol ingestion [21]. Studies in the intact animal have demonstrated that rats on a chronic alcohol diet have enhanced permeability to small molecules (e.g., inulin) as well as to proteins as large as serum albumin [22]. Moreover, their ability to resist a saline challenge is impaired. Understanding this at a molecular level has benefited from the observation that the "alcoholic lung" phenotype of alveolar epithelial cells isolated from alcohol-fed rats persists in culture. In other words, alveolar epithelial cells isolated from alcohol-fed rats and grown in monolayers in culture remain leakier than comparably isolated and cultured cells from control-fed rats [22]. The basis for this is not fully understood, but it is likely that alveolar epithelial cells differentiate in response to chronic stress in the alcoholic lung and that this results in epigenetic modification to genomic DNA that modifies the behavior of these cells [23]. Epigenetics is a nascent field in alcohol research and is only beginning to be explored [24, 25]. Given the persistence of the alcoholic lung phenotype, alveolar epithelial cells from alcohol-fed rats provide a valuable model system to study their pathophysiology.

Tight Junctions and Alveolar Barrier Function

The bulk of the alveolar barrier consists of the cells themselves. The remaining barrier function is due to a series of structures at cell–cell interfaces called tight junctions. Tight junctions serve as a sealing point between polarized epithelial cells and denote the boundary between the apical and basolateral plasma membrane domains (Fig. 8.2). This segregation and sealing function formed by the tight junctions provide two important physiological functions of the epithelium: (1) a physical barrier to small molecules and (2) a series of paracellular ion channels that enable ion diffusion between cells [26].

Tight junctions are formed by a complex that includes transmembrane proteins, cytosolic scaffold proteins, and cytoskeletal proteins. Tight junction permeability is primarily mediated by proteins known as claudins that span the membrane bilayer four times and have both the N- and C-terminal domains oriented inside towards the cytosol. The C terminus of claudins interacts with cytosolic scaffold proteins, most notably zonula occludens-1 (ZO-1) and ZO-2 [27]. ZO-1 and ZO-2 in turn link claudins to the actin cytoskeleton. While almost all claudins interact with ZO-1 and ZO-2, they differ in the extent of actin association, which correlates with incorporation into functional tight junctions. Other transmembrane proteins also regulate tight junction assembly and stability, particularly occludin and tricellulin [28]. Roles for these proteins in the alveolar epithelium are continuing to be elucidated. However, occludin and ZO-1 expression are both down-regulated by the alveolar epithelium in response to alcohol, consistent with the concept that an early and primary defect in the alcoholic lung is impaired alveolar epithelial barrier function [29, 30].

There are over a dozen claudin isoforms expressed by the alveolus; the nine most prevalent are summarized in Table 8.1. Of these, 97 % of claudin mRNA in both

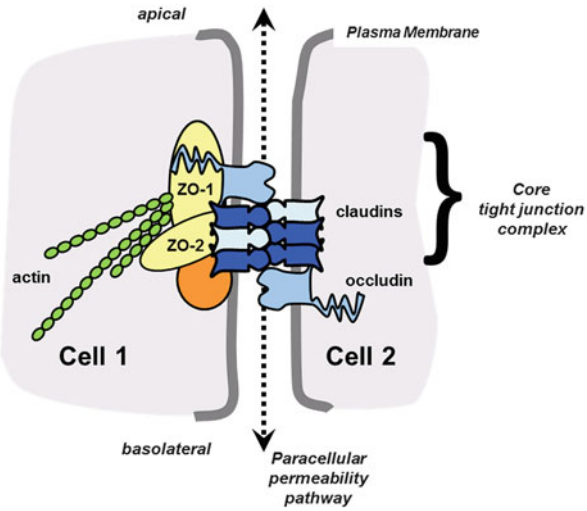

Fig. 8.2 Core tight junction protein complex. Shown are the major functional components of tight junctions known to directly interact. These include transmembrane proteins (claudins, occludin), scaffold proteins (ZO-1, ZO-2), and the actin cytoskeleton. Head-to-head interactions between claudins on adjacent cells form the basis for paracellular channels which restrict permeability. Homotypic interactions (between the same type of claudin) and heterotypic interactions (between different types of claudins) are depicted. The likely presence of additional scaffold proteins is represented by the *orange circle*. Not shown are immunoglobulin-fold transmembrane proteins, which associate with tight junctions and also regulate claudin expression and tight junction permeability, such as Junction Adhesion Molecule A (JAM-A) [116]. Adapted from [26]

type I and type II cells encodes for claudin-3, claudin-4, and claudin-18 [31]. However, type II cells express significantly more claudin-3 than is expressed by type I cells. This difference has functional ramifications, since claudin-3 expression increases alveolar tight junction permeability whereas claudin-4 makes tight junctions less permeable (Fig. 8.3) [32]. One consequence of this difference is that type II–type I cell interfaces are enriched for claudin-3 and are therefore likely to be more permeable than type I–type I cell interfaces. Functionally, the unique permeability of type II–type I cell junctions might provide a paracellular pathway for ion and fluid diffusion that counterbalances ion flux due to channel activity (see below).

Critically, claudin-4 is up-regulated during ventilator-induced lung injury. Since claudin-4 expression correlates well with human lung fluid clearance and is inversely related to the severity of the Acute Respiratory Distress Syndrome (ARDS), this underscores a protective effect of claudin-4 in lung function [33, 34]. Claudin-18 has a longer C-terminal domain and is more tightly associated with the cytoskeleton than claudin-3 or claudin-4, most likely due to increased interactions with tight junction scaffold proteins [32]. Claudin-18 is decreased in response to inflammation [35], consistent with impaired barrier function in ARDS.

As mentioned above, alcohol impairs alveolar barrier function by increasing tight junction permeability. In part this is due to decreases in claudin expression [36].

Table 8.1 Alveolar epithelial claudin expression

	Human		Rat		Mouse
	Fetal [109, 110]	Adult [33, 35, 111]	Type II [31, 34, 39]	Type I [31, 34, 39, 112]	Type II [113, 114]
Claudin-3	RP[a]	P	RP[b]	RP[b]	P
Claudin-4	RP	P	RP	RP	P
Claudin-18	RP	P	RP	RP	P
Claudin-5	RP	P	RP	RP	P
Claudin-7	RP		RP	RP	P
Claudin-10b	R		R	R	P
Claudin-12			R	RP	
Claudin-15			R	RP	
Claudin-19			R	R	

Other claudin mRNAs expressed by rat alveolar epithelial cells are: cldn-9, cldn-11, cldn-20, cldn-22, cldn-23 [31]. Table is adapted from [37]

[a]R—mRNA expression detected, P—protein expression detected

[b]Rat type II cells express ~17-fold more cldn-3 than type I cells, cldn-4 and cldn-18 expression is comparable [31, 34, 39]

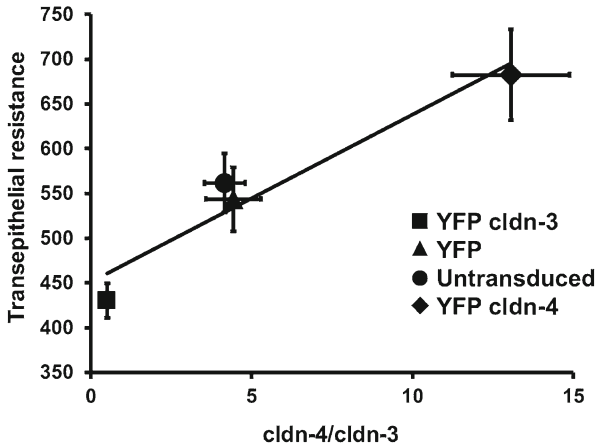

Fig. 8.3 Differential effect of increasing cldn-3 or cldn-4 on alveolar epithelial cell barrier function. Model type I alveolar epithelial cells transduced with YFP-cldn-3 (*filled square*), YFP-cldn-4 (*filled diamond*), YFP-control virus (*filled circle*), or untransfected controls (*filled triangle*) were assessed for the effect of altering claudin expression on barrier function, as determined using transepithelial resistance (TER; Ohm×cm^2) (*Y*-axis). The expression ratio cldn-4/cldn-3 was determined by immunoblot (*X*-axis) demonstrating that there was a linear relationship between cldn-4/cldn-3 ratio and TER ($r^2=0.93$). Cells expressing increased cldn-3 had significantly lower TER than either control cells or cells expressing increased cldn-4 ($p<0.05$). Increasing cldn-4 also significantly increased barrier function ($p<0.05$). Adapted from [37]

Perhaps more critically, the decrease in alveolar barrier function due to alcohol is accompanied by a significant shift in tight junction morphology as determined by immunofluorescence microscopy (Fig. 8.4). In normal cells, claudins are predominantly localized to cell–cell contact sites. By contrast, cells impaired by alcohol

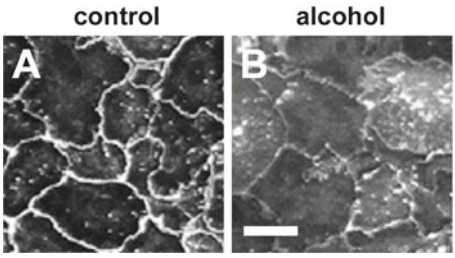

Fig. 8.4 Alcohol impairs assembly of claudins into tight junctions. Model type I alveolar epithelial monolayers were derived from primary cells isolated from either control (**a**) or alcohol-fed (**b**) rats which were cultured for 6 days and then immunolabeled for cldn-7. In contrast with control alveolar epithelial cells, where cldn-7 prominently localized to sites of cell–cell contact (**a**), cells isolated from alcohol-fed rats had impaired claudin assembly (**b**) which correlated with impaired barrier function. Bar—10 μm. Adapted from [37]

show significant morphological disruption of claudin localization, including strand breaks and increased intracellular labeling. Intriguingly, this correlates with an increase in expression of claudin-5, which is normally expressed at low levels in the alveolus [36, 37]. These effects of alcohol on claudin-5 and epithelial barrier function are consistent with the observations that increased claudin-5 correlates with impaired lung epithelial barrier function in cells treated with methanandamide or in cells directly transfected with claudin-5 [38, 39]. However, whether there is a direct mechanistic link between claudin-5 and disruption of tight junction assembly and function in alveolar epithelium remains to be determined.

Ion Channels and Alveolar Fluid Clearance

The primary mode of fluid efflux from airspaces is promoted by ion flux through alveolar epithelial cells that sets up an electrochemical gradient that drives water from airspaces into tissues. The majority of ion flux is due to sodium transport. On the apical surface, sodium transport into cells is mediated by epithelial sodium channels (ENaC), which are the rate-limiting step in alveolar sodium transport [40]. Transport out of cells on the basolateral surface is by Na,K-ATPases that actively pump sodium out of the cell and thereby provide the gradient that promotes transcellular passage of sodium from the alveolar space into the interstitium. Both type I and type II cells express sodium channels, as well as other ion transporters, that contribute to maintenance of lung fluid balance.

In contrast to the effect of alcohol on barrier function, alcohol increases net flux of sodium by having a direct effect on both parts of the sodium transport pathway. This was confirmed by examining the expression of lung Na,K-ATPase in rats fed the Lieber-DeCarli diet [41]. Na,K-ATPase is composed of several subunits, all of which were up-regulated in the alcoholic lung. When treated with the Na,K-ATPase inhibitor ouabain, lungs from alcohol-fed rats developed more edema than control

lungs, indicating that increased Na,K-ATPase compensates for other deficits in maintenance of lung fluid balance induced by alcohol.

In parallel to this increase in Na,K-ATPase expression, ENaC is also up-regulated in the alcoholic lung [42]. Critically, ENaC activity was also increased by direct oxidation mediated through NADPH oxidase activity. This is in line with other studies demonstrating that reactive oxygen species have the capacity to increase ENaC and lung fluid clearance [43, 44]. Together, increased ENaC and Na,K-ATPase in the alcoholic lung provide a pathway for up-regulated fluid clearance that can compensate for deficits in barrier function induced by dietary alcohol. Studies to date have focused on sodium transport; chloride transport must also increase in order to retain electronic neutrality across the alveolar epithelium. Whether this occurs via up-regulation of chloride channels or by chloride transport via the paracellular route remains an open question [40]. Taken together, these observations explained an initial paradox when the phenotype of the alcoholic lung was first being examined experimentally. Specifically, if chronic alcohol ingestion causes such significant defects in alveolar epithelial permeability, why don't alcoholics have pulmonary edema at baseline? The marked up-regulation of active fluid transport mechanisms in the alcoholic lung appear to compensate for the increased paracellular permeability and maintain a relatively "dry" airspace. However, this new equilibrium is far more susceptible to the stresses imposed by acute inflammation such as pneumonia or sepsis, and the alcoholic lung is far more prone to alveolar flooding with proteins and fluids during critical illness.

Therefore, in the otherwise healthy alcoholic, barrier function and fluid efflux are counterbalanced to maintain a non-edematous lung. However, if alveolar barrier function is further impaired due to a second hit, e.g., sepsis or barotrauma, the lung requires an additional increase in sodium transporters in order to prevent alveolar edema. ENaC in the alcoholic lung does have some additional capacity for further up-regulation (e.g., in acute response to endotoxin [42]). However, it is clear that lungs of injured alcohol-fed animals are more susceptible to endotoxin and sepsis induced lung edema than non-alcohol-fed controls [2, 18]. This suggests that in the alcoholic lung, fluid clearance saturates at a level insufficient to compensate for barrier dysfunction which, in turn, exacerbates the severity of ARDS. Therefore, any treatment modality designed to prevent alcohol-associated ARDS must address alveolar tight junctions, since it is not likely to be possible to increase fluid clearance enough to prevent alveolar flooding.

Intercellular Communication

Gap Junctions

Gap junctions provide a means for intracellular communication by forming channels that connect the cytosols of adjacent cells in a tissue (Fig. 8.5). This connection enables the direct diffusion of small cytoplasmic molecules, ions, and water. Metabolites including adenosine triphosphate (ATP) and glutathione also can move through gap junction channels. Several functions have been ascribed to gap

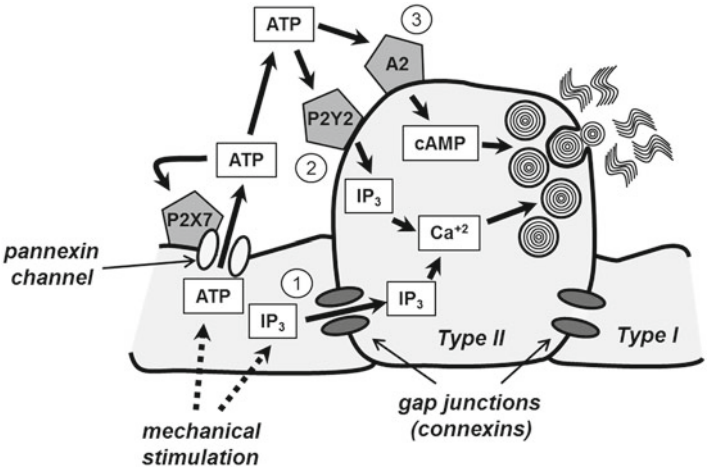

Fig. 8.5 Intercellular communication pathways between alveolar epithelial cells which control surfactant secretion. Gap junctional transmission (1) of either calcium or inositol trisphosphate (IP3) generates increased cytosolic calcium in type II cells, which, in turn, could stimulate surfactant secretion either through protein kinase C activation or through a direct effect of calcium on lamellar body fusion. Increased cytosolic calcium can also be mediated through secretion of ATP through P2X7 receptor-mediated pannexin channels (2) acting on P2Y2 purinergic receptors. Protein kinase A activation through stimulation of A2 purinergic receptors by ATP can also occur (3). Adapted from [46]

junctions including metabolite sharing, electrical coupling, cell growth control, and regulation of cell migration.

The proteins that form gap junctions are known as connexins. In the alveoli, connexin-43 (Cx43) is the major connexin demonstrated to have functional roles [45–49]. Other connexins expressed by the alveolar epithelium include Cx26, Cx32, Cx30.3, Cx40, and Cx46. Specific roles for these other connexins in the alveolar epithelium have not been elucidated. Among these, Cx32 is restricted to type II cells and is absent from type I cells [50, 51]. Moreover, type I cells cannot form functional gap junctions with cells expressing only Cx32; however, type II cells readily communicate through Cx32 gap junction channels [51]. Although type I cells can form functional heterocellular gap junctions with type II cells via Cx43, roles for Cx32 in regulating alveolar signaling remain unknown at present. One possibility is that Cx32 provides a "priority channel" where type II cells can specifically communicate with each other, but this has yet to be determined.

Alcohol treatment of cells in vitro inhibits gap junctional communication [52–55]. Specifically, alcohol significantly inhibits expression of the major lung connexin, Cx43 [52, 53], but has less effect on other connexins [52, 54]. A direct mechanism for the effect of alcohol on gap junctions is by partitioning into membranes and changing the connexin microenvironment [56]. Hormones induced by chronic alcohol ingestion can also have deleterious effects on alveolar gap junctions. Most notably, TGF-β inhibits alveolar Cx43 expression [55]. Moreover, glutathione depletion and increased oxidant stress induced by chronic alcohol

exposure also decrease gap junctional communication [57, 58]. Although a reduction in gap junctions in response to oxidant stress reduces intercellular signaling, it can be beneficial since it decreases the transfer of toxic agents and thus minimizes tissue damage [59, 60]. However, this is at the expense of promoting the transfer of protective agents including glutathione.

Recently, a novel mode of Cx43-dependent interaction in the alveolus was demonstrated by showing that bone-marrow-derived stromal cells (BMSCs) instilled into injured lungs formed functional gap junctions with type II cells in vivo [61]. This interaction was particularly critical for preventing cell injury due to insufficient mitochondrial function. In part, this could be due to direct transfer of metabolites from healthy BMSCs to damaged type II cells. More provocatively, evidence was provided showing direct mitochondrial transfer from the BMSCs to the type II cells, which also appeared to require functional gap junctions. Gap junction permeability is too limited to enable direct movement of mitochondria through channels themselves. Instead, mitochondrial transfer was due to microvesicles that were taken up by type II cells. It is plausible that this gap junction plaque endocytosis could enable mitochondrial transfer since, during this process, the plasma membrane and a small amount of cytosol are taken up by the neighboring cell [62]. Regardless of the specific mechanism, if a mitochondrial transfer pathway is critical for ameliorating lung injury then it is likely to be adversely affected by alcohol, which impairs both gap junctional communication (see above) and mitochondrial function [63].

Gap junctions play an important role in regulating the secretion of pulmonary surfactant produced by type II alveolar epithelial cells. Mechanical distension of type I cells in the alveolus occurs during a physical stretch, for example a deep breath or assisted ventilation, which then initiates a transient increase in cytosolic calcium through stretch-activated channels. The calcium is then transmitted through gap junctions from type I cells to type II cells where it stimulates fusion of lamellar bodies with the plasma membranes to release surfactant [64–66].

Paracrine Signaling

In addition to gap junctional communication, intercellular calcium transients can also be generated by extracellular ATP release and paracrine stimulation of purinergic receptors that can also promote surfactant secretion (Fig. 8.5) [67–70]. This provides a system where multiple pathways can promote intercellular signaling and surfactant release. In type II cells, P2Y2 receptors are the primary targets for paracrine ATP signaling that induce calcium transients through a G-protein-coupled mechanism [71]. Alveolar epithelial cells also express P2X-type purinergic receptors and L-type voltage-gated calcium channels that can initiate calcium transients involved in surfactant secretion [72, 73].

By contrast, type I cells express high levels of P2X7 purinergic receptors [74, 75]. P2X7 receptors differ from other classes of purinergic receptors in that they are

linked to a high conductance pore composed of proteins known as pannexins [76]. Thus, ATP stimulation of P2X7 receptors induces additional ATP release, acting as a feed-forward loop to further amplify purinergic signaling. Consistent with this, cocultures of type I and type II cells are more sensitive to stretch-induced surfactant lipid secretion than type II cells in monoculture [67]. The enhanced lipid secretion by cocultures was due to the sensitivity of type I cells to mechanical stimulation. In response to ~20 % stretch, type I cells released fourfold more ATP than type II cells. This supports the hypothesis that type I cells are mechanosensors and that paracrine stimulation of type II cells by extracellular ATP regulates surfactant secretion.

Pulmonary Surfactant

Pulmonary surfactant is a mixture of proteins and lipids synthesized by type II cells that lines the alveolar airspace. Surfactant prevents atelectasis by reducing airspace surface tension that would otherwise cause lung collapse due to the pressure of fluid filled tissues. The major proteins found in pulmonary surfactant are SP-A, SP-B, SP-C, and SP-D.

Two of these, SP-B and SP-C, are hydrophobic and interact with surfactant lipid components to create a surface active mixture [77, 78]. By contrast, SP-A and SP-D are members of the collectin protein family and serve as part of the innate immune system by opsonizing bacteria, viruses, and other foreign agents [79–82]. Both SP-A and SP-D have carbohydrate recognition domains that bind to polysaccharides, while the collagenous stalk region of the proteins is recognized by phagocytes in the airspaces. However, SP-A and SP-D also play key roles in regulating pulmonary surfactant as well. SP-A promotes surfactant lipid turnover by mediating surfactant uptake by type II cells [83]. SP-D also helps regulate surfactant degradation by macrophages and type II cells, as evidenced by the observation that this function is impaired in SP-D-deficient mice [84, 85].

The majority of surfactant lipid is phosphatidylcholine, with phosphatidylglycerol and phosphatidylethanolamine as the other major phospholipids present [86]. Moreover, most surfactant lipid contains saturated fatty acids, the bulk of which is dipalmitoyl-phosphatidylcholine. Saturated fatty acids are fairly unreactive as opposed to unsaturated lipids present in surfactant and cell membranes that are readily oxidized [87, 88]. Oxidant damage to type II cells alters their ability to synthesize surfactant lipids, which further compromises its surface activity [89, 90].

Given the negative impact of oxidant stress on surfactant function it is not surprising that surfactant production is impaired in the alcoholic lung. Surfactant lipid synthesis, secretion, and function are all impaired by chronic alcohol ingestion in rats as a result of glutathione depletion [17, 18]. Procysteine administered as a redox protective strategy restored the ability of type II cells to produce functional surfactant. Interestingly, *N*-acetyl cysteine was ineffective, since it can only compensate for cytosolic glutathione, whereas procysteine can also replenish mitochondrial glutathione [17]. Exposure to alcohol in utero also has a negative impact on production of several

surfactant lipids, indicating that oxidant stress can occur in the absence of atmospheric oxygen [91]. This is significant, since it suggests that pulmonary complications are a likely complication of fetal alcohol exposure, particularly in preterm infants [92].

Alveolar Wound Repair and Extracellular Matrix

Lung epithelia are susceptible to environmental insults and are in varying states of repair ranging from baseline turnover to recovery from acute injury. Acute and chronic alcohol consumption has been shown to alter the repair of damaged epithelial tissues. Much of what has been learned about wound repair is from studies of patient recovery from skin trauma [93]. Of particular relevance to alveolar epithelium is that the state and remodeling of the extracellular matrix are impaired as a result of alcohol ingestion.

Cells interact with the extracellular matrix as a means of regulating homeostasis and cell proliferation. Normal alveolar cells interact with an extracellular matrix containing several proteins, including laminin and type IV collagen [94, 95]. When the alveolus is injured, the matrix is destroyed and replaced with a provisional matrix of fibronectin and type I collagen that stimulates cell migration and wound healing [96–100]. Interestingly, fibronectin also stimulates alveolar epithelial cells to more rapidly form a high resistance barrier as opposed to cells on a matrix with basal composition [101, 102]. This is due to enhanced spreading of the cells on a fibronectin-enriched matrix. Although initially beneficial to the lung, barriers formed by alveolar epithelial cells on fibronectin over the long term have lower resistance than barriers formed by cells on laminin or collagen [102].

Therefore, healthy alveolar repair requires that the extracellular matrix is remodeled back to a state rich in laminin and type IV collagen. If this does not occur, then persistent fibronectin will cause an exaggerated inflammatory response and fibroproliferation that can permanently damage the alveolar epithelium [103, 104]. In response to chronic alcohol exposure, fibronectin expression in the lung is significantly increased [105, 106]. Increased fibronectin, in conjunction with other aspects of the alcoholic lung (e.g., oxidant stress and increased TGF-β), provides an alveolar microenvironment primed for aberrant repair in response to lung injury.

Summary

In order to withstand the environmental stresses of alcohol, the lung has to compensate for several changes that are not present in the normal lung. Chief among these is increased oxidant stress. To some extent, there is overt damage to the alveolar epithelium as a direct result of oxidant exposure. However, the lung epithelium also adapts through subtle changes in phenotype. Hallmarks of the alcoholic lung are similar to changes that occur during lung injury; however they are more limited in

scale. Therefore, as previously mentioned the otherwise healthy alcoholic has no clinically detectable evidence of alveolar dysfunction. However, these phenotypic changes predispose the alveolus for an exaggerated response when subjected to the added injury of a "second hit" such as ventilator-induced lung injury or sepsis.

Two key aspects of alveolar function are particularly impaired in response to alcohol exposure and have a significant impact on the severity of acute lung injury, namely impaired surfactant production and barrier dysfunction. Most of the effects of alcohol on pulmonary surfactant diminish the surface active lipid component rather than the protein component. However, when coupled with the added impact of alcohol on host defense, even a small effect on collectin family surfactant proteins is likely to be amplified. Given difficulties in using surfactant augmentation therapy in adult lung injury [107], it seems likely that the best approach is to target improving the immune response or attenuating inflammation rather than directly manipulating the surfactant pool in the adult alcoholic lung.

An impaired alveolar barrier is perhaps the more serious consequence of alcohol and likely has a major impact on the severity of acute lung injury because of the effects of alveolar flooding on gas exchange [108]. As discussed earlier, increased fluid clearance can compensate for some deficiencies in alveolar tight junctions. However, the capacity for up-regulated fluid clearance is not unlimited. Progress in understanding how alcohol affects the expression and assembly of alveolar tight junctions has identified several potential therapeutic targets to improve alveolar barrier function. Given that alveolar tight junctions are remodeled in response to alcohol, this suggests that a successful therapeutic approach targeting the alveolar epithelium may require normalizing tight junction protein composition. Alternatively, given the profound effects of alcohol that diminish tight junction stability, pharmacologic approaches to stabilize incorporation of claudins into tight junctions may prove to be a useful approach to minimize alveolar flooding due to barotrauma.

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Chapter 9
Alcohol-Mediated Oxidative Stress in the Airway: The Unique Role of Thiol Depletion

Samantha M. Yeligar, Yan Liang, and Lou Ann S. Brown

Abstract Individuals with alcohol use disorders (AUDs) have increased susceptibility to developing respiratory infections and the Acute Respiratory Distress Syndrome (ARDS). Oxidative stress is a primary contributor to the pathogenesis of these alcohol-related derangements. Sources of alcohol-induced lung oxidative stress include depletion of cytosolic and mitochondrial glutathione (GSH), increases in reactive oxygen species (ROS) and reactive nitrogen species (RNS), and enhanced expression and activity of NADPH oxidases (Nox). Alcohol-mediated oxidative stress in the lung leads to tissue injury and barrier dysfunction, phospholipid peroxidation and DNA oxidation, fibronectin production, apoptosis, and dysregulation of cellular zinc transport and immune function. Since these consequences directly relate to rising healthcare costs and associated hospitalizations of alcoholic patients, therapeutic interventions to attenuate alcohol-induced pulmonary oxidative stress are critical. Treatments recently under investigation in preclinical studies and, in some cases clinical studies, include drugs that activate peroxisome proliferator-activated receptor gamma (PPARγ), dietary zinc supplementation, and treatment with GSH precursors. These interventions are designed to attenuate alcohol-mediated increases in lung oxidative stress with the goal of restoring healthy lung function and thereby decreasing the risk of lung infections and injury in this vulnerable population.

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Abbreviations

AUD	Alcohol use disorder
ARDS	Acute respiratory distress syndrome
GSH	Glutathione
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
Nox	NADPH oxidases
PPARγ	Peroxisome proliferator-activated receptor gamma
$\bullet O_2^-$	Superoxide
H_2O_2	Hydrogen peroxide
•OH	Hydroxyl radical
•NO	Nitric oxide
ONOO-	Peroxynitrite
ELF	Epithelial lining fluid
GSSG	Glutathione disulfide
BAL	Bronchoalveolar lavage
TGFβ1	Transforming growth factor beta 1
SA	Small aggregate
LA	Large aggregate
MMP	Matrix metalloproteinase
TNFα	Tumor-necrosis factor alpha
GM-CSF	Granulocyte/macrophage colony-stimulating factor
ARE	Antioxidant response element
SAMe	*S*-Adenosyl-methionine
NAC	*N*-Acetylcysteine
GBS	Group B Streptococcus pneumoniae

Introduction

Individuals suffering from alcohol use disorders (AUDs) have a greater incidence of the Acute Respiratory Distress Syndrome (ARDS) compared to non-alcoholics [1]. During recent years, ample evidence has emerged describing the link between alcohol abuse and lung oxidative stress. Chronic alcohol consumption causes pulmonary oxidative stress through various mechanisms, including decreasing levels of the critical antioxidant glutathione (GSH), and renders the lung susceptible to injury [2]. Alcohol-induced oxidative stress affects whole lung tissue, multiple lung compartments such as epithelial lining fluid (ELF), and specific cell types such as epithelial cells, neutrophils, and alveolar macrophages. This chapter outlines recent investigations of the sources, consequences, and treatments of lung oxidative stress in the context of chronic alcohol use.

Sources of Alcohol-Induced Oxidative Stress

Chronic alcohol consumption increases lung oxidative stress. Alcohol-mediated pulmonary oxidative stress is generated from numerous sources, such as depletion of cytosolic and mitochondrial GSH, increases in reactive oxygen species (ROS) and reactive nitrogen species (RNS), and enhanced NADPH oxidases (Nox) expression and activity (Fig. 9.1). Several ROS produced in the lung include superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH), and RNS that include nitric oxide (•NO), and peroxynitrite ($ONOO^-$).

In the lung, alcohol is metabolized by cytochrome p450 [3] into its major by-product acetaldehyde, which itself causes ROS production and lipid peroxidation [4]. When acetaldehyde is generated, antioxidants are utilized and depleted. GSH, the most abundant non-protein thiol in the body, is essential for cellular protection through detoxification of ROS. ARDS patients have decreased GSH levels in their ELF [5]. In fact, concentrations of oxidized GSH, known as glutathione disulfide (GSSG), were greater than the levels of GSH in the ELF of patients with ARDS [6]. Compared with non-alcoholic control subjects, ELF concentrations of GSH were significantly decreased in otherwise healthy chronic alcoholics (~580 μM in controls versus ~80 μM in alcoholics) [7]. Further, the percentage of GSSG was greater in chronic alcoholics (~10 % in alcoholics versus ~3 % in non-alcoholics) [7]. In a rat model of chronic alcohol consumption in which rats were fed normal chow but their drinking water contained alcohol (20 %) for >3 weeks, GSH levels were decreased in plasma, lung tissue, and lung lavage fluid, and GSSG levels were increased in lung lavage fluid [8]. Additionally, under normal conditions, GSH

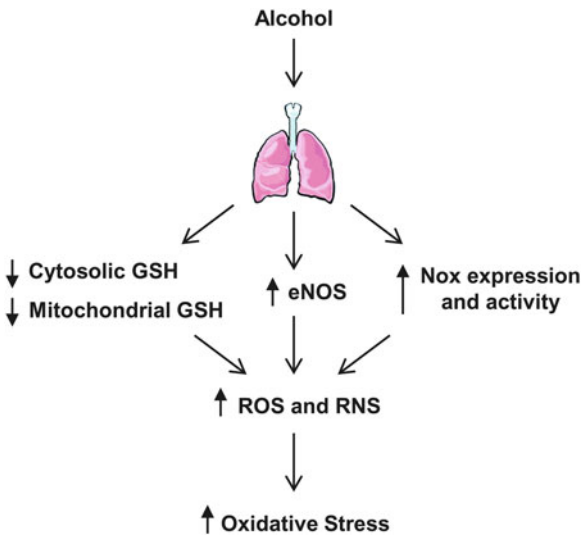

Fig. 9.1 Alcohol-induced sources of oxidative stress in the lung. Chronic alcohol use decreases cytosolic and mitochondrial GSH levels, and increases the expression and activity of endothelial nitric oxide synthase (eNOS) and NADPH oxidases (Nox) in the lung. These alcohol-induced alterations generate reactive oxygen species (ROS) and reactive nitrogen species (RNS)

concentrations in the lung ELF are maintained at very high levels through active transport into this space by alveolar type II cells, with levels exceeding 400 μM, higher than the GSH levels in plasma or in other extracellular fluids [8]. Alcohol ingestion diminished GSH content in alveolar type II cells by 95 % [8] and contributed to alveolar epithelial barrier dysfunction [9]. Rats chronically fed alcohol additionally exhibited depleted GSH levels and increased ROS generation in the mitochondria of alveolar type II cells [2, 10, 11]. Dramatic decreases in GSH/GSSG ratios render the alveolar epithelium and the lung more susceptible to severe injury.

Critical sources of oxidative stress in the alcoholic lung are ROS and RNS. In rats fed liquid diets with or without alcohol (36 % of calories) for 6 weeks, •NO synthesis, metabolism, and release were determined in the lungs and in pulmonary microvascular endothelial cells. Compared to rats fed the control diet, alcohol-fed rats exhibited increases in lung H_2O_2 production, expression and activity of endothelial nitric oxide synthase (eNOS), and levels of protein nitration and oxidation [12]. Pulmonary microvascular endothelial cells from alcohol-fed rats had increased eNOS expression and activity [13] and •NO release [12]. In parallel, mice given alcohol (20 %) in their drinking water for 12 weeks also had increased expression of eNOS in their lungs [14]. Further, human umbilical vein endothelial cells exposed to 0.10 % alcohol in vitro for 3 days showed enhanced H_2O_2 production and eNOS expression [14]. These and other studies have identified that chronic alcohol consumption increases ROS and RNS production within the lung and thereby promotes oxidative and nitrosative stress.

NADPH oxidases (Noxes) are multicomponent, membrane-associated enzymes that utilize NADPH as an electron donor to catalyze the reduction of molecular oxygen to $O_2^{\bullet-}$ and H_2O_2 [15]. Several Nox isoforms, specifically Nox1, Nox2, and Nox4, are expressed in the lung [16–18]. Studies in mice demonstrate that increased expression of either Nox1 or Nox2 is sufficient to up-regulate Nox4 expression [19]. In alcohol-exposed mouse embryos, Nox1, Nox2, and Nox4 constitute critical sources of ROS production [20]. Further, lungs from alcohol-fed mice showed increased expression and activity of Nox1 and Nox4 [14], and alveolar macrophages isolated from alcohol-fed mice demonstrated enhanced expression and activity of Nox1, Nox2, and Nox4, leading to increased ROS generation [19]. These studies show the critical role of Noxes in alcohol-induced reactive species production and collectively they implicate depletion of cytosolic and mitochondrial GSH, increases in reactive species, and enhanced NADPH oxidases (Nox) expression and activity, as key sources of pulmonary oxidative stress in the context of chronic alcohol use. The following section explores the pathological effects of chronic alcohol-mediated oxidative stress on the lung.

Consequences of Alcohol-Induced Oxidative Stress

Pulmonary oxidative stress induced by chronic alcohol consumption leads to a myriad of pathophysiological consequences. These consequences include lung injury and barrier dysfunction, phospholipid peroxidation and DNA oxidation, fibronectin production, apoptosis, and dysregulation of cellular zinc transport and immune

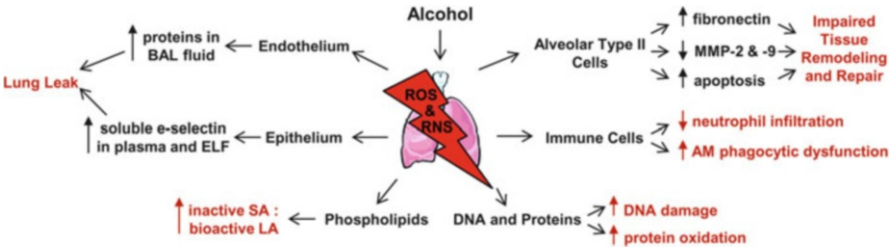

Fig. 9.2 Consequences of alcohol-induced oxidative stress in the lung. Chronic alcohol use primes the lung for severe injury and immune dysfunction. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) production induce pulmonary oxidative stress. Increased protein concentrations in bronchoalveolar lavage (BAL) fluid, enhanced soluble e-selectin in plasma and ELF, decreased active surfactant phospholipids, increased DNA damage and protein oxidation, impaired tissue remodeling and repair, and decreased neutrophil infiltration and alveolar macrophage (AM) function result from chronic alcohol-induced oxidative stress

function. As shown in Fig. 9.2, alcohol-induced oxidative stress leads to severe lung injury and immune dysfunction through increased protein concentrations in bronchoalveolar lavage (BAL) fluid, enhanced soluble e-selectin in plasma and ELF, decreased activity of surfactant phospholipids, increased DNA damage and protein oxidation, impaired tissue remodeling and repair, and immune cell dysregulation.

Alcohol abuse itself does not cause acute lung injury; however, it renders the lung susceptible to dysfunction in response to inflammatory stimuli such as trauma, sepsis, or other clinical conditions that are recognized to cause ARDS [21]. Sepsis is commonly associated with the development of ARDS [22], and chronic alcohol abuse independently increases the incidence of ARDS in critically ill patients [23]. The incidence of ARDS in patients with a history of AUDs and sepsis combined is two- to four-times that of patients without a history of AUDs [21]. Additionally, patients with a history of AUDs and septic shock had more severe non-pulmonary organ dysfunction than non-alcoholics [24].

Alcohol abuse significantly increases the risk of sepsis-induced acute lung injury [25]. In experimental models, alcohol ingestion increases the expression of transforming growth factor beta 1 (TGFβ1) by activating the renin–angiotensin system that, through the actions of angiotensin II, induces oxidative stress and TGFβ1 expression that together contribute to alveolar epithelial barrier dysfunction, and these effects are exaggerated during endotoxemia [25]. The relatively permissive leak of proteins into the airspace of the alcoholic lung [14] not only contributes to the non-cardiogenic pulmonary edema that is the hallmark of ARDS, it may exacerbate the lung injury when these proteins become oxidized and interfere with normal alveolar functions.

Endothelial cell activation is also a critical step in the pathogenesis of ARDS. Soluble e-selectin is an endothelial cell-specific molecule that regulates leukocyte-endothelial cell adhesion and is important in endothelial cell and alveolar-capillary barrier function. Alcoholic patients with ARDS have increased concentrations of soluble e-selectin in both plasma and the ELF, which is consistent with endothelial cell and alveolar-capillary barrier dysfunction [26].

In addition, impaired lung surfactant production in alcoholics [8, 9, 27] is proposed to contribute to their increased susceptibility to ARDS. In the ELF of ARDS patients, there are increased ratios of inactive small aggregate (SA) surfactant phospholipids to bioactive large aggregate (LA) surfactant phospholipids. In an experimental model, chronic alcohol ingestion in rats prior to inducing acute sepsis via cecal ligation and perforation increased lung lavage protein levels, aggravated hypoxemia, and attenuated the pool of functional LA surfactant phospholipids [22].

Chronic oxidative stress induced by alcohol exposure also has detrimental effects on lung DNA and proteins including fibronectin, a matrix glycoprotein implicated in lung injury and repair. In the ELF and alveolar macrophages isolated from the lungs of patients with a history of AUDs, alcohol abuse increased the expression of fibronectin. Alcohol also attenuated the expression of matrix metalloproteinase (MMP)-2 and -9 [29], which are implicated in tissue remodeling. Further, alveolar type II cells isolated from ethanol-fed rats showed increased oxidative stress and fibronectin expression, leading to subsequent lung tissue remodeling and stimulation of pro-inflammatory mediators that prime the alcoholic lung to injury [30]. Alcohol-induced oxidative stress also mediates dysfunction in immune cells, such as neutrophils, monocytes, and alveolar macrophages. In rats administered alcohol (4 g/kg body weight as a 50 % solution) by gastric gavage, there was increased DNA damage and protein oxidation in the lung tissue [28].

Since alveolar type II cell viability is critical for epithelial repair, the effect of chronic alcohol ingestion on the sensitivity of alveolar type II cells to inflammatory mediators that are up-regulated during sepsis is very important. Alveolar epithelial type II cells isolated from alcohol-fed rats have decreased mitochondrial GSH levels along with increased susceptibility to apoptosis and necrosis [11]. Further, chronic alcohol exposure exacerbates the oxidative stress caused by either tumor-necrosis factor alpha (TNFα) or H_2O_2 treatment of rat alveolar type II cells alone via mitochondrial GSH depletion and apoptosis through caspase-3 activation [10, 11]. Overall, there is compelling evidence that chronic alcohol ingestion sensitizes alveolar type II cells to inflammatory mediator-induced apoptosis and necrosis, thereby impairing the ability of these cells to promote repair following damage to the alveolar epithelium.

Individuals suffering from AUDs have increased susceptibility to respiratory infections, and this appears to be due at least in part to impairments in immune function caused by oxidative stress. Acute alcohol intoxication in animals impairs neutrophil migration in response to *Streptococcus pneumoniae*, the most common bacterial cause of community-acquired pneumonia [31]. The BAL fluid from alcohol-exposed animals showed decreases in levels of chemokines that enhance neutrophil adhesion and direct neutrophil migration to sites of inflammation [31]. These chemokines included macrophage inflammatory protein-2, cytokine-induced neutrophil chemoattractant, and neutrophil adhesion molecule expression [31].

Alveolar macrophages are critical to innate and acquired immunity in the lung [32]. The alveolar epithelium secretes granulocyte/macrophage colony-stimulating factor (GM-CSF) into the airway where it is required for alveolar macrophage maturation [33]. Alveolar macrophages exposed to chronic alcohol consumption exhibit

an alternatively activated phenotype, in which they express increased ROS [34] and decreased levels of GM-CSF receptors alpha (GM-CSFRα) and beta (GM-CSFRβ) [33]. Chronic alcohol-induced oxidative stress causes zinc deficiency in alveolar macrophages, through impaired zinc synthesis and transport [33, 35], and phagocytic dysfunction in clearing bacteria [19, 34, 35]. Alcohol-induced alterations in lung immune function described in the studies above likely increase the susceptibility of patients with a history of AUDs to develop respiratory infections.

Treatments to Attenuate Alcohol-Induced Oxidative Stress

The pathological consequences of chronic alcohol-mediated oxidative stress, including increased susceptibility to respiratory infections leading to sepsis and ARDS, contribute significantly to rising healthcare costs and hospitalizations. Therefore, it is imperative that we develop effective therapies that can mitigate alcohol-induced oxidative stress and thereby enhance lung health (and systemic health) as complete abstinence is a difficult goal to achieve for many of these individuals. Further, even individuals who are successful in treatment for their AUDs are likely at increased risk for pneumonia and lung injury for many weeks and perhaps even many months after their last drink. Therapeutic interventions currently being tested to attenuate alcohol-induced oxidative stress including activation of peroxisome proliferator-activated receptor gamma (PPARγ), zinc supplementation, and treatment with GSH precursors (Fig. 9.3) will be briefly discussed here.

Activation of PPARγ with its thiazolidinedione ligand, rosiglitazone, represents a novel strategy to attenuate oxidative stress by down-regulating the expression of Noxes. Previous studies established the ability of rosiglitazone to reduce Nox expression and activity in cultured endothelial cells [36], in the vasculature of diabetic mice [37], and in the lungs of hypoxia-exposed mice [38]. In a mouse model of chronic ethanol consumption with rosiglitazone intervention treatment, PPARγ activation with rosiglitazone attenuated the expression of Nox1 and Nox4, ROS generation, and barrier dysfunction in the lung [14]. These preclinical studies suggest that PPARγ activation, which is already in widespread clinical use in the treatment of diabetes, represents a novel therapeutic strategy that could decrease the risk of lung injury in individuals with AUDs.

Zinc deficiency is one of the most consistently observed biochemical and nutritional manifestations of alcoholic liver disease [39]. Systemic zinc deficiency develops in response to alcohol abuse, even when adequate zinc is present in the diet, through decreased expression of zinc transporters in the intestinal epithelium [33]. Zinc supplementation significantly attenuated alcohol-mediated increases in hepatic fatty acid oxidation, oxidative stress, and liver injury in a mouse model [40]. Decreased zinc levels were also a hallmark of chronic alcohol abuse in the lung [33] leading to dysfunction of alveolar macrophages [35] and impaired antioxidant defenses [41]. Alcohol interferes with zinc bioavailability within the alveolar space [42] and leads to increased oxidative stress in the alveolar

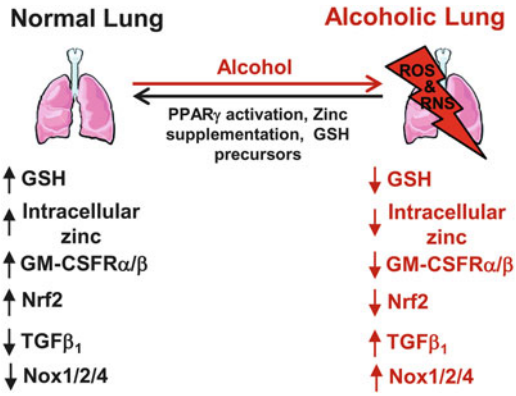

Fig. 9.3 Recently investigated therapeutic interventions for ameliorating alcohol-induced lung oxidative stress. Alcohol-mediated increases in pulmonary oxidative stress cause alterations in glutathione (GSH) levels in plasma and epithelial lining fluid (ELF), intracellular zinc levels in ELF and alveolar macrophages, and alveolar macrophage levels of granulocyte/macrophage colony-stimulating factor receptors (GM-CSFR)α and β, nuclear factor erythroid 2-related factor 2 (Nrf2), transforming growth factor beta 1 ($TGF\beta_1$), and NADPH oxidases (Nox)1/2/4. Therapeutic interventions to attenuate these consequences of alcohol-induced oxidative stress include activation of peroxisome proliferator-activated receptor gamma (PPARγ), zinc supplementation, and treatment with GSH precursors

macrophage through oxidation of the redox thiol pairs glutathione and GSSG [43] as well as cysteine and cystine [35]. In addition to oxidation of thiols, antioxidant defense mechanisms, such as the antioxidant response element (ARE), become activated. The ARE pathway is regulated by its major transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and serves as a cytoprotective program by which oxidative stress created by chronic alcohol abuse can be counteracted. Chronic alcohol ingestion decreased Nrf2 expression and impaired bacterial clearance in rat lungs [35]. Importantly, dietary zinc supplementation in this model attenuated oxidative stress in the alveolar space via up-regulation of Nrf2 expression and restored alveolar macrophage function, as demonstrated by improved lung bacterial clearance of *Klebsiella pneumoniae* [35, 42]. These studies suggest that dietary zinc supplementation could prevent alveolar macrophage oxidative stress and immune dysfunction, reducing the risk of pneumonia and lung injury in patients with the history of AUDs. This subject is explored in far greater detail in a subsequent chapter.

In the alveolar space, GSH is critical for the detoxification of oxidant radicals and the protection of cells in the airway and alveolus from inhaled oxidants [2]. Oxidative stress like that generated during pneumonia or ARDS requires protection by antioxidants such as GSH to circumvent lung injury [2]. The GSH precursors *S*-adenosyl-methionine (SAMe) and *N*-acetylcysteine (NAC) significantly decreased lung edema in alcohol-fed mice exposed to endotoxin [8]. In a guinea pig model of fetal alcohol exposure, fetal ELF and alveolar macrophage GSH

levels were decreased, oxidative stress was increased, and phagocytosis was impaired. When SAMe was added to the maternal drinking water, fetal ELF and macrophage GSH levels were maintained, oxidative stress was diminished, and phagocytosis was restored [44]. Further, NAC treatment of alcohol-fed rats normalized group B *Streptococcus pneumoniae* (GBS) clearance in the lung, prevented the systemic appearance of GBS, and attenuated acute lung injury [45]. Alveolar type II cells from alcohol-fed rats showed decreased mitochondrial GSH levels, cytosolic GSH levels, and surfactant synthesis and secretion. NAC treatment restored cytosolic, but not mitochondrial, GSH levels and did not affect surfactant synthesis and secretion. In contrast, treatment with procysteine, another GSH precursor, restored both the cytosolic and the mitochondrial GSH pools and normalized surfactant synthesis and secretion [27]. These studies suggest a role for GSH precursors as a treatment that could attenuate alcohol-induced oxidative stress and immune dysfunction, at least in the chronic setting such as in individuals undergoing substance abuse treatment. Collectively, treatments with PPARγ ligands, dietary zinc, and GSH precursors attenuate alcohol-mediated increases in oxidative stress and ameliorate lung derangements that contribute to the development of acute lung injury seen in alcoholic patients. Clinical studies are currently necessary to determine whether or not such strategies, alone or in combination, can enhance lung health and decrease the tragic consequences of alcohol-mediated lung disease.

Summary

Increased susceptibility to lung injury and infections in the context of chronic alcohol use is due in large part to oxidative stress, and is reflected by a myriad of derangements including depletion of cytosolic and mitochondrial GSH levels, increased nitrosative stress, and enhanced NADPH oxidase expression and activity. The situation is quite complex as the array of toxic mediators that result from chronic alcohol consumption includes ROS such as $\bullet O_2^-$, H_2O_2, and •OH, as well as RNS such as •NO and $ONOO^-$. As a result, alcohol-induced pulmonary oxidative and nitrosative stress leads to numerous pathological consequences and no single antioxidant strategy is likely to be effective. These consequences include lung injury and barrier dysfunction, phospholipid peroxidation and DNA oxidation, fibronectin production, apoptosis, and dysregulation of cellular zinc transport and immune function. Several therapeutic interventions that have recently been under investigation to determine if they can attenuate alcohol-mediated increases in lung oxidative stress include activation of PPARγ with its ligand rosiglitazone, dietary zinc supplementation, and treatment with the GSH precursors NAC and SAMe. These strategies represent novel therapeutic approaches to treating patients with a history of AUDs.

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Chapter 10
Alcohol and the Adaptive Immune Response in the Airway: Dendritic Cell and Lymphocyte Impairments

Kevin L. Legge and Thomas J. Waldschmidt

Abstract It is well established that alcoholics have an increased incidence of respiratory diseases. Further, chronic alcoholism predisposes for more severe disease following respiratory infections. While many studies have detailed the increase in disease severity during respiratory infections in alcoholics, much less is understood about the underlying mechanisms through which chronic alcohol consumption mediates this increase in disease severity or how alcohol alters pulmonary adaptive immune responses. It is well accepted that chronic alcohol ingestion is immunosuppressive and exerts inhibitory effects on adaptive immunity. However, these studies have largely focused on examining the immunosuppression of cell-mediated immunity in the spleen, skin, and liver. Importantly, for many pathogens the type of immune response generated in the respiratory and intestinal mucosa is distinct from that generated following intravenous, intraperitoneal, or subcutaneous exposures. Therefore, the type and extent of lesions that exist in the respiratory adaptive immune system of alcoholics and the consequences of these lesions, particularly during infections, remain in question. This chapter highlights and discusses the field's current knowledge on chronic alcohol-induced lesions within the pulmonary dendritic cell and T cell compartments and the relationship of these lesions to increased respiratory disease.

Keywords Ethanol • Lung • Adaptive immunity • Respiratory pathogens • Dendritic cell • T cell

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Effects of Chronic EtOH Consumption on the Human Immune System

It is well known that chronic alcohol consumption compromises the human immune system. This fact is best underscored when examining the susceptibility of chronic alcoholics to infectious diseases. Alcoholic patients have a greatly increased risk of infection with extracellular bacteria, intracellular bacteria, and viruses [1–8]. Numerous reports have documented that alcoholics exhibit higher rates of bacterial pneumonia, sepsis, meningitis, and peritonitis [1–8]. Tuberculosis and diseases caused by other intracellular agents are often associated with chronic alcohol abuse, as are elevated rates of hepatitis B, hepatitis C, and HIV infection [1–8]. Collectively, these findings point to multiple lesions in both innate and adaptive immunity.

Examination of alcoholic patients has indeed revealed defects in many aspects of the immune system. Innate immunity is clearly altered, with functional lesions in granulocytes, monocytes/macrophages, and NK cells [1–9]. The human T cell compartment likewise shows abnormalities after chronic alcohol consumption, with evidence of both anergy and hyperactivity. Although patients typically exhibit poor delayed type hypersensitivity (DTH) reactions, analyses of freshly obtained peripheral blood T cells show evidence of persistent activation [1, 4, 7, 10–12]. This is demonstrated by heightened levels of activation markers on both $CD4^+$ and $CD8^+$ T cells, as well as enhanced cytokine production [1, 10–12]. These mixed observations suggest fundamental regulatory defects in the cell-mediated immune response of alcoholics.

Effects of Alcohol Consumption on the Immune System in Experimental Rodent Models

In general, experimental animal models of alcohol consumption can be categorized as acute, short term, or long term. In acute (or binge) models, alcohol is given as a bolus, either intraperitoneally or by gastric gavage, followed by analyses of immune parameters within hours. In short-term models, animals (most commonly, mice) are administered alcohol in a complete liquid diet (e.g., the standardized and widely used Lieber-DiCarli diet) for 1–3 weeks, during which time-dependent assessments of immune function are performed. The majority of rodent studies examining the effects of alcohol on the immune system have utilized short-term liquid diets. Long-term models typically entail months of alcohol intake and are varied in the manner in which the alcohol is given. In some models, alcohol is delivered as part of a complete liquid diet (such as the Lieber-DeCarli diet), whereas in others alcohol is provided in the drinking water along with free access to rodent chow.

Although acute and short-term models have provided substantial information on how alcohol affects immunity, a number of questions arise as to the application of these data to immune dysfunction in chronic alcoholic humans. Not only is there a

significant difference in the time course of alcohol intake (days to weeks in rodents versus years or decades in humans), but a number of studies have suggested immune alterations in acute [13, 14] and short-term [15, 16] rodent models are in fact due to generalized stress. For example, in acute models it has been demonstrated that bolus administration of alcohol leads to rapid induction of corticosteroids [17, 18], agents central to the stress reaction [19]. Likewise, short-term alcohol-containing liquid diets can lead to weight loss (nutritional deprivation) and systemic stress [20]. In both cases, endogenous steroid production likely contributes to the reported immune alterations [13, 14, 17, 18]. Accordingly, results derived from acute and short-term models may be best applied to human immune suppression resulting from binge drinking.

In order to better model immune dysfunction in chronic alcoholics, a number of investigators have devised rodent models of chronic alcohol intake. Ideally, such models involve weeks to months of alcohol consumption without accompanying weight loss (nutritional deprivation). Since rats tolerate alcohol-containing Lieber-DiCarli diets much better than mice, a variety of immune function studies have been performed with this species after long-term alcohol feeding. Although immune lesions were observed, weight loss in rats still occurred in some studies [21, 22] and thereby making interpretations of the results problematic. An alternative approach has been to provide alcohol in the drinking water while allowing the animals to have free access to rodent chow (i.e., the Meadows-Cook model). This approach has proven successful in that mice consume significant levels of alcohol while maintaining normal adult mouse weights, even after 6–8 months of ingestion. [Morning blood alcohol levels in alcohol-fed mice are as high as 400 mg/dL, with lower levels as the day progresses, and alcohol intake per mouse averages 20 g/kg/day.]. Using the alcohol-in-drinking-water model, immune dysfunction in the systemic natural killer (NK) cell, dendritic cell (DC), B cell, and T cell compartments have been documented [12, 23–32]. As detailed below, we and others have similarly found a number of defects in pulmonary DC and CD8 T cells in mice after chronic alcohol intake. Importantly, these occur in animals that maintain normal adult weights and exhibit normal levels of serum corticosterone [23].

Alcohol Consumption and Pulmonary Immunity

As discussed above alcohol abuse is well known to increase susceptibility to pulmonary infections [3, 8]. Indeed, as noted elsewhere in this book, Benjamin Rush in 1785 described that alcoholics were susceptible to yellow fever, tuberculosis, and pneumonia [33]. Subsequent studies have demonstrated that alcohol abuse significantly increases pneumonia-associated morbidity and mortality two- to sevenfold [4, 34–36] and increases the risk of mycobacterial infections [4, 37]. However, chronic alcohol abuse does not only increase susceptibility to bacterial infections in the lung, as alcoholics also have an increased likelihood of respiratory fungal and virus infections [8, 38].

In part these increases in susceptibility have been shown to relate to alcohol-induced changes in pulmonary innate immunity. In particular, alcohol suppresses both alveolar macrophage and neutrophil functions as well as reduces ciliary function and production of surfactant proteins [8, 39, 40]. Indeed, alveolar macrophages in alcohol-treated animals have decreased phagocytosis, bactericidal activity, migration, and cytokine production [3, 8, 41–44]. Likewise, neutrophils in these animals show decreased bacterial killing, recruitment, and phagocytosis [3, 8, 45–47]. Together, these studies suggest that decreased or altered innate immune responses may in part be responsible for the increased severity of respiratory infections in chronic alcoholics. However, how lesions within the respiratory adaptive immune response contribute to the increased susceptibility, morbidity, and mortality in alcoholics remains less well studied. Therefore, the mechanisms by which chronic alcohol alters pulmonary adaptive immune responses and disease outcome are the focus of the later parts of this chapter.

Chronic Alcohol Consumption and T Cell Immunity

Chronic alcohol exposure induces substantial changes in T cell immunity. Indeed, studies have shown that chronic alcoholics are lymphopenic and have marked changes in their peripheral T cell compartments [1, 7, 48]. Similarly, chronic alcohol treatment of mice leads to decreased numbers of cells within the spleen and thymus [23, 49, 50]. Additionally, exposure to chronic alcohol results in T cells with an "activated" marker expression (i.e., increased CD69, CD25, and CD44 expression) that are easily triggered by stimulation to produce the effector cytokine IFNγ [12, 24, 51, 52]. In contrast to the increased activation marker and cytokine expression, these T cells from both human alcoholics and chronic alcohol-fed rat models exhibit a reduced ability to proliferate [53–55]. Further, this lack of proliferation is not rescued by the addition of IL-2 [53–55], suggesting that these T cells are not simply anergized. The effects of chronic alcohol on T cell skewing are varied. Infection of chronic alcohol-fed mice with *Klebsiella pneumoniae*, which normally generates a Th1 response, generates a profound Th2 response and IL-10 production within the lungs, and neutralization of this IL-10 improves the clearance of the bacteria [56]. However, not all studies have shown skewing in the T cell cytokine response after alcohol exposure [57]. Overall however, the available data suggest that chronic alcohol exposure leads to activated T cells that cannot proliferate or properly expand during a pathogen challenge.

Chronic Alcohol Consumption and Dendritic Cells (DC)

To date there have been limited studies investigating the effects of alcohol on DC. Monocyte-derived and bone-marrow-derived DC that have been cultured in alcohol have reduced expression of the co-stimulatory molecules CD40, CD80, and CD86,

reduced allostimulatory capacity, and diminished IL-12 production [58–60]. These changes in DC were not reversible by alcohol removal, suggesting that the exposure stably alters DC [58]. Studies examining DC in chronic alcohol-fed mice have shown that both Langerhans cell and dermal DC numbers in the skin are reduced and that these cells have delayed migration from the skin to the lymph nodes in response to inflammatory stimuli [32]. Further, similar to DC exposed to alcohol in vitro, the migrated skin DC in the lymph nodes of chronic alcohol-fed mice show lower expression of co-stimulatory molecules [32]. In the spleen, chronic alcohol consumption also results in a loss of DC, decreased co-stimulatory molecule expression following inflammatory stimuli, diminished cytokine production, and reduced allo- and peptide-specific stimulatory ability [30, 31, 61, 62]. Together these results suggest that chronic alcohol consumption may alter the co-stimulation, migration, and effector abilities of DC. However, in light of studies that suggest that DC isolated from different tissues can differentially respond to pathogens and drive diverse T cell responses, it has been important to determine which specific changes in pulmonary DC are induced by chronic alcohol consumption.

Pulmonary Adaptive Immunity to Pathogens

In response to pulmonary infections such as from influenza virus, respiratory DC are integral to the induction of adaptive immunity [63–68]. In general, DC are thought to be the primary cells involved in activating naïve T cells in the lymph nodes due to their expression of co-stimulatory and secondary signals required for T cell activation [69–71]. During influenza infections the initiation of adaptive T cell responses is believed to start with the uptake of antigen by respiratory DC that reside in the mucosa and sub-mucosa of the conducting airways as well as on the alveolar surfaces [72, 73]. In response to infection and inflammation, these respiratory DC then mature and migrate from the lungs to the lymph nodes that drain the lung [63–68, 74] where they present the influenza virus-derived peptides and release growth and differentiation factors (e.g., cytokines) needed for activation of naïve T cells [69–71].

DC Antigen Acquisition, Antigen Presentation, and Activation of Naïve T Cells

Based primarily on experiments in vitro, respiratory DC are thought to acquire antigens in the lungs during pulmonary infections by one of two methods; direct infection of the cells with the pathogen [75, 76] or exogenous uptake of antigen in the form of apoptotic bodies from dying infected epithelial cells [77–79]. After acquisition of this antigen the DC degrade the pathogen proteins and then load the processed peptides onto MHC class I and II molecules for presentation to naïve T cells in the lymph

nodes. Mature activated DC provide 3 types of signals to naïve T cells that together lead to the activation of the T cell: (1) MHC class I or II with peptide (viral peptides in the case of influenza virus), (2) co-stimulatory molecules (i.e., CD40, CD80, and CD86), and (3) cytokine production (i.e., IL-12) [69–71]. Together these signals lead to full activation of naïve T cells specific for the peptide antigens presented within the MHC and also lead to T cell effector commitment [69–71, 80]. In contrast, DC that do not provide these three complete signals are thought to induce tolerance rather than activation of T cells [69–71]. Studies have shown that individual DC subsets may drive, through differential cytokine production, distinct T cell responses. For example, DC-produced IL-12 drives IFNγ-mediated Th1 responses and cytotoxic CD8 T cell responses. In contrast, lack of DC-produced IL-12 in the face of IL-4 expression drives Th2 responses. In parallel, DC-produced IL-10 leads to the induction of T regulatory (T_{reg}) cells. This differential skewing largely depends on the DC exposure to pathogens, pathogen products, and the local environment [69–71]. Indeed, infection of DC in vitro with high multiplicities of infection (MOI) of influenza virus leads to low DC IL-12 production and inhibition of influenza-specific T cell responses. In contrast, infection of DC with low MOI leads to abundant IL-12 production and drives robust T cell responses [81]. These results suggest that different types of antigen acquisition and DC cytokine production during pulmonary infections in the presence or absence of alcohol consumption could potentially alter T cell effector responses.

Dendritic Cell Subsets

Different subsets of DC are found within many organs [92–94]. For example, the spleen has four major DC populations: $CD8^+$ DC, $CD4^+$ DC, $CD8^-/CD4^-$ DC, and plasmacytoid DC [82–84]. $CD8^+$ DC make large amounts of IL-12 and are potent cross-presenters of antigens and as such are potent inducers of Th1 and CD8 T cells responses [82]. $CD8^-$ DC, on the other hand, are poor producers of IL-12 and therefore are much better at inducing Th2 immunity [82]. Plasmacytoid DC are best known for their potent production of type I interferon during viral infections and have recently been shown to be able to kill virus infected cells [82]. Each of these DC subsets is thought to be specially equipped to recognize different types of pathogens based upon expression of pattern recognition receptors (PRR) such as the toll-like receptors (TLR) [69–71, 82, 85]. Interactions of these PRR with pathogen byproducts (such as LPS or double-stranded RNA) typically lead to the maturation and activation of immature DC and allow these DC to drive a polarized immune response [69–71, 82, 85]. While the generalized functions including antigen uptake, processing and presentation, as well as TLR and cytokine expression of the individual DC subsets described above often hold for DC with a similar phenotype found within other tissues, those tissues may also hold different subsets or groups of DC that may then differentially respond to pathogens based upon their PRR expression and programming [86]. For example, while splenic DC infected in vitro make type I IFN to influenza virus infections, those isolated from the lungs do not (Legge, unpublished results).

Respiratory DC Subsets

The DC found within the lungs prior to infection broadly fall into three subsets: airway DC, alveolar DC, and interstitial DC (iDC) [73, 87–89] (for differential marker expression of these subsets please see Fig. 10.1). Based upon labeling DC with carboxyfluorescein succinimidyl ester (CFSE) followed by influenza virus infection in situ, we and others [63–68, 90, 91] have demonstrated that the $CD11c^{+}$ $CD11b^{-}MHCII^{+}F4/80^{+}autofluorescent^{neg}$ cells are the major DC subset that migrates from the lungs to the T cell areas of the lymph nodes during influenza virus infections. (Note: alveolar macrophages are also $CD11c^{+}CD11b^{-}$ but are autofluorescent and $MHCII^{dim/neg}$) [73, 87–89]. These markers correspond to the airway and alveolar dendritic cell (aDC) populations.

Our recent studies using a novel in situ CFSE labeling technique have demonstrated that respiratory DC migration from the lungs to the lymph nodes is rapidly augmented in the first 18 h following influenza infection [64]. Interestingly however, such respiratory DC efflux from the lungs to the lymph nodes following influenza virus infection is not continuous, as labeled DC remain prevalent in the lung yet disappear from the lymph nodes within 48 h [64]. Further, the remaining pulmonary DC become resistant to subsequent migration stimuli [64]. Therefore, it appears that antigen trafficking from the lungs to the lymph nodes is largely

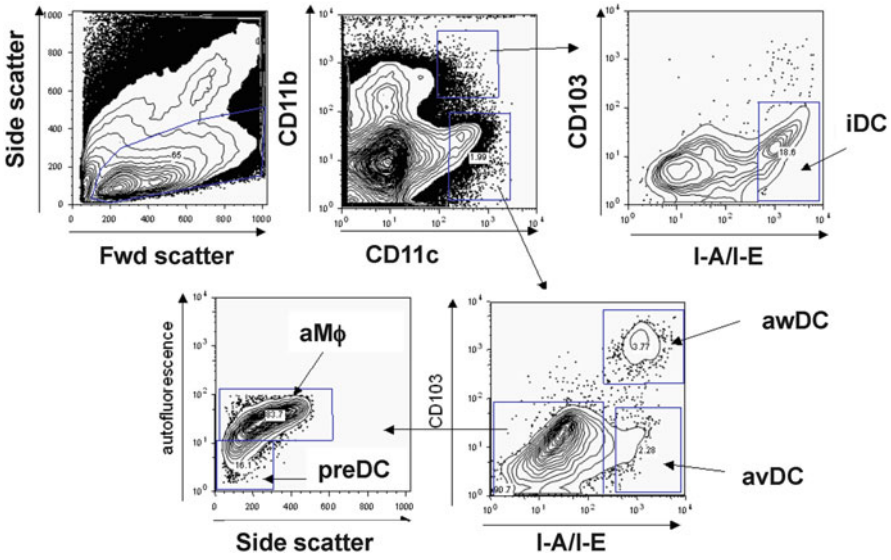

Fig. 10.1 Phenotype of pulmonary dendritic cells (DC). Shown is the gating strategy for DC within the lungs. Interstitial DC (iDC) are $CD11c^{+}CD11b^{+}$I-A/I-E^{+} cells. Airway DC (awDC) are $CD11c^{+}CD11b^{-}$I-A/I-$E^{+}CD103^{+}$ cells. Alveolar DC (avDC) are $CD11c^{+}CD11b^{-}$I-A/I-$E^{+}CD103^{-}$ cells. Precursor DC (preDC) are $CD11c^{+}CD11b^{-}$I-A/I-E^{-}autoflourescent^{-} cells. Note: Alveolar Macrophages (aMac) are $CD11c^{+}CD11b^{-}$I-A/I-E^{-}autoflourescent^{+} cells. I-A/I-E=MHC class II

confined to the first 48 h following influenza infection. Overall, our studies as well as those of others suggest that airway DC appear to be integral to the induction of T cell immune responses to pulmonary pathogens and, due to their localization and pulmonary "education," may play a dominant role in the determination of the magnitude and effector phenotype of the CD8 T cell response generated.

Alcohol-Induced Increases in Pathogen-Associated Morbidity and Mortality

Viruses

To date, studies on how alcohol may affect influenza virus infections have been quite limited. Using an experimental "binge" model, Cotte et al. showed that alcohol ingestion immediately preceding or following influenza infection increases influenza-associated mortality [2]. Further, alcohol consumption led to both increased plateaus in influenza virus titers and sustained high levels of virus in the lungs even out to day ten following infection [2]. While this study suggests that alcohol increases the severity of influenza virus infections, it did not examine how chronic alcohol consumption alters influenza susceptibility and adaptive immune responses.

Using the Meadows-Cook model, we have studied how chronic alcohol ingestion affects influenza A viral infections [23, 92–94]. When 6-week-old BALB/c or C57BL/6 mice were placed on chronic alcohol consumption for 4, 6, or 8 weeks and then infected intranasally with a $0.01LD_{50}$ dose of influenza A virus, they showed substantial increases in both morbidity and mortality compared to comparably infected mice that did not consume alcohol [93]. Note: in healthy mice this influenza A virus inoculum only leads to moderate to low weight loss (morbidity) and a mortality rate of only ~1 %. Importantly, our results showed that alcohol ingestion induced substantially greater weight loss and death (50 % of the alcohol-fed mice succumb to the infection). Additionally these mice exhibited increased and sustained viral titers as well as a severe pulmonary pathology characterized by increased neutrophilia and robust edema [93]. Taken together, our results suggest that, consistent with the clinical observations in alcoholic humans, chronic alcohol ingestion greatly increases the morbidity and mortality of pneumonia.

Similar to the effects of chronic alcohol ingestion on the susceptibility to influenza virus infections, a recent study has demonstrated an alcohol-induced increased susceptibility to and disease severity during respiratory syncytial virus (RSV) infections [95]. In this model, chronic alcohol consumption increased pulmonary neutrophilia, edema, hemorrhage, and sustained pulmonary inflammation for an additional 2 days. Further, similar to influenza infection, alcohol-fed mice infected with RSV failed to clear virus with appropriate kinetics, as virus was detectable in the lungs for at least 5 additional days when compared to similar infected water-fed control mice.

Bacterial Infections

Given the profound lesions and enhanced disease severity during virus infection of chronic alcohol-fed mice and the epidemiology data showing enhanced bacterial pneumonia in chronic alcoholics, we and others have explored pulmonary disease and immunity to bacterial infections during chronic alcohol exposure. Similar to its effects in the context of a subsequent virus infection, chronic alcohol ingestion significantly increases pulmonary neutrophils and pathogen burden during pulmonary *Pneumocystis carinii* infections [96]. Likewise, alcohol ingestion increases mortality during experimental *Klebsiella pneumoniae* infection [97] and organism burden during *Mycobacterium tuberculosis* infections [98]. Similar to previous studies using *Streptococcus pneumoniae* [99], our own recent studies using a *Streptococcus pyogenes* (GAS) lung infection model show that alcohol ingestion amplifies morbidity, neutrophilia, lung pathology, and mortality (~30 % vs. 0 %). Further our results suggest that alcohol increases and sustains the pulmonary bacterial burden in the lungs and may facilitate spread of GAS systemically.

All together, the common theme of respiratory infections in the context of chronic alcohol ingestion suggests that alcohol increases the overall morbidity and mortality by increasing and sustaining the pathogen burden, increasing pulmonary neutrophilia, and increasing lung inflammation and edema.

Alcohol-Induced Inhibition in Pulmonary DC Numbers and Alteration of Migration

As the above-described changes in disease severity in alcohol-fed mice could be related to altered adaptive immune responses, we have also determined how chronic alcohol ingestion affects respiratory DC. Similar to reports describing decreased numbers of DC in other tissues following chronic alcohol exposure [30–32, 58–62], our studies have demonstrated that 4 weeks of alcohol consumption decreases the total number of DC found in the lungs. These observations suggest that chronic alcohol exposure may inhibit the number of DC in the lungs available for initiating adaptive T cell immune responses following challenge with a respiratory pathogen. Consistent with this idea, we have observed a similar decrease in the number of DC present within the lungs following influenza virus infection in mice fed alcohol for 4–8 weeks. Specifically, the numbers of alveolar and airway DC, interstitial DC, and alveolar macrophages found in the lungs on day 6 post influenza virus infection are substantially decreased (see Fig. 10.2). In contrast, pulmonary interstitial macrophage numbers appear to be unchanged. Likewise, other investigators have observed a similar decrease in pulmonary DC during RSV infections [95]. This reduced responsiveness of pulmonary DC from alcohol-fed mice does not appear to be specific to virus infections, as a similar decrease in expansion/recruitment of alveolar, airway, and interstitial dendritic cells in the lungs is found after challenge

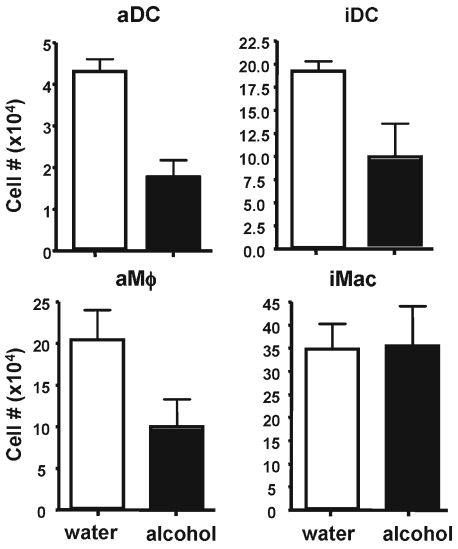

Fig. 10.2 Chronic alcohol exposure decreases the number of pulmonary DC. Groups of BALB/c mice fed for 4 weeks ± alcohol in their drinking water were infected with influenza virus and on day 6 post infection the lungs were removed and the number of airway DC and alveolar DC (aDC), interstitial DC (iDC), alveolar macrophages (aMac), and interstitial macrophages (iMac) enumerated by flow cytometry

of the lungs with TLR ligands (see Fig. 10.3). In addition to the decrease in numbers of DC present within the lungs, chronic alcohol ingestion further reduces the ability of the pulmonary dendritic cells to mature in response to inflammatory stimuli. As shown in Fig. 10.4, unlike DC in control mice, the pulmonary DC from alcohol-fed mice fail to up-regulate CD40 and CD80 expression upon TLR stimulus. These data suggest that chronic alcohol ingestion may compromise or at least delay the maturation of pulmonary dendritic cells in response to infections, which would in turn inhibit or at least delay the induction of adaptive immune responses to pulmonary pathogens.

As described above, the migration of DC from the lungs to the draining lymph nodes is a necessary part of initiating influenza-specific CD8 T cell responses. Therefore, our subsequent studies have determined if chronic alcohol consumption alters the migration of DC from the lungs to these lymph nodes. Our findings demonstrate that DC migration from the lungs to the lymph nodes in chronic alcohol-fed mice is delayed and never reaches the magnitude observed in control mice after influenza virus infections (see Fig. 10.5). This suggests that at least one component of the reduced influenza-specific CD8 T cell response [93] observed during chronic alcohol ingestion may relate to improper activation of the naïve influenza-specific CD8 T cells by dendritic cells within the lymph nodes.

Taken together, the above results demonstrate that chronic alcohol ingestion substantially reduces the number of DC within the lungs and alters the effector ability of DC to respond to respiratory pathogen challenge.

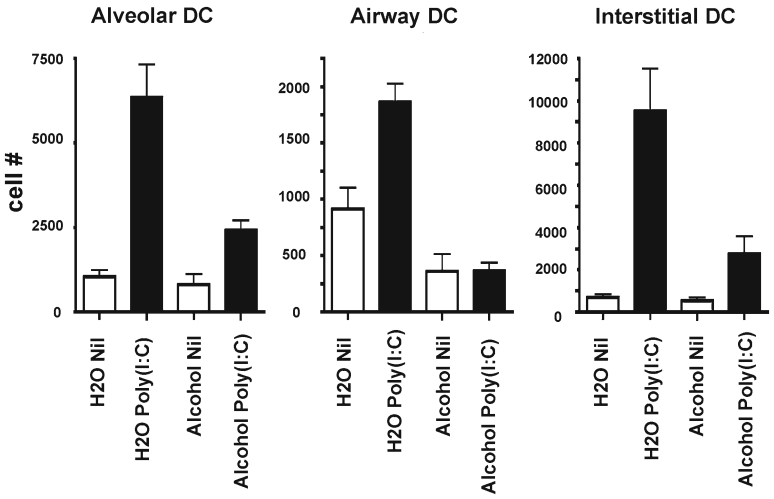

Fig. 10.3 Chronic alcohol ingestion decreases the expansion/recruitment of DC into the lungs following an inflammatory stimulus. Mice fed for 4 weeks ± alcohol in their drinking water were administered either media (Nil) or the inflammatory mediator poly(I:C), which is a mimic of the dsRNA found with many viruses, intranasally. Eighteen hours post polyI:C treatment, fewer alveolar, airway, and interstitial dendritic cells were found in the lungs of the alcohol-fed mice. These findings suggest that the recruitment and/or differentiation of these lung resident dendritic cells from precursors is substantially inhibited by alcohol

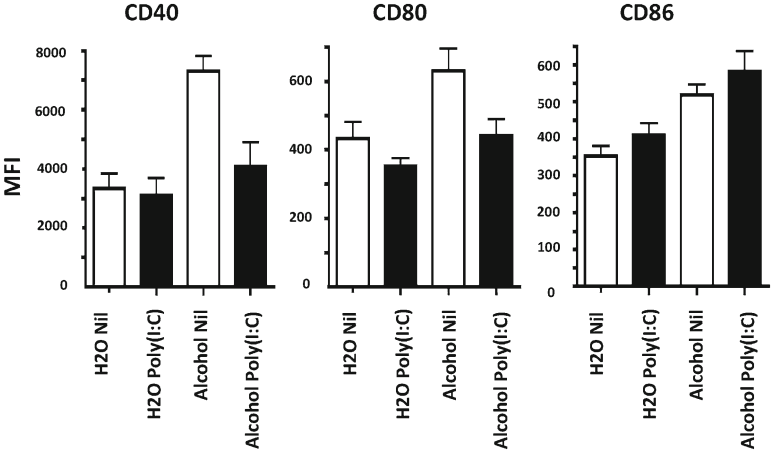

Fig. 10.4 Alcohol decreases the up-regulation of co-stimulatory molecules on DC in the presence of maturation stimuli. Mice fed for 4 weeks ± alcohol in their drinking water were administered either media (Nil) or the inflammatory mediator poly(I:C), which is a mimic of the dsRNA found with many viruses, intranasally (same experimental protocol as in Fig. 10.3). Eighteen hours post polyI:C treatment, DC from alcohol-fed mice failed to up-regulate the expression of CD40 and CD80. These findings suggest that the maturation of pulmonary DC during influenza virus infection may be delayed or inhibited

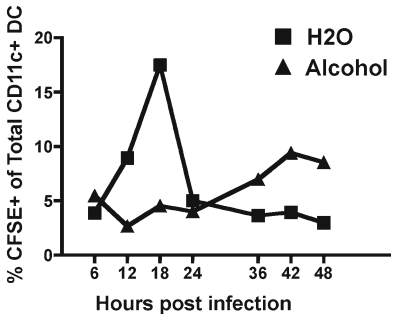

Fig. 10.5 Chronic alcohol consumption delays and inhibits the migration of dendritic cells from the lungs to the lymph nodes during influenza virus infections. Dendritic cells within the lungs of mice fed for 4 weeks ± alcohol in their drinking water were labeled with carboxyfluorescein succinimidyl ester (CFSE) and their migration from the lungs to the lymph nodes was measured at the indicated times after influenza virus infection by determining the % of $CFSE^+$ dendritic cells among total dendritic cells (i.e., $CD11c^+$ cells) within the lymph nodes. As shown in the figure, alcohol ingestion delayed entry of dendritic cells into the lymph nodes (note in particular the relative difference at 18 h) and decreased the absolute number of dendritic cells that entered the lymph nodes

Alcohol-Induced Inhibition of Pathogen-Specific CD8 T Cells

Given the alcohol-induced increase in disease and pathogen titers described above, coupled with the fact that primary influenza virus infections are largely cleared by influenza-specific CD8 T cells, one could infer that alcohol induces changes in the pulmonary CD8 T cell response [93]. When the overall CD8 T cell response was examined, our studies have demonstrated that chronic alcohol ingestion decreases, prior to any subsequent infection, the total number of CD8 T cells within the lymph nodes, which is similar to results observed in the spleen [23]. Further, chronic alcohol ingestion reduces numbers of total CD8 T cells in the lungs in response to influenza virus infection and this in turn translates into reduced numbers of influenza-specific CD8 T cells [93]. These findings suggest that influenza-specific cytotoxicity would be reduced, resulting in the observed increase in influenza virus titers and increased disease in alcohol-fed mice [93]. Similar to the inhibition of the CD8 T cell response to influenza virus, other studies have demonstrated an alcohol-induced decrease in recruitment of CD8 and CD4 T cells into the lungs during *P. carinii* and *M tuberculosis* infections [27, 98].

Studies have suggested that many of the defects that alcohol imposes on the immune system worsen with the duration of alcohol ingestion [93]. Therefore, in order to next determine if the inhibition of T cell immunity is maintained, lost, or strengthened with prolonged alcohol exposure, we infected mice that had been conditioned with alcohol ingestion for 4 or 8 weeks. While alcohol ingestion for 4 weeks reduced the total pulmonary CD8 T cell response to influenza by ~40 %, this inhibition was increased to ~65 % by 8 weeks of alcohol ingestion [93]. These results suggest that the defects in pulmonary T cell immunity increase with the

duration of alcohol ingestion, and this has obvious implications in the clinical setting where alcohol abuse may be intermittent or daily, depending on the individual's specific patterns of abuse.

Dysregulation of the Influenza-Specific CD8 T Cell Response

Studies have also been undertaken to determine if the chronicity of alcohol exposure alters the effector ability of pulmonary pathogen-specific CD8 T cells. Interestingly, the influenza-specific CD8 T cell response in alcohol-fed mice after 8 weeks showed a defect in addition to the above-described reduction in the total number of cells. Specifically, T cells from these alcohol-fed mice were dysregulated and did not make IFNγ in response to stimulation with influenza virus peptides [93]. This result suggests that the influenza-specific CD8 T cell response had increased defects as exposure to alcohol progressed. Additionally, this defect in IFNγ production suggests that other intrinsic effector responses, such as cytotoxicity, may potentially be defective in the chronic alcoholic lung, an idea supported by our more recent studies that demonstrate T cells from alcohol-fed mice exhibit reduced degranulation to influenza peptides. The extent of the defect in CD8 T cell effector function remains unknown at this time but, given studies that have demonstrated this dysregulation can be overcome by stimulating the T cells in vitro by treatment with phorbol esters and ionomycin [93], it is likely that the stimulating environment is defective in vivo, as previously discussed.

Summary

In conclusion, the currently available data suggest that chronic alcohol ingestion decreases the number of DC in the lung as well as their recruitment and effector functions. These changes in DC translate into profound inhibitory/suppressive effects on the development and effector functions of CD8 T cells during respiratory infections. Overall, these alcohol-induced changes in the adaptive immune response contribute to poor control of the respiratory pathogen, whether it is viral, bacterial, or even fungal in nature, and ultimately increases the overall morbidity and mortality of the acute infection. In this context, alcohol-induced derangements in dendritic cell function and their ability to coordinate and amplify a robust adaptive immune response complement the myriad of other defects in airway structure and function, as well as other innate immune deficiencies that characterize the "alcoholic lung". Therefore, strategies to mitigate the devastating effects of alcohol abuse on the lung may need to target all of these defects, including those in both innate and adaptive immunity. Whether or not alcohol-induced derangements in dendritic cell function are mediated by factors common to its effects on other lung functions such as oxidative stress is unknown and will no doubt be the subject of future investigation. In fact, if common mechanisms are indeed producing the "alcoholic lung" phenotype, then there is the clear hope that effective treatments for the millions of individuals suffering from alcohol use disorders can be developed.

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Chapter 11
Alcohol Impairment of Granulocyte Function During Lung Infection

Gregory J. Bagby, Kyle I. Happel, and J. Nicholas Melvan

Abstract Heavy alcohol consumption increases the incidence and severity of bacterial pneumonia and other infections. Neutrophil recruitment into the lung is a critical early host response to infection. This chapter focuses on the defects in neutrophil function and production in the alcohol-abusing host. Most of the preclinical literature on this subject has been produced using acute, intoxicating doses of alcohol. These models identify that alcohol-induced suppression of neutrophil recruitment and production are strongly associated with an increased severity of lung infection. Mechanisms responsible for this alcohol-induced granulocyte suppression include decreasing pro-inflammatory cytokine, CXC chemokine, and granulopoietic growth factor production. Neutrophil and/or granulocyte progenitor cell responsiveness to these mediators is also suppressed by alcohol. Additionally, studies indicate that neutrophil functions such as phagocytosis and pathogen killing may also be impaired by severe alcohol intoxication or chronic consumption. Human studies, though fewer in number, identify similar defects imparted by intoxicating concentrations of alcohol. Taken together, present knowledge supports the conclusion that defects in neutrophil recruitment, function and production are pivotal consequences of alcohol abuse and render the host susceptible to a multitude of respiratory infections.

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Introduction: Granulocytes and Lung Host Defense

As discussed in Chap. 4, excessive alcohol consumption increases the incidence and severity of bacterial pneumonia. This effect is multifaceted and associated with worsened clinical outcomes. The earliest effector cells to arrive from the blood into the infected lung are neutrophils (aka PMN or granulocyte; terms used interchangeably). These granulocytes are recruited down a gradient of inflammatory mediators to the site of infection and kill microbes by both intracellular and extracellular processes. As with most other cells of the host defense system, neutrophils originate in hematopoietic tissues such as bone marrow and use the blood to migrate into the lung to fight infection. Often referred to as the "professional phagocytes," neutrophils are critically important for the ingestion, degradation, and removal of pathogens during pneumonia.

This chapter focuses on mechanisms by which alcohol intoxication impairs normal neutrophil function during bacterial pneumonia. Three important aspects of alcohol-induced neutrophil suppression will be discussed: (1) recruitment to the site of infection, (2) killing of pathogens, and (3) bone marrow production and release. Each topic is prefaced by a brief description of the normal neutrophil response to bacterial pneumonia in order to provide a context for understanding how alcohol interferes with neutrophil recruitment, pathogen killing, and production.

Granulocyte Recruitment

Following a complex process of microbial recognition and cytokine elaboration, granulocytes migrate from the circulation into sites of microbial invasion. At these foci of infection, neutrophils transverse out of the vascular compartment and through the interstitial space where they ultimately enter into the intra-alveolar environment. This process occurs through sequential steps of cell rolling, firm adhesion, transmigration, and diapedesis. As granulocytes circulate, they continuously sample the walls of endothelial cells in search of adhesion molecules. These adhesion molecules, including P-selectins, E-selectins, and integrins (ICAMs), are upregulated by endothelial cells in response to pro-inflammatory stimuli produced at sites of infection [1]. As neutrophils roll across the luminal endothelial surface, granulocyte surface receptors such as the glycoproteins P-selectin, L-selectin, and glycoprotein ligand-1 (PSGL-1) engage the endothelial adhesion molecules. As these endothelial ligands are recognized by granulocyte receptors, membrane tethers form and intracellular signaling mechanisms are activated, thereby initiating the

transition from granulocyte rolling to firm adhesion. PSGL-1 redistribution from microvilli tips to uropod is considered a key component of the transition from selectin to integrin-mediated adhesion [2–4]. Increased time of PSGL-1/P-selectin interaction increases the likelihood of transmigration [2–4]. Firm arrest of granulocytes at the endothelial surface is mediated by β-2 integrins, LFA-1, and Mac-1, expressed on the granulocyte surface. As neutrophil β-2 integrins engage ICAMS, these complementary ligands cluster at the granulocyte–endothelial interface. After firm adhesion, granulocytes undergo cytoskeletal reorganization. Structural changes of the neutrophil membrane enable neutrophil sequestration within the lung vasculature during infection [5]. Cytoskeleton changes and pseudopodia formation enable neutrophil transmigration through endothelial cell borders at permissive sites called matrix protein low expression regions [6, 7].

Acute Alcohol Suppresses Neutrophil Recruitment

Studies have repeatedly demonstrated that neutrophil recruitment is suppressed by acute alcohol intoxication. This was first described by Kenneth Pickrell in 1938 [8]. His seminal study showed that blood alcohol concentrations (BAC) of 0.5–0.7 % severely decreased neutrophil migration into subcutaneous tissue inoculated with *Streptococcus pneumoniae*. Alcohol-treated rabbits were unable to clear the infection and died. Control animals however cleared the bacteria within hours and survived. Pickrell also showed a similar defect in neutrophil recruitment into the lungs of rabbits receiving an intrapulmonary challenge of *S. pneumoniae*. Comparable results were reported by Lushbaugh in the early 1940s [9] and Louria and Almy in 1963 [10]. Impaired neutrophil recruitment into abrasion-injured skin without infectious challenge has also been observed in human volunteers with BAC less than 0.1 %. The authors concluded that alcohol-suppressed leukocyte recruitment might increase susceptibility to infection, including pneumonia [11]. Gluckman and MacGregor [12] also reported impaired neutrophil recruitment in volunteers with BACs of 0.10 and 0.24 %. In the 1980s Astry et al. [13] conducted experiments in rodents to determine the effects of alcohol on pulmonary host defense against bacterial pneumonia. In their study, animals received aerosolized *S. aureus* or *P. mirabilis*. Alcohol intoxication decreased bacterial clearance in association with a dramatic decrease in pulmonary neutrophil recruitment. This was especially true at intoxicating doses producing stupor and ataxia. As demonstrated in studies by Boe et al. [14], pulmonary neutrophil recruitment in alcohol-treated rats was decreased for up to 18 h after *S. pneumoniae* challenge but was increased by 40 h post-challenge, likely as a result of increased bacterial burden in the intoxicated group. Taken together, these studies demonstrate that decreased neutrophil recruitment into infected tissue, including the lung, is an early and important consequence of alcohol intoxication.

Alcohol impairment of neutrophil recruitment does not appear to involve intrinsic deficits in neutrophil chemotaxis or ability of cells to migrate toward chemoattractants. Chemotaxis is quantified experimentally by determining how

many cells migrate across a semipermeable, artificial membrane toward a chemotactic stimulus. Studies have reported that alcohol decreases neutrophil chemotaxis, but typically only at very high concentrations of alcohol. Spagnuolo and MacGregor [15] found that alcohol concentrations >0.8 % were required to decrease chemotaxis of human neutrophils. They also compared chemotaxis between neutrophils obtained from subjects before and after they consumed alcohol to achieve a BAC of 0.065 and 0.2 %. When neutrophils were removed from an intoxicated environment, chemotaxis was not impaired. Nilsson et al. [16] did not see an effect of alcohol on spontaneous or FMLP-stimulated chemotaxis even at very high (1 %) alcohol concentrations. Moreover, in studies by Boe et al. [17], an alcohol-induced decrease in neutrophil chemotaxis toward the exogenous chemokines CINC/KC or MIP-2 was not evident at alcohol concentrations as high as 0.46 %, a concentration at the upper limit of physically tolerable doses. Hence, these studies in vitro suggest that neutrophil chemotaxis in the absence of cell barriers is not impaired at clinically relevant alcohol concentrations. However, these studies did not study chemotaxis across endothelial barriers.

Several studies show that acute alcohol attenuates the increase in adhesion molecule expression on neutrophils and endothelial cells during infection, which are key steps in neutrophil migration across vascular barriers into foci of infection. MacGregor and coworkers [12, 18, 19] observed an alcohol dose-dependent decrease in neutrophil adherence to nylon fiber columns, to cultured endothelial cells, and to the vasculature in vivo. Neutrophils exposed to alcohol in vitro exhibited suppressed ability to up-regulate the β-2 integrin adhesion molecules, CD11b and CD18, that are necessary for binding to vascular endothelial cells [20, 21]. Likewise, Zhang reported an attenuation of surface adhesion molecule expression by neutrophils obtained from alcohol-treated animals following intratracheal lipopolysaccharide (LPS) challenge [22]. These results suggest that alcohol may interfere with mechanisms involved in neutrophil adhesion, which is a critical early step in transmigration.

Cytokine and Chemokine Response

Early Response Cytokines

The rapid recognition of bacterial pathogens, cytokine activation of host defenses, and recruitment of immune effector cells to sites of microbial invasion are all essential processes involved in pulmonary host defense. Alveolar macrophages are the first phagocytes to detect and respond to the microbial invasion of distal airspaces. While alveolar macrophages contribute to phagocytosis of pathogens, their principal role in host defense against pulmonary infection is through the elaboration of inflammatory cytokines and chemokines to activate the adaptive immune response. These soluble mediators orchestrate key cellular processes including granulocyte recruitment and activation [23]. Among the key cytokines involved in

pulmonary host defense are tumor necrosis factor-alpha (TNF-α), interleukins (IL), chemokines, interferons (IFN), and colony stimulating factors. TNF-α, IL-1β, and IL-6 orchestrate the early innate response to microbial invasion [24].

Rodent studies have shown that following intratracheal instillation of LPS, the major cell wall component of gram-negative bacteria, intrapulmonary levels of TNF-α increase within the first hour of exposure. This response plateaus in the lung within 2–3 h and steadily decreases thereafter [25, 26]. TNF-α and IL-1β synergistically initiate immune responses via activation of the nuclear factor-kappa B (NF-κB) transcription factor pathway, which in turn stimulates production of other cytokines [27, 28]. IL-6 is another key early response cytokine that begins to increase within the first 2 h of bacterial exposure and peaks after 6 h [29]. Responses to IL-6 are principally mediated through STAT3 activation [28, 30]. Early response cytokines activate a myriad of other cellular processes necessary for host defense including the production of chemokines, integrins, opsonins, other pro- and anti-inflammatory cytokines, and colony stimulating factors [28].

Alcohol and Early Response Cytokines

In response to intrapulmonary infection, alveolar macrophages produce an array of pro- and anti-inflammatory cytokines. Acute alcohol intoxication promotes anti-inflammatory over pro-inflammatory cytokine responses. Pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), chemokines (IL-8, MIP-2, CINC/KC, LIX, MCP-1), and colony stimulating factors (G-CSF and GM-CSF) are decreased by alcohol intoxication. In contrast, the production of anti-inflammatory and pro-fibrotic cytokines such as IL-10 and transforming growth factor-β (TGFβ) are increased.

TNF-α is a pro-inflammatory cytokine and a key initiator of pulmonary host defense that is produced by macrophages early during infection. Although TNF-α is not a direct neutrophil chemoattractant, it stimulates adhesion molecule expression, production of additional pro-inflammatory cytokines including CXC chemokines, and the production of granulocyte-colony stimulating factor (G-CSF). As illustrated in Fig. 11.1, suppression of TNF-α by alcohol has been shown in animals infected intratracheally with *K. pneumoniae* to occur in association with decreased neutrophil delivery to the intrapulmonary compartment and decreased bacterial clearance [31]. Alcohol-induced suppression of the TNF-α response to LPS was first reported by our group in 1989 [32, 33]. In these studies, we showed that an intoxicating dose of alcohol suppresses the TNF-α response to both systemic and intratracheal LPS challenge. Suppression of the systemic TNF-α response in plasma following intravenous delivery of LPS was dose-dependent, with a 50 % decrease in circulating TNF-α concentrations at blood alcohol levels between 75 and 175 mg/dl [34]. Alveolar macrophages, isolated from acutely intoxicated animals or treated with alcohol in vitro, also show depressed production of TNF-α in response to LPS stimulation [35, 36]. Similar impairment of neutrophil recruitment was observed when animals received a neutralizing TNF-α antibody [37].

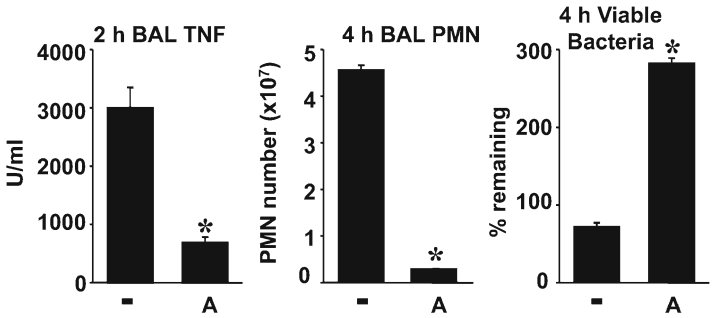

Fig. 11.1 Effect of alcohol intoxication on pulmonary host defense against *K. pneumoniae*. Alcohol-treated rats were injected i.p. with 5 g/kg ethanol 30 min before intratracheal *K. pneumoniae*. Data expressed as mean ± SEM. $*p < 0.05$ compared to vehicle-treated control. Adapted from Nelson et al. [111]

Two mechanisms have been identified for alcohol's effect on TNF-α production: (1) suppression of TNF-α mRNA expression and (2) impairment of posttranslational TNF-α processing. Alcohol attenuates TNF-α mRNA expression in macrophages in response to infectious stimuli. Szabo and colleagues [38, 39] have found that alcohol decreased nuclear translocation of NF-κB, a transcription factor important in TNF-α gene transcription, and reduced steady-state expression of TNF-α mRNA in alcohol-treated human monocyte/macrophages. Their studies suggested that alcohol-stimulated IL-10 production was in part responsible for the attenuation of TNF-α mRNA expression. Another underlying mechanism was impaired toll-like receptor-4 (TLR-4) clustering within lipid rafts. TLR-4 is the principle LPS receptor expressed on the surface pulmonary host defense cells. Decreased LPS-TLR4 signaling results in significant impairment in TNF-α mRNA transcription [40, 41]. However, decreased TNF-α protein secretion has also been observed under conditions in which lung or alveolar macrophage TNF-α mRNA content is not attenuated by alcohol [36, 42]. These findings suggested a second posttranscriptional or posttranslational mechanism causing the alcohol-induced suppression of TNF-α. Subsequently, Zhao et al. [43] reported that alcohol inhibits TNF-α-converting enzyme (TACE), a protein responsible for TNF-α shedding. Such an effect could in part be responsible for the observed decrease in the appearance of TNF-α in extracellular fluid or media after LPS stimulation despite normal levels of TNF-α mRNA expression.

Chemokines

Chemokines are small (8–14 kDa), soluble proteins that provide chemotactic signals to circulating blood cells, recruiting them to sites of infection and/or cellular injury. These soluble signals can be produced by alveolar macrophages, airway epithelium, lymphocytes, mast cells, fibroblasts, smooth muscle cells, platelets, mesothelial cells, and endothelial cells, as well as by granulocytes themselves, in

response to pulmonary infection [44]. Stimulus for their production can include a variety of triggers including pattern recognition ligands, IL-1β, TNF-α, C5a, leukotriene B4, and IFNs. Chemokines are divided into four classes based upon the position of cysteine residues; CXC, CC, C, and CXC3. The CXC class is further divided by the presence or absence of a glu-leu-arg (ELR) amino acid motif immediately preceding the first cysteine residue [45]. ELR + CXC chemokines (so called "alpha chemokines") are produced in early responses to microbial recognition and are the predominant chemokines responsible for neutrophil recruitment into the lung [46]. In rodents, this subgroup includes keratinocyte-derived chemokines (KC, CXCL-1, growth-related gene (GRO)-α), macrophage inflammatory protein-2 (MIP-2, CXCL-2/3, GRO-β, CINC), LPS-induced CXC chemokine (LIX), IL-8 (CXCL-8), and lungkine (CXCL-15) [45, 46]. In humans, CXCL1–3 and CXCL5–8 bind CXCR1 and CXCR2. In rodents, CXCL1–3, CXCL5, and CXCL6 bind to CXCR2 [47]. Rodent studies have shown that production of ELR + CXC chemokines MIP-2, CINC, and LIX are rapidly enhanced in response to the early response cytokines TNF-α and IL-1β. LIX is expressed by Type II alveolar cells and is up-regulated with LPS challenge [48]. CINC production is rapidly induced during inflammatory challenge but can also be transported out of the lungs into the systemic circulation [49, 50]. This activity of CINC closely mimics its human homolog, IL-8, the major cytokine for neutrophil recruitment [51, 52]. Other chemoattractants include C5a, platelet activating factor, leukotriene B4, and fMLP [47].

Chemokines and Alcohol

Blood monocytes treated with alcohol in vitro, or whole blood obtained 4 h after alcohol consumption by human volunteers, exhibit attenuated IL-8 production in response to LPS and other infectious agents. This suppression is association with a decreased NF-κB response [53]. In animal studies, acute alcohol administration has also been shown to attenuate the increase in MIP-2 following intratracheal LPS challenge [22]. In animals challenged intratracheally with *S. pneumoniae*, alcohol intoxication resulted in an early decrease of CXC chemokines MIP-2 and KC/CINC in association with delayed neutrophil recruitment, impaired bacterial clearance, and increased lethality [14]. Alcohol intoxication also has been shown to attenuate intratracheal LPS-induced LIX, a CXC chemokine shown to be primarily produced by lung alveolar epithelial cells [48, 54]. Alcohol suppression of the CXC chemokine response to inflammatory stimuli is consistent with studies that show antibody neutralization of CXC chemokines attenuates neutrophil recruitment [55, 56]. In other studies, neutrophil chemotaxis in vitro to lung lavage fluid from alcohol-treated *S. pneumoniae*-infected animals was decreased compared to chemotaxis to lung lavage fluid from non-intoxicated animals [17]. Addition of exogenous CINC and MIP-2 to the lung fluid from alcohol-treated animals to control levels partially corrected neutrophil chemotoxis. These data demonstrate the importance of CXC chemokines to neutrophil recruitment and that alcohol-induced suppression of CXC chemokines is likely an important mechanism for the development and/or the severity of pneumonia in alcohol abusers.

Alcohol Impairs Neutrophil Cytokine and Chemokine Responsiveness

In addition to decreased production of pro-inflammatory cytokines and chemokines, a number of findings indicate that impaired neutrophil responsiveness to these and other inflammatory stimuli also plays a role to decrease neutrophil recruitment during alcohol intoxication. As discussed earlier, neutrophils exposed to alcohol in vitro exhibit suppressed ability to up-regulate β-2 integrin adhesion molecules, CD11b and CD18 [19–21]. As these adhesion molecules are an important prerequisite for neutrophil binding to vascular endothelial cells, it is not surprising that alcohol exposure in vitro also impairs binding to cultured endothelial cells in response to inflammatory stimuli [12, 57]. In such studies, alcohol is the manipulated variable and the concentrations of agents that initiate an inflammatory response are controlled. These experiments identify that alcohol suppresses the responsiveness of neutrophils to the inflammatory stimulus. Recently Oh and Diamond [4] studied the effect of alcohol in vitro on the interactions of neutrophils and endothelial cells under flow conditions designed to mimic the dynamics of vascular blood flow. They found that alcohol-suppressed neutrophil binding to endothelial cells following stimulation with IL-1 and also demonstrated impairments in both rolling velocity and firm attachment. While movement across endothelial cells was not studied, decreased firm attachment would be expected to impair migration towards an infectious foci during infection. Moreover, studies in vivo have been performed to examine migration toward inflammatory signals in the absence and presence of alcohol intoxication. In one study, alcohol given by gavage to produce an intoxicated state suppressed neutrophil recruitment into skin injected with fMLP [58]. Boe et al. [17] also examined the effect of alcohol intoxication on neutrophil recruitment towards a recombinant CXC chemokine (instilled intratracheally) in rats. In this study, neutrophil recruitment into the intrapulmonary compartment in alcohol-treated animals was suppressed by 75 % in response to recombinant MIP-2 when compared to control-fed animals. Therefore, under conditions where the chemotactic stimuli were identical, neutrophil migration is still markedly suppressed by alcohol. These results indicate that, in addition to alcohol's ability to decrease chemokine production, it also impairs processes required for neutrophil responsiveness to inflammatory signaling.

Granulocyte Pathogen Killing

The antimicrobial function of granulocytes at sites of infection can be divided into intra- and extracellular pathogen destruction. Phagocytosis of invasive pathogens is facilitated by opsonization with immunoglobins and complement. Intracellular microbicidal activity requires the coordinated activation of intracellular enzymes, antimicrobial proteins, and cytoplasmic granules mobilized into phagosomes. The enveloping granulocyte membrane contains critical proteins involved in activating intracellular degradation including β-2 integrins, nicotinamide adenine dinucleotide

phosphate (NADPH) oxidase-related flavocytochrome b558, formyl peptide receptor, the fusogenic proteins SCAMP (secretory carrier membrane protein) and VAMP-2 (vesicle-associated membrane protein-2), and urokinase type plasminogen-activating receptor [59]. These intracellular proteins help mediate respiratory bursts of oxidized radicals, degranulation of microbicidal granules, and release of lytic enzymes, all of which function to break down phagocytosed pathogens. Moreover, during a robust inflammatory response, granulocytes also participate in extracellular pathogen destruction through the formation of neutrophil extracellular traps (NETs) [60, 61]. Following inflammatory stimulation, high concentrations of reactive oxygen species cause the condensation of nuclear chromatin, dissolution of the nuclear envelope, phagosome breakdown and granular enzyme mixing, and expulsion of intracellular contents that acquire an extracellular volume several-fold greater than the cell volume of the granulocyte itself [60]. NETs can be formed in the presence or absence of programmed cell death, and enable granulocytes to continue applying their antimicrobial products on trapped pathogens even after they die [62, 63]. Not surprisingly, impairments in intracellular and extracellular mechanisms of pathogen destruction increase susceptibility to systemic infection in immunodeficient hosts [64].

Alcohol and Neutrophil Pathogen Destruction

The effects of alcohol on neutrophil phagocytosis and pathogen killing are unclear. Several studies indicate that alcohol does not lessen pathogen destruction by granulocytes. Pickrell's 1938 study [8] concluded that alcohol's immunosuppressive effects are principally mediated through impaired neutrophil recruitment and not phagocytosis or killing. In his studies, neutrophils were recruited into the peritoneal cavity of rabbits with a noninfectious stimulus in advance of a bacterial challenge. Alcohol was then injected intravenously at the same time that *S. pneumoniae* was administered into the peritoneal cavity. Neutrophils that were already in the peritoneal cavity of intoxicated and control rabbits engulfed and killed bacteria equally well. Other investigators have reached similar conclusions that alcohol does not impair phagocytosis under conditions where neutrophil adhesion and migration are compromised [15, 65]. Brayton et al. [11] conducted experiments in vitro to assess bacterial phagocytosis during alcohol exposure. Blood neutrophils were isolated and incubated with *S. albus* in the absence and presence of 0.2–0.4 % alcohol. Phagocytosis did not differ between control and alcohol-treated neutrophils. Further, neutrophils collected 1 h after alcohol administration in vivo did not exhibit reduced phagocytosis compared to controls. These investigators also found that alcohol added to mixtures of bacteria and neutrophils had no effect on bacterial killing.

In contrast, other studies have shown that acute alcohol exposure impairs functional activities of neutrophils and potentially compromising bacterial killing. However, many of these effects require very high alcohol concentrations. Hallengren and Forsgren [66] observed impairment of phagocytosis and killing of *S. aureus* by human neutrophils in vitro only at alcohol concentrations that are rarely seen

clinically (0.64 %). At more clinically relevant concentrations, phagocytosis and killing were actually increased compared to non-alcohol-treated controls. Our group has found decreased phagocytosis of latex microspheres in vitro by blood neutrophils from alcohol-intoxicated rats challenged intratracheally with LPS [22, 67]. When phagocytosis was studied following intratracheal LPS, alcohol intoxication in vivo decreased microsphere engulfment by neutrophils in whole blood but not by neutrophils recovered from the lung by lavage. Although neutrophils studied in blood and lung lavage fluid represent non-recruited and recruited cells, respectively, the likely differences in the effects of alcohol appeared to depend on the presence or absence of alcohol during phagocytosis. Supporting these findings, Tamura et al. [68] found human neutrophil degranulation and bactericidal activity against *S. aureus* were inhibited in the presence of as little as 0.2 % alcohol.

Some investigations indicate that pathogen killing by neutrophils might be impaired by chronic alcohol consumption despite normal recruitment to the lung. In rodent models, 1 week of alcohol consumption resulted in failure to clear bacteria from the lungs, bacteremia, and increased mortality despite robust neutrophil numbers in the lung [69]. These data indicate that animals on a chronic alcohol liquid diet have substantial numbers of neutrophils in the lung, but bactericidal activities may be compromised. Increased neutrophil recruitment after chronic alcohol ingestion is in contrast to the suppressed neutrophil recruitment seen after acute alcohol intoxication. However, this increase in intrapulmonary neutrophil concentration was not evident until 24 h after *S. pneumoniae* challenge in these chronic alcohol-treated animals. This finding is similar to that observed in acute alcohol studies and points to increased bacterial burden in alcohol-treated animals at this time point [14]. Other studies revealed that 1 week of alcohol ingestion impairs neutrophil killing of selected strains of pneumococcus in vitro, but not the killing of other strains [70]. Interestingly, clearance of these same strains was impaired in vivo. These findings indicate that chronic alcohol may impair the killing only of specific strains of bacteria. One potential explanation is offered by the observation that chronic alcohol consumption did not suppress *S. pneumoniae* phagocytosis by neutrophils in vitro, but impaired *S. pneumoniae*-induced oxygen radical production and lysozyme release [71].

Granulocyte Production

Granulocyte Production During Pneumonia

In healthy hosts, the bone marrow produces 120 billion granulocytes per day, compensating for their relatively short 6–10 h lifespan in the circulation [72–74]. This production allows the bone marrow to support blood neutrophil turnover and maintains a large storage pool that is available for release into the circulation on short notice. The number of circulating granulocytes is estimated to only be 1–2 % of the number of granulocytes in the bone marrow [72–74]. Under normal conditions,

60 % of all leukocytes in the bone marrow are granulocyte precursors [75]. Thus, the marrow has a large storage pool of "potential granulocytes" that can be mobilized quickly during infection [76, 77].

Three colony stimulating factors, including granulocyte-colony stimulating factor (G-CSF), granulocyte/macrophage-colony stimulating factor (GM-CSF), and interleukin-3 (IL-3 or multi-lineage colony stimulating factor) are important regulators of granulocyte production and maturation [78]. Of these, G-CSF, a lineage specific growth factor, is the principle cytokine of granulocyte production, maturation, and function [79]. Neutralizing anti-G-CSF antibody given to normal animals produces a neutropenia within 24 h. G-CSF receptors (G-CSFRs) begin to be expressed early in granulocyte development during the myeloblast stage, and receptor density increases as myeloblasts differentiate into mature granulocytes [80]. G-CSFR-mediated intracellular signaling promotes the proliferation, survival, and terminal differentiation of granulopoietic precursors while simultaneously enhancing the release of both mature granulocytes and immature myeloid progenitors from the bone marrow [74, 81].

The production and maturation rate of granulocytes increases significantly in response to infection [82]. As neutrophil recruitment to infected tissues can exceed the numbers found in the blood, their replacement via production in the bone marrow is critical. Hence, granulocyte turnover is rapidly accelerated during the host response to pulmonary infection. During infection, the commitment of hematopoietic stem and progenitor cells becomes biased towards granulocyte production over all other blood cell lineages in order to reinforce phagocyte host defense [77, 83, 84]. The bone marrow retains an incredible reserve for increased granulocyte production during infection. Production can approach 10–15 times the basal rate [85]. During the marrow granulopoietic response to bacterial pneumonia, large numbers of granulocytes are mobilized from the bone marrow into the bloodstream, resulting in marked neutrophilia and an increased ratio of immature to mature granulocytes.

During infection, G-CSF is the principle cytokine stimulating granulopoiesis. Circulating G-CSF concentrations are low in healthy hosts, but can increase more than 20-fold during infection [86]. Enhanced G-CSF production during infection can be initiated by inflammatory stimuli such as LPS or TNF-α and IL-1β generated from macrophages. Studies by our group have shown that animals challenged intravenously with *E. coli* produce a robust increase in G-GSF, a response that is attenuated by pretreating animals with anti-TNF IgG [87]. G-CSF production is also stimulated by IL-17 produced by T helper-17 cells [79]. Moreover, IL-17 induces expression of stem cell factor and G-CSF by bone marrow stromal cells [88]. IL-17 contributes significantly to neutrophil commitment and host defense [89, 90]. Combined expression of IL-17 with GM-CSF can cause a 28-fold expansion in granulocyte production [91], whereas blocking IL-17 or G-CSF function can result in a 50–70 % reduction in neutrophil numbers [92]. Thus, innate and adaptive immune mechanisms to support continued G-CSF production may be necessary for maintaining the host response until the resolution of pulmonary infection.

As part of the pro-inflammatory cytokine response to intrapulmonary infection, G-CSF production increases within the lung. Unlike TNF-α, which remains restricted to the lung in order to orchestrate the local inflammatory response, G-CSF along with CXC chemokines enter the circulation and travel to the bone marrow where they stimulate granulopoiesis [76, 93, 94].

Alcohol Suppresses Neutrophil Production

In addition to alcohol's suppression of innate host defense within the lungs, the production of neutrophils by hematopoietic tissues is significantly inhibited by acute alcohol intoxication. Principal targets for alcohol-induced suppression of granulocyte production are G-CSF production, the G-CSF/IL-17 axis, and downstream G-CSF signaling. Acute alcohol administration has been shown to suppress the systemic G-CSF response to *E. coli* bacteremia [87]. This attenuated G-CSF response is likely to account for the decreased granulopoiesis observed in response to bacterial infections of the lung in the alcohol-compromised host [95, 96]. Our studies have shown that within 3 h of bacterial exposure in the lung, G-CSF concentrations increase significantly [76]. G-CSF concentrations in both the lung and systemic circulation peak and begin to plateau between 12 and 18 h of infection [93]. Impaired IL-17 production by alcohol may be partially responsible for the observed decrease in G-CSF. For example, alcohol consumption has been shown to attenuate the normal increased production of IL-17 in the lung following *Klebsiella pneumoniae* infection [97]. Others have shown that such IL-17 deficiency impairs up-regulation of colony stimulating factors and increases mortality from pneumonia [89]. The inhibitory effects of alcohol on the G-CSF/IL-17 axis may be in part through targeting activating signals like IL-23 produced by antigen presenting cells [98].

Within 24 h of bacterial pneumonia, newly produced granulocytes are exported out of the bone marrow into the peripheral circulation [95]. We have seen a significant increase in bone marrow granulocyte production within 24–48 h of systemic bacterial infection [77]. Alcohol intoxication impairs granulopoietic precursor proliferation and inhibits increased granulocyte production by the bone marrow [77, 95]. Subsequently, fewer neutrophils can be found in the lungs during the host response to pneumonia following alcohol intoxication [96]. Our studies show that intoxicated animals begin to succumb to infection 2–4 days after intra-tracheal inoculation of *S. pneumoniae* [14]. Therefore, the association between impaired neutrophil production and decreased lung bacterial clearance may be in part responsible for the increased mortality observed in alcohol-treated animals.

Impairment of granulopoietic precursor proliferation by alcohol may also occur through the suppression of intracellular G-CSF signaling pathways. In a dog model, administration of alcohol for 14 days in the absence of infection was sufficient to deplete granulocyte precursors in the bone marrow and produce a

neutropenic state [99]. Using a mouse model, we have found that plasma G-CSF concentrations 10 h post intra-tracheal inoculation of pneumococcus are no different following acute alcohol intoxication [95]. Therefore, as G-CSF levels themselves are not affected by alcohol, we predicted that intracellular signaling pathways necessary to respond to G-CSF signaling could also serve as targets of alcohol-induced suppression of granulopoiesis. Indeed we found that acute alcohol down-regulates proliferative signaling through MAP kinases and enhances anti-proliferative STAT3 signaling [95, 100]. Therefore, both suppression of G-CSF production and the response of hematopoietic cells to G-CSF are likely involved in suppression of granulopoiesis by acute alcohol intoxication during the host response to pneumonia.

Augmenting Alcohol-Induced Suppression of Granulocyte Recruitment

If suppression of the pro-inflammatory cytokine response, cellular responsiveness to cytokines and chemokines, and/or neutrophil production are responsible for the alcohol-induced decreased neutrophil recruitment to or function in the infected lung, then supplementing these cytokines could abrogate these deficits. Several prophylactic and therapeutic cytokine strategies have been demonstrated to augment pulmonary host defense in the alcohol-intoxicated host.

Pretreatment with G-CSF for 2 days prior to infectious challenge results in peripheral neutrophilia and increased neutrophil recruitment in response to intrapulmonary gram-negative infection with *K. pneumoniae* [101]. In this study, neutrophil recruitment in alcohol-treated animals was increased to that of non-intoxicated controls not receiving G-CSF. Non-intoxicated animals receiving G-CSF demonstrated greater neutrophil recruitment into infected lungs than did intoxicated rats. The G-CSF-induced increase in pulmonary neutrophil recruitment in alcohol-treated animals was associated with improved bacterial killing and decreased mortality (from 100 % in intoxicated rats not receiving G-CSF to 10 % in those receiving G-CSF). Interestingly, other investigators found similar pretreatment with G-CSF improved survival of control rats but not rats receiving an alcohol-containing liquid diet for 1 week before inoculation with *S. pneumoniae* into the lungs [102]. Whereas G-CSF pretreatment primarily acts through increased neutrophil production to cause a neutrophilia, this growth factor also enhances neutrophil function. Zhang et al. [22, 103] reported that an attenuation of adhesion molecule expression in alcohol-treated animals challenged with LPS was partially prevented by G-CSF pretreatment. Therefore, the overall evidence suggests that G-CSF supplementation augments pulmonary host defense by enhancing both granulocyte production and function.

The distinct T helper cell class known as Th17 cells is a more recently described facet of the immune system and is an important component of the pulmonary host defense response to bacterial infection [104, 105]. Upon recognition of bacterial

pathogens, antigen presenting cells in the lung, such as dendritic cells and alveolar macrophages, secrete the dimeric cytokine IL-23. This cytokine induces the expression of IL-17 by CD4+ T cells, gamma delta T cells, natural killer cells, and natural killer T cells [106]. The IL-17 receptor is widely expressed in the lung by many cell types, including respiratory epithelial cells. In response to IL-17 signaling, these stromal cells amplify the lung's neutrophil chemokine expression by secreting large amounts of KC and MIP-2, which are orthologues of human IL-8, as well as G-CSF. The importance of this pathway in pulmonary host defenses can be seen in animals that are deficient in the IL-17 receptor, in which universal mortality is observed during experimental pulmonary challenge with *Klebsiella pneumoniae* [90]. IL-17 receptor deficient animals demonstrate a failure to increase circulating PMN numbers, an effect associated with insufficient lung G-CSF and MIP-2 expression. In a mouse model of alcohol exposure, Shellito et al. found impaired expression of IL-17 during *Klebsiella pneumonia* infection was associated with decreased neutrophil recruitment and increased mortality [97]. Conversely, pretreatment of the lung with adenovirus encoding IL-17 resulted in a dramatic increase in production of IL-17 despite alcohol intoxication. This treatment induced marked neutrophil influx into the lungs and restored survival in alcohol-treated mice. Although the precise mechanism by which alcohol intoxication impairs pulmonary IL-17 expression is unknown, a subsequent study found that acute alcohol exposure directly and dose-dependently inhibits alveolar macrophage IL-23 expression [98]. This effect is independent of anti-inflammatory IL-10 induction, a known effect of alcohol exposure. Similar work has shown that alcohol exerts a dose-dependent inhibition of IL-23 in bone marrow-derived dendritic cells in response to LPS, further supporting the pivotal effects of alcohol on this cytokine's role during acute infection [107]. Indeed, IL-23 itself has been shown to be a potentially useful immunomodulatory therapy in alcohol abusers, as lung pretreatment with IL-23 gene therapy is effective in augmenting pulmonary control of *M. tuberculosis* in a murine model of this virulent pathogen [108].

Another successful strategy to overcome alcohol-induced immunosuppression is to increase the pro-inflammatory cytokine response in the intoxicated host. IFNγ primes macrophages to increase cytokine secretion in response to infectious stimuli. Kolls et al. [109] pretreated rats intratracheally with an adenovirus encoding IFNγ (ADIFNγ). This therapy primed lung host defenses prior to intrapulmonary LPS or *K. pneumoniae* challenge. IFNγ treatment resulted in more TNF-α production in response to LPS compared to rats receiving a control adenovirus. As a consequence, neutrophil recruitment into the LPS-challenged lungs of alcohol-treated animals was increased compared to the lungs of intoxicated rats pretreated with the control vector. When the lungs of intoxicated rats were instilled with *K. pneumoniae*, the bacteria were cleared faster in the AdIFNγ group than in animals treated with the control adenovirus. While this strategy was used to increase TNF-α production, the use of neutralizing anti-TNF-α did not attenuate the efficacy of AdIFNγ. These data prompted the investigators to conclude that the benefit was not TNF-α dependent.

Specifically, it is likely that pretreatment with AdIFNγ increases other components of the pro-inflammatory response to infection, such as CXC chemokines, that are more directly capable of neutrophil recruitment.

As CXC chemokines are directly responsible for neutrophil recruitment, and acute alcohol administration attenuates their increase during lung infection, our group has studied intratracheal injection of two rat chemokines, MIP-2 and CINC, into control or alcohol-intoxicated animals after lung *K. pneumoniae* challenge [110]. In alcohol-treated rats, intrapulmonary injection of MIP-2 and CINC 20 min after lung inoculation with *K. pneumoniae* partially restored neutrophil recruitment.

To summarize, immunomodulatory experiments with G-CSF, IFNγ, IL-17, or CXC chemokines indicate that the immunosuppressive effects of alcohol on cytokine production and release are at least in part responsible for alcohol's adverse effects on neutrophil recruitment. However, neutrophil recruitment is not completely restored with the supplementation of these cytokines. These findings suggest that impaired neutrophil recruitment in the alcohol-intoxicated host also involves decreased responsiveness of neutrophils to recruitment signals or other mechanisms of immunosuppression.

Summary

Heavy alcohol consumption increases the incidence and severity of bacterial pneumonia and other infections. Neutrophil recruitment into the lung is a critical early host response to infection. This chapter focused on the defects in neutrophil function and production in the alcohol-abusing host. Most of the preclinical literature on this subject has been produced using acute, intoxicating doses of alcohol. These models identify that alcohol-induced suppression of neutrophil recruitment and production are strongly associated with an increased severity of lung infection. Mechanisms responsible for this alcohol-induced granulopoietic suppression include decreasing the production of pro-inflammatory cytokines, CXC chemokines, and granulopoietic growth factors. Neutrophil and/or granulocyte progenitor cell responsiveness to these mediators is also suppressed by alcohol. As a result, the alcohol-intoxicated host fails to recruit and produce sufficient neutrophils because of both reduced mediator production and action on neutrophils and other cells involved in their migration into the lung and production in bone marrow. Additionally, studies indicate that neutrophil functions such as phagocytosis and pathogen killing may also be impaired by severe alcohol intoxication or chronic consumption. Human studies, though fewer in number, identify similar defects imparted by intoxicating concentrations of alcohol. Taken together, these studies strongly support the conclusion that defects in neutrophil recruitment, function and production are pivotal consequences of alcohol abuse and render the host susceptible to a multitude of respiratory infections.

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Chapter 12
Disruption in the Dynamic Balance Between Transforming Growth Factor-β and Granulocyte/Macrophage Colony-Stimulating Factor Signaling Within the Alveolar Space of the Alcoholic Lung: Impact on Epithelial and Macrophage Function

David M. Guidot and Ashish J. Mehta

Abstract The mammalian lung is a remarkably complex and delicate organ that has evolved to serve the principal function of exchanging expired carbon dioxide for inspired oxygen to help fuel aerobic metabolism throughout the body. The fundamental gas exchange structure within the lung is the alveolus and its surrounding capillary network. Intrauterine lung development is exquisitely regulated and progresses through stages, with the formation of alveoli occurring at the end of gestation and in the immediate postnatal period. The signaling molecules that comprise the pluripotential superfamily that includes transforming growth factor-β (TGFβ) are critically involved in the branching morphogenesis and later alveolarization that are vital for normal lung development, but their expression and activity wane rapidly in the postnatal period in the healthy state. In contradistinction, the relative influence of granulocyte/macrophage colony-stimulating factor (GM-CSF) emerges in the immediate prenatal period and throughout normal lung health as the dominant regulator of alveolar functions including the maintenance of the tight epithelial barrier, the formation and recycling of surfactant, and the maturation of the alveolar macrophage, which is the unique resident host immune cell within the lower airways. There is now abundant experimental evidence that chronic alcohol ingestion disrupts the dynamic balance between TGFβ1 and GM-CSF within the lung with profound consequences for alveolar epithelial and macrophage function. In fact, the aberrant expression and activity of TGFβ1 and the consequent dampening of GM-CSF signaling within the lower airways appears to be a fundamental factor that

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drives the "alcoholic lung phenotype." This chapter reviews the fundamental roles of these two molecules and their signaling pathways and illustrates the evolving recognition that disruption of their dynamic balance by alcohol renders the lung susceptible to a wide range of pathologies.

Keywords Transforming growth factor beta (TGFβ) • Granulocyte/macrophage colony-stimulating factor (GM-CSF) • Alveolar macrophage • Alveolar epithelium • Phagocytosis • Tight junctions

Introduction

The complex structure of the lung is designed to serve its primary function of exchanging oxygen from the inspired atmospheric gas with carbon dioxide that is produced by metabolic pathways throughout the body and is eliminated with the expired gas during ventilation. The fundamental gas-exchange units within the lung are the alveoli, which are the terminal small airways lined by a unique epithelium contained within a delicate extracellular matrix and surrounded by a dense capillary network that together enable the efficient bidirectional transfer of inhaled oxygen from the airspace to the hemoglobin in circulating red blood cells and the parallel transfer of carbon dioxide from the blood to the alveoli for elimination by exhalation. Each alveolus is 200–300 μM in diameter, and their total surface has been estimated to be approximately that of a tennis court. This enormous surface area is essential for efficient gas exchange, and the structural and functional integrity of the alveoli depends on several remarkable characteristics of this unique microenvironment.

First, the alveolar epithelium provides a tight and relatively impermeable barrier that is essential to create a stable air–liquid interface even though the entire cardiac output is continuously flowing just microns away through the alveolar capillary network. The alveolar epithelium comprises two cell types. The first is a cuboidal cell named the alveolar epithelial type II (ATII or AT2) cell [1], which is a specialized cell that among its many functions produces and secretes surfactant, a complex structure of phospholipids and proteins that decreases surface tension on the alveolar surface and thereby facilitates cyclical inflation and deflation of the alveoli at very low pressures during ventilation. The ATII cell is also responsible for maintaining the local redox potential within the highly oxidizing microenvironment of the alveoli where local oxygen concentrations are the highest than anywhere in the body. A cardinal feature of this role in maintaining antioxidant defenses is the synthesis and secretion of the tripeptide glutathione into the alveolar space. In the normal healthy adult lung, the concentration of glutathione within the alveolar epithelial lining fluid is in the range of 500–1,000 μM, which is 100–200 times its concentration in plasma. The second cell type that forms the alveolar epithelial surface and barrier is the alveolar epithelial type 1 (ATI or AT1) cell, which is a terminally differentiated cell that is derived from the ATII cell through a process that has been termed

transdifferentiation [1]. The ATI cell is flat and provides the primary surface for gas exchange. In fact, although the ratio of ATI and ATII cells within the lung is approximately 1:1, the ATI cells comprise approximately 95 % of the total alveolar surface area. The intercellular tight junctions between these cells sharply limit the paracellular passage of water and solutes from the capillary and interstitial compartments into the alveolar space [2]. In parallel, the relatively small amounts of fluid that do leak into the alveolar space are efficiently transported back across the epithelial barrier by both ATI and ATII cells using a coordinated system involving the active transcellular pumping of sodium and the consequent passage of water back into the interstitial compartment [3–6] where it is ultimately cleared by lymphatic drainage. As a consequence, a thin layer of epithelial lining fluid with its surface covered by surfactant is continuously maintained within the alveoli and provides the unique air–liquid interface that is critical for efficient gas exchange. The earlier chapter on the effects of alcohol on the alveolar epithelium [Koval] describes these specialized barrier functions in detail and how they are perturbed by chronic alcohol ingestion.

As the airways are covered with a large epithelial surface that is constantly exposed to the external environment, the protection of the delicate alveoli from inhalational injury by both biological and non-biological agents is of paramount importance. As discussed in the chapter by Sisson and Wyatt, there are exquisitely effective mechanisms within the upper and conducting airways that limit access of most of these agents to the alveolar space. However, noxious particles that are inhaled and evade these defenses in the larger airways must be cleared efficiently and rapidly before they can cause damage. In parallel, infectious agents such as bacteria that are either aspirated into the airways or enter the alveolar space from the alveolar capillaries must likewise be recognized and cleared before they can cause significant infections. Further, surfactant phospholipids and proteins that become oxidized and/or otherwise dysfunctional, even through normal "wear and tear" in the alveolar space, must be cleared and recycled into "fresh and functional" surfactant by the ATII cells. These complex tasks are served by the alveolar macrophage, a terminally differentiated cell that is derived from peripheral blood mononuclear cells and is unique to the alveolar space. This cell is among the body's most potent phagocytes ("large eater" in Latin) and is capable of ingesting particulate matter such as inhaled dust, bacteria, and damaged surfactant; in fact, these cells were called "dust cells" by early investigators. As the sentinel that patrols the alveolar space, the alveolar macrophage acts as the principal host defense cell within the lower airways but can also activate an adaptive immune response when pathogens are recognized. Specifically, it can recruit neutrophils and lymphocytes to the alveolar space by secreting chemokines including tumor necrosis factor-α, interleukin-12, interleukin-18, and interferon-γ [7–9]. In many ways, the alveolar macrophage is not only the primary sentinel that protects the alveolar space from extrinsic and intrinsic stresses but also the primary herald that signals to cells outside of the alveolar space that their presence is needed.

Previous chapters have elucidated the incredibly complex effects of alcohol on the lung's epithelial barrier as well as on airway immunity, including its profound inhibition of alveolar macrophage function. The focus in this chapter is to highlight

one of the fundamental mechanisms by which alcohol impairs both alveolar epithelial and macrophage function. Specifically, there is relatively recent and rapidly evolving experimental evidence that chronic alcohol ingestion causes a relative increase in the actions of transforming growth factor-β (TGFβ) and a consequent dampening of the relative actions of granulocyte/macrophage colony-stimulating factor (GM-CSF) within the alveolar space. To understand how a relative shift in the influences of TGFβ and GM-CSF creates the "alcoholic lung phenotype" within the alveolar space, we must first briefly review the normal actions of, and the dynamic balance between, these two pluripotent signaling molecules.

Transforming Growth Factor-β and the Lung

TGFβ, named for its ability to transform the phenotype of normal fibroblasts in culture, is the titular member of a large superfamily of ~30 growth factors that includes diverse polypeptides such as the activins, inhibins, bone morphogenetic proteins, Mullerian inhibiting substance, and others [10]. Within this superfamily is the subfamily of TGFβ, which includes three isoforms ($TGF\beta_1$, $TGF\beta_2$, and $TGF\beta_3$) [11]. The sequence homology for $TGF\beta_1$ among various mammalian species (human, rat, mouse, pig, and monkey) is >97 %, indicating remarkable evolutionary conservation. Among the three isoforms there is considerable homology as well, and in most cell systems studied the isoforms have essentially the same effects [12]. Therefore, although a distinct gene encodes each isoform and the isoforms are not expressed uniformly in all cell types, they appear to have more or less the same biological effects and are often referred to collectively as simply TGFβ. Within the lung, $TGF\beta_1$ is the dominant isoform that is expressed and the one that has received the most scientific attention in studies of lung biology. $TGF\beta_1$ is a pluripotent cytokine that influences tissue injury and repair. Although first identified for its proliferative effects on mesenchymal cells, it inhibits epithelial growth and function and may even promote apoptosis in these cells.

TGFβ exerts its effects by binding to specific receptors on the surface of target cells. However, its biological function is regulated at the level of activation from a latent form. Specifically, TGFβ is synthesized and secreted as a 25 kDa homodimer that is non-covalently associated with a latency-associated peptide that is part of the originally synthesized pro-peptide that undergoes proteolytic cleavage [13]. The TGFβ latency-associated peptide complex is called the "small latent complex." This complex is usually bound to a larger peptide of variable length called latent TGFβ-binding protein, of which four family members have been identified [13, 14]. The latent TGFβ-binding protein is covalently bound to the latency-associated peptide, and the entire combination of TGFβ, latency-associated peptide, and latent TGFβ-binding protein forms the "large latent complex." TGFβ is inactive when associated with latency-associated peptide, either in the large or in the small latent complex, as it cannot bind to its receptors on the cell surface. The latent TGFβ-binding proteins are a subfamily of the extracellular microfibrillin proteins, fibrillin 1 and fibrillin 2 [14], that target TGFβ for association

with the extracellular matrix. The TGFβ latency-associated peptide complex is released from the matrix by proteolytic cleavage of the latent TGFβ-binding protein and subsequent release of the small latent complex. In addition, some secreted TGFβ is associated only with the latency-associated peptide and may be soluble. However, free TGFβ is not present in significant amounts in biological fluids, at least under normal circumstances, as it is bound by a variety of other proteins such as α_2-macroglobulin. Therefore, multiple regulatory mechanisms maintain TGFβ in an inactive form under normal healthy conditions.

The study of TGFβ in complex biological systems has been somewhat hampered by difficulties in distinguishing the latent from the active form, particularly in tissues. Rifkin and colleagues developed a bioassay for active TGFβ that uses a mink lung epithelial cell line in which the promoter for the plasminogen activator inhibitor-1 (PAI-1) gene is linked to a luciferase reporter [15]. Biological samples are co-incubated with these cells, and luciferase activity correlates with the amount of biologically active TGFβ present. Although this assay has been used to assess the amount of active TGFβ in fluids such as lung lavage fluid, the detection of active TGFβ in tissues has proven to be problematic, as the extraction of tissue lysates activates the latent complex. Brunner and colleagues modified the original Rifkin assay by placing frozen sections of rat tissue (i.e., no extraction methods used) directly over the mink lung cells in culture [16]. This assay is only semiquantitative but can be used to provide some evidence as to whether tissue-bound TGFβ is bioactive. However, the study of TGFβ and its role in complex processes such as tissue injury and repair has been problematic because of this fundamental problem in distinguishing the latent from the active form within tissues. However, there is strong experimental evidence that only the active form of TGFβ is released into the alveolar space during acute inflammatory insults such as endotoxemia, which enables its levels within this space to be quantified using more sensitive and accurate assays such as ELISA [17].

TGFβ can be released and activated from the latency-associated peptide by a myriad of factors identified thus far, including oxidants [18, 19], nitric oxide and/or reactive nitrogen species [19, 20], at cell surfaces by thrombospondin-1 from platelets or by cell-associated plasmin [11], and via interactions with specific integrins such as αvβ6 [21] or matrix glycoproteins [22, 23]. Upon activation, TGFβ can interact with specific receptors on the surface of its target cells [12, 13, 24]. There are three classes of TGFβ receptors, RI, RII, and RIII [11]. The RIII receptors appear to serve only to facilitate the association of TGFβ with the RII receptors, which upon activation by binding to TGFβ recruit, bind, and transphosphorylate the RI receptor [11]. The subsequent intracellular signaling cascade involves phosphorylation of a unique family of intracellular molecules known as Smads [11, 12], which ultimately affect transcription of a wide array of TGFβ-responsive genes. The intracellular signaling is complex, including in epithelial cells where it may involve pathways other than the Smads, such as Ras-dependent and Ras-independent pathways that go through MAP kinase signaling [12]. The end result of TGFβ signaling on a target cell likely depends on a myriad of factors as TGFβ can act as a proliferative or an antiproliferative factor depending on the target cell and the local

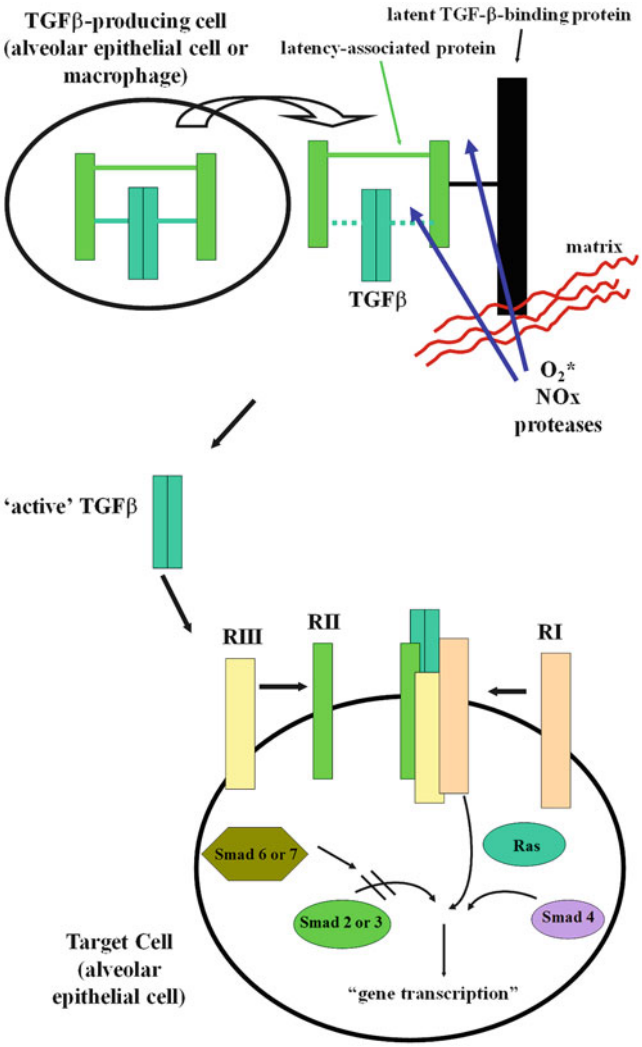

Fig. 12.1 Simplified schema for the expression, activation, and signaling of transforming growth factor beta (TGFβ). Cells synthesize and secrete TGFβ in a complex with latency-associated peptide, and this complex is bound to a latent TGFβ-binding protein that localizes the complex to the extracellular matrix. The TGFβ can be released from the complex by oxidants, reactive nitrogen species, proteases, and other mechanisms, allowing it to act on target cells by binding membrane receptors. TGFβ signal transduction uses a unique family of proteins known as Smads as well as other factors such as Ras to alter transcription of a variety of genes in a cell-specific and condition-specific manner

conditions. Although much has been learned about TGFβ in the past decade, it remains largely unknown how specificity in signal transduction and responses are governed in a cell-specific and condition-specific manner. A simplified schema of TGFβ expression, activation, and signaling is depicted in Fig. 12.1.

The aforementioned "dual roles" for TGFβ, specifically both proliferative and antiproliferative effects, have made studies of its biological actions fascinating as well as daunting. Specifically, TGFβ has such diverse effects and in so many contexts that it has been challenging to elucidate its discrete roles in both physiology and pathophysiology. Within the lung it is well established that TGFβ is absolutely critical for lung development. However, even in this context it has apparently contradictory roles at different stages. For example, whereas there is consistent experimental evidence that TGFβ regulates branching morphogenesis in early lung development, it appears that TGFβ acts as both a positive and a negative regulator of alveolarization in late lung development. One essential function for TGFβ in alveolarization is in stimulating the transdifferentiation of AT2 epithelial cells into AT1 epithelial cells. As discussed previously, the AT2 cell is the progenitor of the AT2 cell, and its transdifferentiation is essential to develop the enormous surface area of the alveoli required to make the transition to air breathing at birth. However, TGFβ can also stimulate alveolar epithelial cells to undergo epithelial-to-mesenchymal transition or "EMT," which may be necessary in the context of lung repair following injury (although this is at present poorly understood) but is clearly detrimental to normal alveolarization in the prenatal and postnatal period. In parallel, although TGFβ may have a role in alveolarization during late lung development and perhaps even in the early postnatal period, there is virtually no significant TGFβ expression in the healthy mature lung, and at least experimentally it is clear that TGFβ impairs alveolar epithelial barrier function [17, 21, 25]. During the acute response to an injury such as pneumonia or trauma, TGFβ expression is robust at the local site of the inflammatory response and its ability to loosen the epithelial barrier and even promote EMT is likely important to an appropriate injury response. However, once the acute inflammation has resolved and the lung undergoes normal repair, TGFβ expression wanes once again. Therefore, it appears that there is an exquisite balance that governs its functions in a particular context, and, while crucial for tissue development and function, TGFβ has also been implicated as a pathophysiological agent in a wide range of diseases. In particular, aberrant signaling by TGFβ has been identified as a likely mediator of bronchopulmonary dysplasia (BPD) in the premature newborn as well as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis which are primarily seen in much later stages of life [26].

Granulocyte/Macrophage Colony-Stimulating Factor and the Lung

Granulocyte/macrophage colony-stimulating factor or GM-CSF is a 23-kDa glycosylated monomeric peptide that is secreted by multiple cell types, including the alveolar epithelial type II cell [9]. It was first identified in mouse lung cell-conditioned medium and was named for its ability to stimulate the growth of granulocytes and macrophages from cultured hematopoietic progenitor cells. The cloning of this protein permitted a variety of studies in vitro and in vivo to characterize its functions, and it was subsequently found to stimulate the production of

eosinophils, erythrocytes, megakaryocytes, and dendritic cells in addition to granulocytes and macrophages. GM-CSF has been widely used clinically to improve bone marrow recovery following chemotherapy. Therefore, although it had first been isolated from lung tissue the prevailing view evolved that its functions were largely restricted to bone marrow lineage development.

The construction of GM-CSF knockout mice produced startling findings that had not been predicted based on the initial studies of its functional roles. Specifically, mice with targeted ablation of the GM-CSF gene had unexpectedly normal bone marrow maturation and normal circulating levels of all blood cell lines [27]. However, these mice were found to develop a lung disease similar to human pulmonary alveolar proteinosis (PAP) [27], which is a relatively rare disorder characterized by massive accumulation of surfactant phospholipids and proteins in the airspaces, leading to progressive respiratory failure and death in most cases if untreated. It is interesting that alveolar macrophages from patients with PAP are dysfunctional when examined in vitro, and in parallel, macrophages from GM-CSF-deficient mice are deficient in TNFα secretion, respiratory burst, and bactericidal activity [28]. Importantly, site-directed expression of GM-CSF in the alveolar type II cells of GM-CSF-deficient mice (by reinserting the gene and coupling it to the surfactant protein C promoter) completely eliminated the pulmonary defect [29]. Further, GM-CSF treatment by inhalation corrects the PAP defect in GM-CSF-deficient mice [30]. Taken together, these and related studies demonstrate that the most important site of GM-CSF activity is within the alveolar airspace, where it induces maturation of alveolar macrophages via a process that has been termed "priming." Although patients with the acquired or the idiopathic form of PAP do not have an apparent genetic defect in GM-CSF, they have autoantibodies directed against GM-CSF and therefore have a functional deficiency of GM-CSF signaling in the lung. For additional information the reader is referred to an excellent review on this topic [8]. In parallel, GM-CSF acts in an autocrine and/or a paracrine manner within the airway and induces alveolar epithelial barrier integrity and surfactant secretion. In fact, there are receptors for GM-CSF on the epithelial surface of conducting airways including the trachea and the bronchi, suggesting that it may be critical for epithelial barrier integrity throughout the entire airway and not just within the alveolar space [31]. Not surprisingly, whereas TGFβ is expressed abundantly in the developing lung in utero, including at the earliest stages of development, GM-CSF is expressed only late in lung development. It is just prior to birth and thereafter when it assumes a critical role in maintaining the postnatal "air breathing" lung in which a tight alveolar epithelial barrier and a dynamic surfactant layer at the air–liquid interface within the alveolar space are absolutely essential for normal lung function.

GM-CSF receptors share a common beta subunit chain with other cytokine receptors but also have a unique alpha subunit chain. The GM-CSF receptor beta chain (GM-CSF Rβ) is common to the interleukin-3 and interleukin-5 receptors [9, 32], whereas the alpha chain (GM-CSF Rα) is unique to the GM-CSF receptor. Neither chain has any catalytic activity, but the β chain is constitutively associated with the tyrosine kinase JAK2. The α chain binds with low affinity to GM-CSF, and this

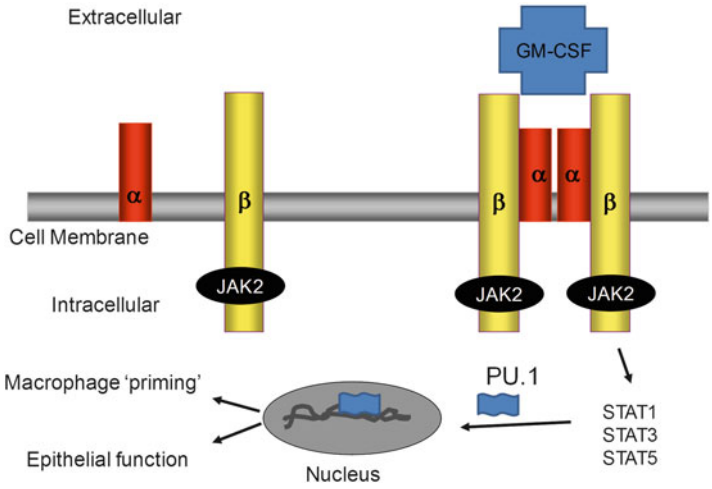

Fig. 12.2 Schematic illustration of GM-CSF signaling within the alveolar compartment. GM-CSF is synthesized by alveolar epithelial cells and secreted into the alveolar space where it can prime macrophages in a paracrine manner as well as stimulate epithelial function in an autocrine manner. The active GM-CSF receptor requires the coordinate clustering of two alpha chains (GM-CSFRα) that bind the soluble GM-CSF on the cell surface and two beta chains (GM-CSFRβ) that are associated with JAK2, a kinase necessary to initiate a series of intracellular signaling steps involving the STAT family that terminate in the translocation of the master transcription factor, PU.1, into the nucleus. PU.1 then activates a program of gene expression that either primes and then activates the alveolar macrophage or induces a range of alveolar epithelial cell functions including the formation of tight junctions that are critical to maintaining a tight alveolar barrier

facilitates formation of a high-affinity six-polypeptide complex composed of two α chains, two β chains, and two JAK2 chains. Activation of JAK2 following GM-CSF binding initiates a series of intracellular signaling pathways that are both in parallel and in series [32] and that ultimately leads to activation of the nuclear transcription factor PU.1. PU.1 is in the ets family of transcription factors and is a "master" transcription factor in the proliferation and differentiation of myeloid cells. PU.1 is expressed in alveolar macrophages of normal mice, but its expression is lost in GM-CSF-deficient mice [9]. Interestingly, restoration of GM-CSF expression in the type II cells of these mice also restores PU.1 expression in the alveolar macrophage [9]. GM-CSF-mediated nuclear binding of PU.1 activates an array of genes that are required to "prime" monocytes to mature into functional alveolar macrophages that are proficient in the phagocytosis of pathogens, surfactant phospholipid clearance, cell adhesion, and inflammatory signaling [9, 33, 34]. In fact, the constitutive expression of PU.1 (using a retroviral vector) completely reverses the PAP defect in alveolar macrophages of GM-CSF-deficient mice [34]. Therefore, GM-CSF-dependent expression of PU.1 appears to be absolutely required for terminal maturation and function of the alveolar macrophage in the normal host. A schema illustrating the signaling mechanisms of GM-CSF is shown in Fig. 12.2.

Alcohol Perturbs the Normal Dynamic Balance Between TGFβ and GM-CSF in the Lung

The recognition that the relative influences of TGFβ and GM-CSF are in a dynamic equilibrium has evolved as experimental models of the "alcoholic lung" phenotype have elucidated the mechanisms by which alcohol alters alveolar epithelial and macrophage function. As discussed above, in many ways these growth factors have somewhat diametrically opposed effects on these cellular constituents within the alveolar space. Within the healthy mature lung, the expression of TGFβ is very low and there is no detectable active TGFβ within the alveolar space. In contrast, GM-CSF is readily detectable within the alveolar space where it is synthesized and secreted in continuous fashion by the alveolar epithelium.

Although TGFβ plays a relatively lesser role in the healthy adult lung, it is rapidly activated in response to a wide variety of stresses. Its physiological roles in the context of acute lung inflammation are likely to increase epithelial permeability at the local site, modulate the immune response in a time- and context-dependent manner, and transform fibroblasts into myofibroblasts and thereby promote a scar formation where necessary. However, aberrant expression and activation of TGFβ has been implicated as a pathophysiological mechanism involved in diverse lung diseases from bronchopulmonary dysplasia in the premature neonate to pulmonary fibrosis in adults.

Soon after the landmark epidemiological study showing an association between alcohol abuse and the acute respiratory distress syndrome [35], our research group identified that in experimental animal models chronic alcohol ingestion dramatically induced the expression of latent TGFβ in the lung and markedly increased the release of activated TGFβ into the alveolar space [17, 25] where it increases alveolar epithelial permeability and promotes diffuse lung edema. In fact, the ability of soluble factors within the alveolar space to alter epithelial permeability in the inflamed alcoholic lung could be completely attributed to the actions of TGFβ [17].

The mechanisms by which chronic alcohol ingestion induces the expression of TGFβ within the lung and in turn promotes its activation during acute inflammatory stresses such as sepsis are not entirely clear, but several interdependent mechanisms have been identified in experimental modes. It had been recognized previously that alcohol abuse in humans is associated with hypertension, and activation of the renin–angiotensin system has been postulated as the underlying mechanism. Interestingly, blockade of the renin–angiotensin system in alcohol-fed animals with either an angiotensin-converting enzyme inhibitor (that complexes the conversion of angiotensin I to angiotensin II) or an angiotensin II receptor blocker completely inhibits the induction of TGFβ expression in the lung and decreases the release of active TGFβ into the alveolar space during endotoxemia [25]. In fact, blocking the renin–angiotensin system prevented alcohol-induced glutathione depletion within the alveolar space [25], possibly by inhibiting alcohol-induced expression and/or activation

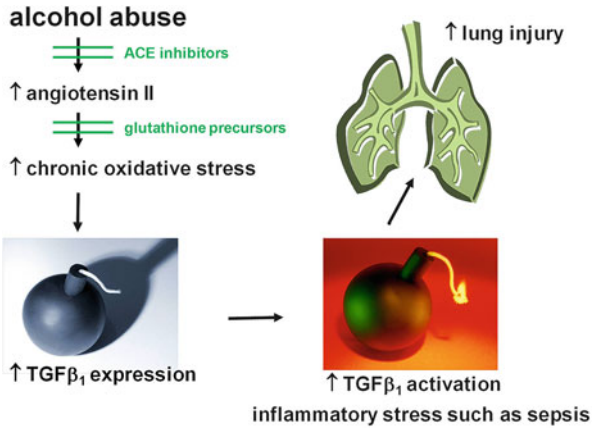

Fig. 12.3 Pathophysiological mechanisms by which alcohol induces TGFβ and promotes lung injury. Experimental evidence indicates that chronic alcohol ingestion activates the renin–angiotensin system. As the lung is a primary source of angiotensin-converting enzyme (ACE), this leads to increased production of angiotensin II which in turn increases the local production of reactive oxygen species that contribute to the oxidative stress and glutathione depletion in the alcoholic lung. As a consequence, there is an aberrant and robust induction of TGFβ within the lung. Most of this alcohol-induced TGFβ remains in the latent or inactive form, and therefore the otherwise healthy alcoholic may have no apparent lung dysfunction. However, in the event of an acute inflammatory stress such as sepsis, this TGFβ becomes activated and released into the alveolar space where it causes epithelial barrier disruption and increases the severity of acute lung injury

of NADPH oxidase within the lung [36]. Consistent with these observations, alcohol-induced expression of TGFβ could also be blocked by supplementing the diets of alcohol-fed animals with glutathione precursors [25]. Taken together, these experimental observations suggest the pathophysiological scheme depicted in Fig. 12.3.

Although confirmation of this pathophysiological pathway in humans will require further studies, at present there is preliminary evidence that TGFβ levels are increased in the airways of critically ill alcoholics as compared to nonalcoholics and that the alveolar macrophages of even otherwise healthy alcoholics have increased expression of TGFβ (Lou Ann Brown, personal communication). Consistent with the latter observation, more recent experimental evidence suggests that alveolar macrophage-derived TGFβ can degrade the alveolar epithelial barrier via cell-to-cell interactions in which the TGFβ is activated at the epithelial surface (Tiana and Pratibha paper). This pathway therefore raises the intriguing possibility that chronic treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (widely used to treat cardiovascular disease), alone or in combination with glutathione precursors (such as the widely available supplement *S*-adenosylmethionine), could mitigate the effects of chronic alcohol abuse on the lung.

This pathological alcohol-induced expression and activation of TGFβ in the lung are paralleled by profound dampening of the GM-CSF signaling within the alveolar space. Our laboratory first identified the impact of chronic alcohol ingestion on GM-CSF-dependent alveolar epithelial cell and macrophage function in a relatively

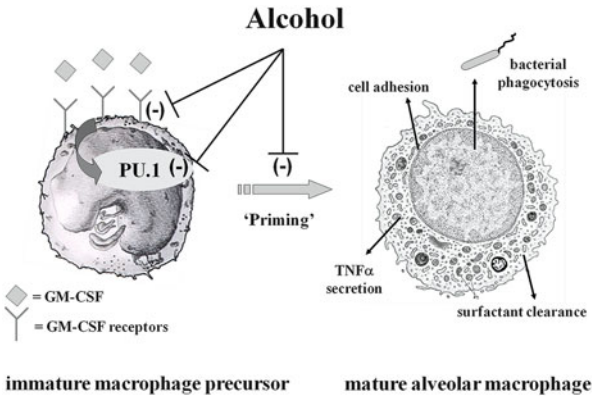

Fig. 12.4 Proposed mechanisms by which alcohol abuse inhibits alveolar macrophage maturation and innate immune functions that are critical to respond to pathogens within the alveolar space. In an experimental model, although chronic alcohol ingestion has no effect on GM-CSF protein levels in the alveolar space, it decreases the cell surface expression of the GM-CSF receptor in alveolar macrophages. In parallel, chronic alcohol ingestion decreases the expression and nuclear binding of the GM-CSF master transcription factor, PU.1. As a consequence, GM-CSF priming and maturation of the precursor cell into a fully functional alveolar macrophage is inhibited. Importantly, recombinant GM-CSF treatment delivered to the airway in alcohol-fed rats rapidly (within 48 h) restores GM-CSF receptor expression, PU.1 expression and nuclear binding, and innate immune function in the alveolar macrophage [39]. This same pathophysiological sequence, including rapid resolution in response to recombinant GM-CSF treatment, also underlies alcohol-mediated alveolar epithelial barrier dysfunction in the same experimental model [37]. Reprinted from the author's review paper in the American Journal of Physiology: Lung Cellular and Molecular Physiology (292: L813–L823, 2007) and reprinted here with permission

indirect fashion. Specifically, we first determined that recombinant GM-CSF delivered via the upper airway restored alveolar epithelial barrier function and fluid transport in alcohol-fed rats, even during endotoxemia [37]. Importantly, although that study showed that GM-CSF treatment decreased endotoxin-mediated lung injury even in control-fed rats, the magnitude of the efficacious response was clearly greater in the alcohol-fed rats. The efficacy of recombinant GM-CSF turned out to be more than a serendipitous finding, as these initial observations led to the discovery that chronic alcohol ingestion decreases the expression of GM-CSF receptors in the airway epithelium and macrophages and in turn dampens intracellular signaling to the GM-CSF master transcription factor, PU.1. As a consequence, GM-CSF-dependent functions in each cell type are impaired. These observations in experimental models have recently been confirmed in the human condition. Specifically, alveolar macrophages isolated from young and otherwise healthy alcoholics have significantly decreased expression of GM-CSF receptors [38]. Remarkably and to date via unknown mechanisms, recombinant GM-CSF treatment restores GM-CSF receptor expression and signaling and normalizes both alveolar epithelial barrier function [31] and alveolar macrophage immune function in experimental models [39]. These experimental findings are summarized in schematic form in Fig. 12.4.

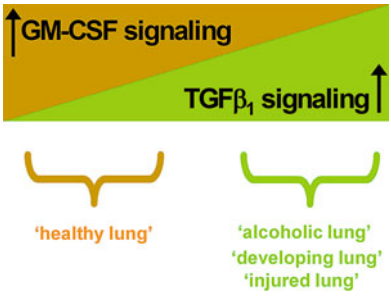

Fig. 12.5 The relative shift from the influence of GM-CSF to TGFβ signaling in the alcoholic lung. In the healthy adult lung the relative influence of GM-CSF, which through its discrete signaling pathway promotes alveolar macrophage activation and alveolar epithelial barrier formation, is dominant over the influence of TGFβ. However, in the alcoholic lung the shift towards the relative influence of TGFβ signals a decrease in epithelial barrier and changes the activation of the alveolar macrophage. Therefore, the alcoholic lung in some respects recapitulates the developing lung in utero and the normal lung responding to a localized acute injury such as pneumonia. Specifically, a relative shift in this balance is physiological in the correct context, but in the adult lung the aberrant and diffuse expression of TGFβ creates a dangerous vulnerability to infection and/or acute lung injury

In parallel, more recent experimental studies have identified that alcohol ingestion interferes with the absorption of dietary zinc in the intestine and its transport into the alveolar space and that dietary zinc supplements restore GM-CSF receptor expression and phagocytic function in the alveolar macrophage [40]. Although the precise mechanism(s) by which chronic alcohol ingestion decreases GM-CSF receptor expression and signaling within the alveolar space are unknown, unpublished observations suggest that TGFβ causes internalization of the GM-CSF receptor in alveolar macrophages (Pratibha Joshi, personal communication). Whether alcohol-induced dampening of GM-CSF signaling is mediated by this mechanism alone or in combination with zinc deficiency or other as yet unidentified mechanisms, there is rapidly growing evidence that the alcoholic lung undergoes an insidious shift from the normal dynamic balance in which the influence of GM-CSF greatly dominates that of TGFβ to one in which TGFβ assumes a pathological role (and may even be directly responsible for inhibiting GM-CSF signaling). In fact, such a shift in the balance between TGFβ and GM-CSF is part of the program of lung development in utero and is likely a critical facet of the normal response to localized lung injury and repair in the postnatal lung. In this context, chronic alcohol abuse may in a perverse manner recapitulate the developing lung and/or the injured lung by shifting the balance away from GM-CSF signaling to that of TGFβ, as depicted in Fig. 12.5.

The alcoholic lung is proving to be a remarkably complex perturbation of normal lung function, and every new experimental observation makes the overall pathophysiological scheme impossible to lay out in a linear fashion. In contrast, multiple molecular, organellar, and cellular derangements appear to interact in a matrix fashion in which seemingly disparate factors conspire to change lung function in ways that render it susceptible to infection and injury. Therefore, there is no one "master switch" or discrete derangement that can explain the complexities of the alcoholic lung.

However, the dynamic imbalance between TGFβ and GM-CSF can certainly be implicated as playing an important role in the phenotypic and functional changes within the alveolar space that confer this remarkable vulnerability. Perhaps more importantly, there is growing experimental evidence as well as intriguing observations from clinical studies that this dynamic imbalance can be manipulated and therefore may be a target for novel therapeutic interventions. This may be of particular value in the chronic setting before pneumonia and/or acute lung injury occurs. For example, as discussed previously angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are already widely used to treat cardiovascular diseases including hypertension and congestive heart failure. As they completely inhibit the aberrant expression of TGFβ and the development of the "alcoholic lung phenotype" in experimental animal models [25], it is reasonable to predict that they could mitigate the pathophysiological effects of alcohol abuse on the lung in humans. Even if their use were limited to individuals with alcohol-use disorders and other medical indications (such as hypertension), they might decrease the risk of serious lung complications in many individuals. In parallel, dietary supplementation with glutathione precursors and/or zinc, which again are remarkably effective in experimental models including decreasing TGFβ expression [17] and restoring GM-CSF receptor expression and signaling [40, 41], is a potentially simple and inexpensive therapy that could enhance lung health in individuals who suffer from alcohol-use disorders. In fact, clinical studies are already under way to determine whether or not interventions such as dietary supplementation with *S*-adenosylmethionine and/or zinc can enhance alveolar macrophage function in subjects seeking treatment for alcohol addiction.

Clearly the perturbations in cellular and organ function that characterize the alcoholic lung are complex and likely cannot be explained entirely by the pathophysiological shift in the relative influences of TGFβ and GM-CSF. However, it is remarkable how many of the experimental features are intimately related to, if not directly caused by, this disequilibrium between these two cardinal signaling molecules within the alveolar space. Therefore, the elucidation of this alcohol-mediated shift towards a TGFβ-driven alveolar microenvironment has not only shed light on the mechanisms by which alcohol abuse renders individuals susceptible to pneumonia, acute lung injury, and other lung diseases, but it has also identified novel therapeutic targets that can now be tested in clinical studies. The goal is clearly not to make it safer to abuse alcohol but rather to limit the devastating pulmonary and perhaps even the systemic consequences of chronic alcohol abuse in individuals who are seeking treatment for their addiction. In this context, it is imperative that we identify mechanisms to mitigate the devastating effects of alcohol abuse on the lung and other target organs as history reminds us that many of us will use and abuse alcohol regardless of any social, cultural, or religious prohibitions. This is in fact entirely consistent with the approach to many lifestyle-related health problems. For example, we treat hypertension, dyslipidemias, and diabetes mellitus in obese individuals with the metabolic syndrome because we recognize that weight loss through diet and exercise may be the fundamental treatment but for many is difficult if not impossible to attain. Therefore, we have an obligation to develop therapies to mitigate the medical complications of alcohol abuse that can be applied in parallel with cognitive and behavioral therapy for their underlying addiction.

Summary

Although the mechanisms by which chronic and excessive alcohol consumption render the lung susceptible to a wide range of infectious and inflammatory injuries are complex and we have only recently begun to elucidate them, there is growing evidence that alcohol disrupts the normal dynamic balance between GM-CSF and TGFβ signaling in the lung. The relative shift towards the influences of TGFβ over GM-CSF in the alcoholic lung has profound consequences for epithelial function as well as for host defense responses by the macrophage. In this context, therapeutic strategies to restore a "healthier" balance in which GM-CSF signaling resumes its dominant influence may limit the detrimental expression of the "alcoholic lung phenotype" and decrease the risk of pneumonia and lung injury in these vulnerable individuals.

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Chapter 13
Alcohol-Mediated Zinc Deficiency Within the Alveolar Space: A Potential Fundamental Mechanism Underlying Oxidative Stress and Cellular Dysfunction in the Alcoholic Lung

Ashish J. Mehta and David M. Guidot

Abstract Zinc is one of the most abundant trace elements in the human body, and its presence is essential for numerous biological processes including enzymatic activity, immune function, protein synthesis, and wound healing. Given these important roles, zinc has a sophisticated transport system to regulate its homeostasis. Determination of zinc status, however, is difficult to determine as serum levels are closely maintained and are not an accurate reflection of total body zinc or metabolism at the organ level. Fortunately, the discovery of zinc-specific fluorescent dyes has allowed for a much better assessment of zinc status in the respiratory system and has revealed that alcoholism perturbs this highly developed zinc metabolism such that its distribution to the lung and alveolar space is significantly decreased. As a result, this pulmonary zinc deficiency impairs function in the alveolar macrophage, which is the primary host immune cell within the lower airway. Experimental models have demonstrated that correction of this zinc deficiency restores immune function to the alveolar macrophage as best reflected by improved bacterial clearance in response to infection. While the precise mechanisms underlying alcohol-induced zinc deficiency are still under investigation, there is experimental evidence of several important connections with granulocyte–macrophage colony-stimulating factor and oxidative stress, suggesting that alteration of zinc homeostasis may be a fundamental mechanism underlying the cellular pathology

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seen in the alcohol lung phenotype. This chapter reviews zinc homeostasis and offers insight into our understanding of zinc deficiency in the setting of alcoholism and the potential of zinc as a therapeutic modality in the vulnerable alcoholic host.

Keywords Zinc • Glutathione • Alveolar macrophage • Pneumonia • Phagocytosis • Alcohol • Granulocyte/macrophage colony-stimulating factor (GM-CSF)

Basics of Zinc Homeostasis

Zinc is the second most abundant trace metal in the human body (behind iron), and it plays a crucial role in normal homeostasis within multiple organ systems. As it is one of 16 essential trace elements, humans require a relatively small amount of zinc, but it must be ingested from exogenous sources since the body cannot produce it endogenously. According to the Office of Dietary Supplements (ODS), the primary source of zinc for most individuals in the United States is protein-dense foods such as red meat and poultry. The food source with the most zinc is oysters, one serving of which has over ten times the zinc content as a serving of red meat [1]. However, other foods may be fortified with zinc, and it is also available as a dietary supplement and as an active ingredient in certain over-the-counter cold remedies. The Recommended Dietary Allowance (RDA) for zinc is 11 mg/day for adult males and 8 mg/day for adult females [2]. Only about 20 % of ingested zinc is actually absorbed into the bloodstream, and the rest remains in the gastrointestinal tract where it is excreted in the feces [3]. In the bloodstream, the form of zinc that is found in the plasma is one that is primarily bound to plasma proteins, mostly albumin.

Regulation of Zinc Transport and Storage

Zinc is important in various cellular processes. Most recognized is its role as a cofactor for the catalytic activity of over 300 enzymes [4] including multiple enzymes that are critically involved in antioxidant defenses. In parallel, zinc plays a key role in regulating gene expression as thousands of transcription factors are the so-called zinc finger proteins [5]. In fact, it has been estimated that approximately 10 % of the human genome encodes proteins that require zinc for their functional activity. Not surprisingly, due to its widespread effects zinc metabolism influences a wide array of important bodily functions including protein synthesis, wound healing, immune function, and DNA synthesis [6]. Given these far-reaching effects in the body, proper zinc homeostasis requires a sophisticated transport and regulatory system to ensure that it is readily available for the various cellular processes that require its presence.

There are two families of transmembrane zinc transport proteins that control its movement in and out of cells and subcellular compartments [7]. These are the Zip and Znt families of transporters, which have opposite or contrasting functions in

regulation of zinc homeostasis. The Zip family of transporters, of which there are 15 in humans, are zinc import proteins that function to increase the cellular concentration of zinc by its uptake from the extracellular space or by the release of zinc from intracellular vesicles. In contrast, there are nine known Znt transporters in human beings, and they function to decrease the cytoplasmic concentration of zinc by promoting its efflux from the cell or its influx into intracellular vesicles [8]. Studies have shown that the primary site of regulation for these transporters occurs in the intestine, where zinc is absorbed. For instance, in states of zinc deficiency, upregulation of Zip transporters occurs to ensure that more dietary zinc is absorbed and less is excreted in the feces [9]. In addition, metallothionein is a zinc storage protein and an indicator of overall cellular zinc status. Production of metallothionein is highly dependent on the availability of dietary minerals including zinc but is not specific to zinc alone.

The Role of Zinc in the Immune Response

In addition to its important role in enzymatic activity, zinc sufficiency is critical for normal immune function. Various studies have shown that zinc deficiency causes atrophy of the thymus, which results in lymphopenia and compromises overall T lymphocyte response and cell-mediated immunity [10]. Experimental models of zinc deprivation also result in defects of the bone marrow and affect the B-cell lineage as well [11]. Clinically, zinc deficiency has recently been shown to play an important role in patients with immune system disorders such as common variable immunodeficiency (CVID) [12]. While depletion of zinc in these patients does not actually cause the immune syndrome, its deficiency can certainly exacerbate the already impaired host response. Comparable to its role in CVID, zinc deficiency has been reported in both patients with human immunodeficiency virus (HIV) [13] as well as in experimental models of HIV infection [14, 15]. In addition to these observed derangements in acquired immunity, zinc deficiency has been shown to cause various deficits in innate immune function as well. Specifically, studies have shown that zinc depletion results in dysfunction of macrophages [16, 17]. This is of particular consequence in the respiratory system where alveolar macrophages are constantly exposed to environmental pathogens and debris and are the first line of defense against various pulmonary infections including pneumonia. Further evidence for the role of zinc in the immune response may be derived from studies of people with the prototypical zinc metabolism disorder, acrodermatitis enteropathica, which is a rare genetic disease characterized by an inability to uptake zinc due to the lack of an important zinc transport protein. Patients with acrodermatitis enteropathica have been used to describe the hallmarks of severe zinc deficiency, and these individuals demonstrate several important defects in host defense [18].

The burden of nutritional zinc deficiency is much more evident in Third World countries where poverty-stricken individuals have limited access to necessary food sources required to formulate a well-balanced diet. This disposition is especially

apparent among young children. Studies show that children in several African countries are much more prone to the development of pneumonia, and this deficiency has been linked specifically to zinc deficiency [19, 20]. International health initiatives have led to the development of organized campaigns to advertise the effectiveness of dietary zinc supplementation in areas where children are most vulnerable. Similarly, in the United States, inadequate dietary zinc intake has been described in children living in families of low socioeconomic status, in infants with low birth weight, and among teenagers who become pregnant [10, 21]. Interesting experimental models have verified the key role of zinc deficiency specifically in pulmonary immune function. In these experiments, researchers zinc-deprived a group of mice and immunized them to pneumococcus, a common cause of bacterial pneumonia. After allowing for immunity to develop, they inoculated the mice with the pneumococcal organism into the lung and subsequently measured pneumonia severity. They demonstrated that pneumonia is much more severe in mice that are zinc-deprived and further that immunization is ineffective at preventing infection in the setting of zinc deficiency [22, 23]. In other words, zinc-deprived mice are not only unable to acquire immunity in response to vaccination, but they also develop a much more severe infection compared to mice that were not zinc-deprived. Taken together these studies illustrate that zinc is not only intimately connected with the global immune response but also crucial for immunity specifically in the pulmonary system.

Determination of Zinc Status

Currently, the only clinically available test to diagnose zinc deficiency is a serum zinc level. This measurement is performed by numerous commercial laboratories and can be accomplished with a simple blood test. While this is a straightforward diagnostic evaluation, the utility of the result has been questioned since plasma zinc only accounts for about 0.1 % of total zinc stores. Thus, serum measurements of zinc are an unreliable indicator of total body zinc, and blood levels of zinc often do not appreciably drop until zinc deficiency reaches a critical level. Further, various biological conditions and stressors can affect serum zinc levels. Studies show that tissue levels of zinc may be decreased even when serum levels are normal. Specifically, a recent study among alcoholic subjects revealed that alcoholics had evidence of zinc deficiency in the lung, but all had serum zinc measurements that were within the normal range. Further, serum zinc levels may be decreased in states of inflammation even in states of zinc sufficiency [24]. While the mechanisms for these findings are not entirely clear, studies have shown that inflammatory cytokines can upregulate the Zip family of transporters and may explain the transient decrease in serum zinc levels that has been observed with the acute-phase response [25]. Therefore, zinc-deficient states may exist with normal serum zinc levels, and plasma zinc deficiency can occur when total body zinc stores are normal. Given the inherent unreliability of serum zinc as a surrogate marker of zinc status, alternate methods have been developed to better assess zinc deficiency in the research setting.

Various zinc-specific dyes have been developed to evaluate zinc in different cell types and biological fluids. These dyes typically come in two forms: a membrane-impermeable form that is able to measure zinc in extracellular fluids and a membrane-permeable form to assess intracellular zinc levels. One particular dye, FluoZin-3, is manufactured by Invitrogen and has been used in various recent studies evaluating pulmonary zinc status. FluoZin-3 is a membrane-impermeable fluorochrome with a very high affinity for zinc but a low affinity for other cations such as magnesium and calcium. Therefore, it is an ideal way to measure extracellular zinc levels in biological specimens such as lung lavage fluid. The membrane-permeable form, FluoZin-3AM, has similar zinc-binding properties and has been used to measure intracellular zinc levels in different pulmonary cell types including alveolar epithelial cells and alveolar macrophages. Utilization of such methods has allowed for a much greater and more precise understanding of zinc metabolism in the respiratory system than evaluating serum zinc levels alone.

Zinc Metabolism in the Respiratory System

Given the clinical observations that zinc deficiency leads to an increased susceptibility to pneumonia and other pulmonary infections, groups of researchers began to investigate zinc metabolism at the organ level. Using the zinc fluorophore Zinquin, groups led by Truong-Tran [26] and Carter [27] were the first to image pools of zinc in respiratory cells. They were able to demonstrate the presence of zinc in the apical region of ciliated airway epithelial cells as well as in alveolar epithelial cells. In these same regions, there were important zinc-dependent enzymes such as caspase-3 and Cu/Zn superoxide dismutase. Zinc inhibits caspase-3, an enzyme that activates apoptosis, and superoxide dismutase has very important antioxidant function within the lung. Further, these researchers demonstrated that pools of zinc in these cell types were strongly decreased by treatment with zinc chelators, and there was a parallel increase in apoptosis and oxidative stress. Subsequent researchers have demonstrated that several important zinc transporters are also localized to the lung [7, 28] and are involved in regulation of zinc metabolism within the respiratory system. Taken together, these early studies highlight the presence and importance of zinc in the respiratory system.

The Effect of Alcohol Exposure on Overall Zinc Homeostasis

The presence of zinc deficiency among alcohol abusers with hepatic dysfunction has been recognized for over 50 years [29]. Studies show that individuals with alcoholic cirrhosis (as well as cirrhosis from other etiologies) have both serum zinc deficiency as well as decreased tissue levels of zinc [30]. However, much less is known about zinc levels in the alcoholic patient who has not developed liver disease, but studies

suggest that there are alterations in zinc metabolism [31, 32]. The mechanisms responsible for creating zinc deficiency in the alcoholic subject are not entirely clear. While the majority of Americans have no difficulty meeting the RDA for zinc, studies have shown that the vast majority of alcoholics are not able to meet this goal [32]. This is not surprising when taken in the context of the generally poor dietary habits that exist in this population. However, even with sufficient intake, experimental studies have demonstrated that the absorption of zinc is impaired by chronic alcohol ingestion [33], and there is also an increase in urinary zinc excretion among alcoholics [34]. An experimental model evaluated different zinc transporters in the small intestine in order to better understand the effects of zinc absorption in the setting of chronic alcohol ingestion [17]. While zinc deficiency would normally increase expression of Zip proteins to increase intestinal absorption of dietary zinc, this study demonstrated that alcohol exposure directly decreased Zip4 expression in the small intestine, thereby decreasing zinc absorption even in a state of zinc deficiency. Finally, one group of investigators showed that albumin has a much lower affinity for zinc in subjects with cirrhosis [35], a finding that may additionally contribute to the observed zinc deficiency in this population. While our overall understanding of the mechanisms involved in creating zinc deficiency among alcoholics is limited, it is likely that multiple mechanisms act in concert.

The Effect of Alcohol Exposure on Zinc Homeostasis in the Lung

In addition to its widespread medical consequences in other organ systems, chronic alcohol exposure causes various derangements in the respiratory system. Clinically, patients who abuse alcohol have a predisposition towards the development of pulmonary infections. The key factors associated with alcoholism that contribute to this increased susceptibility include a change in oral bacterial flora, diminished gag and cough reflexes that occur during inebriation, dysfunction of the cilia in the airway that assist with secretion clearance, and impairment of the alveolar macrophage, which is the primary immune cell in the lower respiratory system. Given the overwhelming evidence that zinc metabolism is crucial for the immune response and the significant findings that illustrate that chronic alcohol exposure alters zinc homeostasis, it is a logical progression to implicate zinc deficiency as a primary contributor to immune dysfunction and susceptibility to pulmonary infection in this vulnerable population.

Experimental models of alcoholism have established that extracellular zinc in lung lavage samples is decreased about 30 % in animals on an alcohol-fed diet compared to control-fed diet. In these models, chronic alcohol exposure directly causes alterations in zinc metabolism since the diets are otherwise similar from a caloric standpoint and overall zinc content. This is an important finding since studies of human subjects implicate poor nutritional status as an important cause of zinc and other nutritional deficiencies that accompany alcoholism. These findings contend

that merely "recommended" nutritional intake may not be enough to combat zinc deficiency with alcohol abuse. In this same experimental model, dietary zinc supplementation is able to reverse zinc deficiency in the alveolar space and improve alveolar macrophage immune function. The recommendations for zinc replacement for individuals with documented zinc deficiency are about five to ten times greater than the RDA for zinc, which is comparable on a milligram per kilogram basis to the dose of zinc supplementation used in this animal model of chronic alcohol ingestion. More recent evidence in human subjects has shown that alcoholic subjects have a similar 30 % decrease of intracellular zinc in alveolar macrophages compared to matched nonalcoholic subjects, validating the findings in experimental models of chronic alcohol ingestion [24].

In addition to alcohol-mediated decreased zinc levels in the alveolar space, there is also alteration of zinc transporter metabolism. It is expected that zinc import proteins, or Zip transporters, would be upregulated in the setting of zinc deficiency. However, alcohol decreases several important Zip proteins in alveolar macrophages, including Zip1 and Zip4 [17]. These findings demonstrate that alveolar macrophages are further impaired in their ability to uptake zinc during this state of global zinc deficiency. This is parallel to the effect of alcohol on decreasing important zinc transporters in the small intestine where zinc is absorbed from the diet and illustrates that alcohol has direct effects in altering zinc metabolism in multiple organ systems including the lung.

While zinc levels are clearly decreased in experimental alcohol-fed animals, these models have further demonstrated that zinc deficiency in the setting of chronic alcohol ingestion contributes to both alveolar epithelial barrier dysfunction and alveolar macrophage immune impairment. These novel investigations argue that zinc deficiency may be a unifying mechanism for the alcohol lung phenotype and explain alcohol-induced susceptibility to pneumonia and acute lung injury.

Effect of Zinc Deficiency on Alveolar Epithelial Barrier Function

Clinically, alcoholics have greater than twofold increase in the risk of developing acute lung injury and acute respiratory distress syndrome (ARDS), and when these devastating conditions do occur alcohol abusers have a higher severity of illness and greater mortality than their nonalcoholic counterparts [36–38]. While the exact mechanisms for these findings are still under investigation, studies have shown that alcohol abuse impairs alveolar epithelial barrier function, resulting in greater fluid leak and an increased propensity to develop lung edema [39–42]. Granulocyte–macrophage colony-stimulating factor (GM-CSF) has been implicated, at least partly, in alcohol-induced epithelial barrier dysfunction. GM-CSF is a 23-kDa peptide that is secreted by several cell types, including alveolar epithelium, and has many important functions in the lung. Chronic alcohol ingestion has been shown to decrease GM-CSF receptor expression and signaling in the

alveolar epithelium, causing impairment in GM-CSF-dependent epithelial barrier formation [43]. Importantly, in experimental models of chronic alcohol ingestion, recombinant GM-CSF treatment is able to normalize epithelial barrier function [44] and improve expression of tight junction proteins that are altered by alcohol exposure [45]. More recently, it was determined that treatment of zinc deficiency with supplementation in vitro and in vivo improves GM-CSF receptor expression and reverses alveolar epithelial barrier dysfunction [17]. Specifically, epithelial barrier integrity was assessed by measuring paracellular leak of radiolabeled sucrose in established monolayers of epithelial cells. These experiments were performed in both alcohol-treated alveolar epithelial cell lines and cells that were isolated from alcohol-fed animals, and zinc supplementation was performed in vitro for cell lines and added to the diet of alcohol-fed animals. Further, in this same model, dietary zinc supplementation increased gene and protein expression of important tight junction proteins that are affected by chronic alcohol exposure. While the precise connection between zinc deficiency and GM-CSF signaling remains under exploration, these novel findings argue that zinc deficiency may be a fundamental mechanism in alcohol-induced epithelial barrier dysfunction and better explain the predisposition alcoholics have to the development of acute lung injury and ARDS.

Effect of Zinc Deficiency on Alveolar Macrophage Function

Pneumonia and aspiration are the most common pulmonary risk factors for the development of acute lung injury and ARDS, and alcoholics have an increased susceptibility to both conditions. The risk of aspiration is primarily related to diminished consciousness associated with intoxication, and the risk for pneumonia (and other pulmonary infections) is the result of weakened host immunity. The alveolar macrophage is the primary immune cell in the lower airways and the first line of defense against invading pathogens. Several experimental studies have demonstrated that chronic alcohol ingestion impairs alveolar macrophage immune function [46–48]. Just as GM-CSF is important for epithelial barrier function, its signaling is crucial for alveolar macrophage differentiation, maturation, and function. GM-CSF signaling occurs through receptors on the cell surface of numerous cell types, including alveolar macrophages. In parallel to its effects on the alveolar epithelium, chronic alcohol exposure decreases expression of GM-CSF receptors and signaling through its master transcription factor PU.1 in alveolar macrophages [48]. Further, recent evidence has shown that treatment of alcohol-induced zinc deficiency with dietary zinc supplementation improves GM-CSF receptor expression and restores alveolar macrophage immune function [17]. In this particular experimental model, dietary zinc supplementation increases expression of both the GM-CSFα binding subunit and the GM-CSFβ signaling subunit. These findings add to a growing body of evidence implicating zinc deficiency as an important mediator of immune dysregulation in the alcoholic lung.

Role of Oxidative Stress

Early observations of the alcohol lung phenotype consistently demonstrated an increase in oxidative stress in the lower airways caused by alcohol abuse [49–51]. Oxidative stress had previously been demonstrated in other tissues affected by alcohol abuse such as the liver [52]. More specifically, extensive research has implicated alcohol-mediated depletion of glutathione (GSH) as a primary driver of oxidative stress in the liver [53] and respiratory system [51]. GSH is a tripeptide that is generated primarily by the liver and is employed in various detoxification pathways and oxidant-mediated cytokine generation [54]. While the exact mechanism has yet to be elucidated, studies have shown that alcoholism decreases GSH levels in the liver even when cirrhosis is not present [53], and treatment with GSH precursors restores oxidant balance and prevents the development of alcohol-induced liver injury in experimental models [55]. GSH is an important cellular antioxidant in the alveolar space, and GSH precursors have a similar therapeutic value in reversing alcohol-mediated pulmonary defects associated with redox stress [50, 56–58].

Oxidative stress is the result of disparity in production and neutralization of reactive oxygen species (ROS), which occurs when there is an imbalance in the oxidized and reduced forms of a redox pair. Synthesis of GSH requires cysteine (Cys), and recent experimental evidence has suggested that Cys and its oxidized counterpart cysteine (Cyss) operate at the extracellular level and GSH and its oxidized form glutathione disulfide (GSSG) function as an important intracellular redox pair [59]. Redox pairs are one means of combating oxidative stress, but there are other important defenses as well. For instance, the antioxidant response element (ARE) is a genetic program that is triggered in the setting of oxidative stress. ARE signaling occurs through its master transcription factor Nrf2, and the ARE/Nrf2 pathway is a more diverse and comprehensive host defense mechanism against redox stress. Studies have suggested that the ARE is an important means of host protection against oxidative stress specifically in alcoholism [60–62].

Appropriate zinc metabolism is vital for the production and activity of numerous antioxidants, and zinc deficiency alone impairs host defense against redox stress [4]. Indeed, zinc deficiency in settings independent of alcoholism has been shown to induce a state of oxidative stress in the respiratory system [63, 64]. More recently, alcohol-induced zinc deficiency has been shown to increase oxidative stress in the lungs and decrease signaling through Nrf2 [65], illustrating a novel mechanism by which alcoholism may exacerbate oxidative stress in the lower airways. More importantly, zinc supplementation was able to restore redox balance and improve Nrf2 activity in these experimental models. Further, specialized assays illustrate that Nrf2 and PU.1 cooperatively bind to DNA, thereby providing important experimental evidence that the GM-CSF and ARE pathways are linked in a zinc-dependent fashion. These observations imply that zinc deficiency is a fundamental mechanism by which alcohol abuse induces oxidative stress and cellular dysfunction within the alveolar space. Further, these experimental models have provided a strong basis to begin exploring the potential therapeutic role of dietary zinc supplements in a vulnerable alcoholic population.

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Part III
Special Circumstances

Chapter 14
The Impact of Alcohol Abuse on Multiple Organ Dysfunction in the Surgical Patient

Katharina Chalk and Claudia Spies

Abstract Individuals with an alcohol-use disorder (AUD) admitted to trauma and/or surgical services in hospital show a two- to fourfold higher rate of postoperative complications, leading to longer hospital stays and increased mortality. Chronic alcohol consumption damages the central and peripheral nerve system and can cause a severe state of physiological withdrawal called the "alcohol withdrawal syndrome" that when manifested during their management in the intensive care unit (ICU) is associated with more adverse outcomes, both in the ICU and after hospital discharge. Often unrecognized, decreased beta-endorphin levels in these individuals may cause their pain to be more pronounced. In parallel, the risk for cardiac complications including arrhythmias and circulatory insufficiency in the postoperative period is increased up to fivefold in individuals with an AUD. Further, alcohol-induced hepatic insufficiency when present leads to higher levels of toxic substances, including ammonia and lactic acid, and promotes insulin resistance and decreased cellular glucose uptake. In addition, disturbances in haemostasis, including decreased platelet function and impaired hepatic synthesis of coagulation factors, are associated with (if not causative of) a twofold increased risk of postoperative bleeding complications. Finally, alterations in the neuroendocrine axis and in the immune system result in a three- to fivefold increased risk of nosocomial infections including pneumonia, wound infections and urinary tract infections. As a result of these myriad pathophysiological mechanisms, surgical patients with an AUD require increased attention to promote a good response to treatment and to minimize their risk of developing multiple organ failure.

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Keywords Alcohol withdrawal syndrome • Cardiac function • Haemostasis • Coagulation • Nosocomial infection • Mortality • Intensive care • Outcome

Abbreviations

ACE	Angiotensin-converting enzyme
ADH	Antidiuretic hormone
AUD	Alcohol-use disorder
AUDIT	Alcohol-use disorder identification test
AWS	Alcohol withdrawal syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CAD	Coronary artery disease
CAGE	Cut down, annoyance, guilty, eye opener
CAM-ICU	Confusion Assessment Method for the ICU
CDT	Carbohydrate-deficient transferrin
CHD	Coronary heart disease
CIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol-Revised scale
DDS	Delirium Detection Score
DIA	Daily alcohol intake
EtG	Ethylglucuronide
EtOH	Ethanol
ICDSC	Intensive Care Delirium Screening Checklist
IFN	Interferon
IL	Interleukin
ITS	Intensive care unit
GABA-A	Gamma-aminobutyric acid type-A
GGT	y-Glutamyl transpeptidase
MCV	Mean corpuscular volume
NMDA	*N*-Methyl-D-aspartate
PBMC	Peripheral blood monocytes
PEA	Pulseless electrical activity
PEth	Phosphatidylethanol
RASS	Richmond Agitation–Sedation Scale
TNF	Tumour necrosis factor

Relevance of Alcohol Abuse in the Surgical Patient

An underlying alcohol-use disorder (AUD) in individuals undergoing surgical procedures and/or in those who are critically ill is of immense clinical and economic relevance. It is estimated that the prevalence of AUDs in the surgical setting is

Table 14.1 Differential diagnosis for AWS

*I*nfection
*W*ithdrawal
*A*cute metabolic or electrolyte
*T*rauma
*C*NS
*H*ypoxic
*D*eficiencies
*E*ndocrine disturbances
*A*cute vascular
*T*oxins/drugs
*H*eavy metals
=I WATCH DEATH

approximately 20 %. In selected diagnostic contexts such as trauma or cancers of the aerodigestive tract the underlying prevalence of an AUD may be over 50 % [1]. A relevant diagnosis of an AUD that increases the risk of perioperative complications has been defined in most studies as a daily average consumption of 60 g of alcohol. Physicians tend to underestimate the prevalence of AUD particularly in women and in younger patients [1]. Therefore, it is important that we increase the preoperative detection rate as the treatment of alcohol-related diseases and complications can be time consuming and requires a multidisciplinary team approach.

Common co-morbidities in AUD patients include essential hypertension, hepatic insufficiency or even cirrhosis, chronic pancreatitis, nutritional deficiencies and a significantly increased risk for selected cancers [2]. Up to 75 % of individuals with an AUD show cognitive dysfunction of variable degrees, and ~9 % develop a serious complication such as delirium [3]. In addition, in many cases underlying chronic and irregular heavy drinking has a negative impact on the outcome in cases of cardiovascular disorders, stroke and diabetes mellitus [4].

Alcoholics are a "high-risk" population for surgery, In part because of the associated co-morbidities at baseline but also due to the various effects of chronic alcohol use on the organism that lead to two- to fivefold higher rates of postoperative complications [5]. The chronic dependence and damage to the central nervous system can cause the severe state of the "alcohol withdrawal syndrome" (AWS) after surgery. Typical symptoms are hallucinations, cognitive disorders, sympathetic hyperactivity, depression anxiety and seizures [6]. The severity of AWS is approximately fourfold higher after surgery compared to the rates observed in psychiatric patients [6, 7]. The synergistic activation of the transmitter systems of inflammatory states during critical illness, which alone are associated with high rates of delirium, combined with alcohol-withdrawal delirium, likely contributes to the high incidence of the AWS in the surgical population. Various factors complicate the differential diagnosis of AWS, including inflammation, infection, mechanical ventilation, organ replacement therapies (such as haemodialysis or haemofiltration) and the use of centrally acting drugs. The differential diagnosis has been organized into the acronym and mnemonic "I WATCH DEATH" (see Table 14.1) and is important to bear in mind when caring for this susceptible population. It is also important to

remember that the diagnosis of the AWS is one of exclusion and that other acute causes of cognitive impairment and delirium such as bleeding, metabolic and electrolyte disorders, infection, hypoxia, pain and focal neurological disorders must first be excluded [6]. Intensive care unit (ICU) patients with severe AWS may exhibit prolonged ventilator dependence and prolonged ICU length of stays [3, 8]. Further, their cardiac function may be sub-clinically compromised [5, 9]. Alcohol can provoke a suppression of thrombopoiesis with a resultant reduced platelet count as well as disturbances of platelet aggregation and low thromboxane synthesis. These disturbances in haemostasis lead to an elevation of the postoperative bleeding complication rate of up to 50 % [3].

Alcohol-induced alterations in cellular and humoral immune function can also dysregulate the response to surgical stress. The T-cell-mediated response, interleukin (IL)-2 expression, tumour necrosis factor (TNF)-α secretion and interferon (IFN)-γ production and its cytolytic activity are all down-regulated by alcohol [5, 10]. As a consequence, individuals with an underlying AUD have impaired immunity and a higher rate of postoperative infections [5, 8, 10, 11].

As noted already, alcohol abuse is associated with a prolonged ICU length of stay; a alcoholic patients undergoing tumour resection of the upper digestive tract spent an average of 8 more days (median) in the ICU than did non-alcoholic patients [8], and this is likely just one factor that contributes to morbidity rates that are two- to fourfold higher in male surgical patients whose premorbid alcohol consumption was >60 g/day [5, 12, 13]; interestingly, consumption below that limit did not appear to increase the risk of perioperative complications in those studies. The complex challenges of treating ICU patients with an underlying AUD often requires more work by the nursing staff as well [14], as these individuals often have a higher severity of illness and associated mortality risk (50 % versus 26 %) compared to critically ill patients without an AUD [15]. As a dramatic example, in a study of patients undergoing elective tumour surgery, 7 % of chronic alcoholics died in the ICU whereas all of the non-alcoholic patients survived their ICU stay, and the long-term outcomes after 3 months were generally poorer in the alcoholic patients [8].

Alcohol Withdrawal Syndrome

AWS in the ICU can be a severe life-threatening state (lethality when untreated is ~15 % and when treated is ~2 %) and occurs in ~25 % of intensive care patients after weaning off sedation [6]. AWS may follow abrupt cessation of drinking, can cause autonomic hyperactivity within hours of the last drink and usually peaks within 24–48 h. There is a wide spectrum of symptoms such as sweating, nausea, vomiting, anxiety, tachycardia and agitation [6, 16, 17]. Auditory, visual and tactile hallucinations may appear within 8–48 h of abstinence and last for 1–6 days. Neuronal excitation manifests within 12–48 h post abstinence, and ~15 % may show seizure activity, which are usually single, short and generalized tonic-clonic seizures. Up to 5 % of hospitalized patients with AWS develop delirium tremens.

Delirium tremens may appear within 2–5 days post abstinence and is the most serious manifestation of alcohol withdrawal. It represents a state of exaggerated sympathetic activity in combination with symptoms such as delirium, confusion, disorientation, dehydration, electrolyte imbalance and cardiac arrhythmias [17].

Pathophysiology of the Alcohol Withdrawal Syndrome

Alcohol interacts with multiple complex neurotransmitter systems in the brain.

Acute alcohol intake inhibits the *N*-methyl-D-aspartate (NMDA) receptors, reducing the release of the excitatory transmitter glutamate, and has an agonistic effect on gamma-aminobutyric acid type-A (GABA-A) receptors. Activation of the GABA-A receptor leads to anxiolytic and sedative effects as well as ataxia and other defects in motor coordination. However, chronic alcohol intake also has depressant effects on the central nervous system that affect neurotransmission and autonomic activity by decreasing GABA-A receptor function and up-regulating NMDA receptors [6, 17]. As a consequence, affected neurons become less sensitive to GABAergic transmission, which enhances the sedative effects of alcohol and leads to tolerance [16]. In contrast, abrupt alcohol withdrawal leads to increased NMDA receptor function, reduced GABAergic transmission and dysregulation of the dopaminergic system [17]. The differential effects of alcohol on various neurotransmitter systems and their variations in vulnerability to the withdrawal of alcohol lead to a somewhat chaotic onset and spectrum of various symptoms. For example, visual and tactile hallucinations seem to be triggered by an increased dopamine transmission, whereas seizure activity appears to be caused by an imbalance between GABA and NMDA transmitters as well as altered cellular calcium influx [17]. During acute physiological stresses such as in the surgical setting, the hypothalamus–pituitary axis is also affected. High levels of corticotrophin-releasing factor and a decrease in ß-endorphin have been reported after stress induction as well as after alcohol withdrawal, and these effects have been suggested to predispose patients to subsequent relapse and a return to their alcohol abuse [11, 18]. Finally, as discussed above the severity of AWS is likely aggravated by inflammatory processes that are inherent to acute illness [19, 20].

Prevention and Treatment of the Alcohol Withdrawal Syndrome

The National Trauma Registry of the American College of Surgeons conducted a retrospective review of 6,431 charts from July 1999 to February 2004. Patients presenting with AWS had more severe medical complications such as respiratory failure, pneumonia, urinary tract infection, sepsis, tracheostomy and endoscopic gastrostomy; more hospital stays; and more global medical costs compared with non-AWS patient [21]. This study highlights the importance of identifying patients at risk for this syndrome in order to establish AWS prophylaxis. A significant

association between AWS and volume of daily alcohol consumption and craving intensity was also found, which suggests the involvement of mechanisms of volume regulation, such as the arginine-vasopressin system, in the neurobiology of alcohol craving and AWS [22]. This might be an interesting aspect for the therapy of AWS.

Possible drugs for AWS prevention have been investigated in several trials and include benzodiazepines, intravenous ethanol, clonidine, clomethiazol and haloperidol [23, 24]. All of these drugs are considered by experts to have some degree of effectiveness in preventing AWS but clearly can have complications. Clomethiazol is not advised for critically ill patients as it was found to be associated with higher rates of tracheobronchitis [20]. A clinical trial investigated four different preventative treatment regimens of flunitrazepam–clonidine, chlomethiazole–haloperidol, flunitrazepam–haloperidol and ethanol in 197 alcohol-dependent patients with similar rates of withdrawal prevention and ICU length of stay found for each treatment [25]. Based on the evidence discussed in a recent systematic review of AWAS by Ungur et al., ethanol or benzodiazepines can be advised for AWS prevention in ICU patients with alcohol dependence; however, it should be noted that an opposing view is evident in a review of the subject by Awissi et al. in which the authors contend that the efficacy of such treatments in AWS prophylaxis remains unproven [23, 24].

As previously discussed, the differential diagnosis of an altered sensorium in the ICU patient is extensive, and a diagnosis of the AWS should only be made when all other possibilities, including bleeding, hypoxia, pain and focal neurologic lesions (see again Table 14.1), have been excluded [6]. To detect AWS symptoms there are various tools available [26]. The best validated psychometric scores are the Clinical Institute Withdrawal Assessment for Alcohol-Revised scale (CIWA-Ar) and for identifying delirium the Confusion Assessment Method for the ICU (CAM-ICU) and the Intensive Care Delirium Screening Checklist (ICDSC). The more easily applied Delirium Detection Score (DDS), which is derived from the CIWA-Ar, has also been validated for use in the ICU (Table 14.2) [20]. In addition, the Richmond Agitation–Sedation Scale (RASS) for sedation monitoring is useful to differentiate between agitated and sedative states [27, 28]. The transition between prophylaxis and therapy might be fluid, and therefore it is of major importance to establish frequent and regular delirium monitoring in the ICU as recommended in the guidelines [26, 29].

The treatment of AWS or delirium of any other cause should be started immediately if any monitoring suggests that the delirium is pathological [30, 31]. An inadequate or late therapy of the AWS could worsen the symptoms. Symptom-triggered and standardized application of benzodiazepines (oral or intravenous) such as lorazepam, diazepam or chlordiazepoxide is the standard first choice in AWS but not in other causes of delirium [23, 24, 26]. Adjuvant therapies with neuroleptics such as haloperidol may be necessary when the AWS is associated with significant hallucinations, and in selected cases autonomic instability may be tempered with clonidine or dexmedetomidine. In mild cases of AWS or during weaning from its treatment, carbamazepine alone can be sufficient. These medications complement one another in mitigating the neurotransmitter imbalances during alcohol withdrawal, specifically, GABAergic (benzodiazepine, chlomethiazole), dopamine (haloperidol, risperidone, quetiapine) and noradrenergic (dexmedetomidine, clonidine) transmission [6, 27].

Table 14.2 Delirium detection score (DDS) validated for the use at ICU

	Symptoms	Points
1	*Orientation*	
	Orientated to time, place and personal identity, able to concentrate	□ 0
	Not sure about time and/or place, not able to concentrate	□ 1
	Not orientated to time and/or place	□ 4
	Not orientated to time, place and personal identity	□ 7
2	*Hallucinations*	
	None	□ 0
	Mild hallucinations at times	□ 1
	Permanent mild-to-moderate hallucinations	□ 4
	Permanent severe hallucinations	□ 7
3	*Agitation*	
	Normal activity	□ 0
	Slightly higher activity	□ 1
	Moderate restlessness	□ 4
	Severe restlessness	□ 7
4	*Anxiety*	
	No anxiety when resting	□ 0
	Slight anxiety	□ 1
	Moderate anxiety at times	□ 4
	Acute panic attacks	□ 7
5	*Myoclonus/convulsions*	
	None	□ 0
	Myoclonus	□ 1
	Convulsions	□ 7
6	*Paroxysmal sweating*	
	No sweating	□ 0
	Almost not detectable, only palms	□ 1
	Beads of perspiration on the forehead	□ 4
	Heavy sweating	□ 7
7	*Altered sleep-waking cycle*	
	None	□ 0
	Mild, patient complaints about problems to sleep	□ 1
	Patient sleeps only with high medication	□ 4
	Patient does not sleep despite medication at night, tired at day time	□ 7
8	*Tremor*	
	None	□ 0
	Not visible, but can be felt	□ 1
	Moderate tremor (arms stretched out)	□ 4
	Severe tremor (without stretching arms)	□ 7
Delirium		≥8 □
No delirium		<8 □

A symptom-oriented, score-based approach is essential to treating AWS, particularly in the ICU, as it is associated with shorter treatment durations, lower doses of medications and fewer complications than when fixed-dose, fixed-schedule regimens are employed [6, 30]. Although orally or even intravenously administered

alcohol has been used in the past and has some pharmacological rationale, this treatment for AWS is now considered obsolete and is rarely used.

The symptoms of AWS can be reduced with the prophylactic use of benzodiazepines, α2-agonists or antipsychotics. When using antipsychotics such as haloperidol and/or when using α2-agonists (such as clonidine) the QTc-time should be monitored. In parallel, in order to prevent Wernicke–Korsakoff encephalopathy an adequate substitution of thiamine (200 mg/day parenterally) for at least 3–5 days should be considered [32]. Specific signs and symptoms of this devastating and in many cases irreversible syndrome include eye motility disturbances, impaired reflexes, decreased consciousness, disorientation, apathy, somnolence, ataxia, dysphagia and hyperhidrosis.

Cardiovascular Complications of Alcohol Abuse

Chronic alcohol consumption has direct effects on the heart and the neurohumoral axis that can lead to the cardiac dysfunction. High levels of alcohol consumption (over 90–100 g/day) increase the risk of sudden cardiac death and arrhythmias. Arrhythmias, particularly atrial fibrillation and ventricular extrasystoles, can occur even after a single episode of binge drinking and have been termed the "holiday heart syndrome" [33]. In the postoperative period alcoholics have up to five times more cardiac complications than do non-alcoholics [5, 33], and the presence of atrial fibrillation is even more relevant to their long-term prognosis than is the presence of ischemic heart disease [34] and may reflect an underlying alcohol-induced cardiomyopathy [14]. In parallel, hypokalaemia commonly accompanies AWS and further increases the risk for cardiac arrhythmias. Further, hypoxaemia after major surgery can also cause cardiac arrhythmias and other cardiac complications, and up to 18 % of AUD patients were reported to have postoperative episodic hypoxaemia that may reflect altered sleep physiology [35].

Acute alcohol intoxication can cause electrolyte imbalances, hypoglycaemia, thermal dysregulation and cardiovascular disturbances including tachycardia and hypotension. A generalized vasodilation and hypovolaemia caused by blockade of the actions of antidiuretic hormone (ADH) that include increased diuresis likely contribute to the hypotension. In parallel, the combination of the vasodilating and sedative effects of alcohol may lead to hypothermia and associated life-threatening cardiac complications such as pulseless electrical activity (PEA) arrest.

The mainstay of therapy for alcohol-mediated cardiac dysfunction, and for AUDs in general, is sustained abstinence from alcohol. In fact, this alone may completely reverse alcohol-induced cardiomyopathy [36]. Even a significant decrease in consumption can have salutary effects, as improvements in left ventricular function have been noted when ingestion is reduced to less than 60 g/day. In parallel with abstinence from alcohol, in selected cases it can be beneficial to treat pharmacologically with an angiotensin-converting enzyme (ACE) inhibitor or a ß-adrenergic blocker.

Alcohol and Rhythm Disorders

The mechanism of dysrhythmias in AUD patients with hepatic steatosis showed late ventricular potentials on the signal-averaged electrocardiogram [37]. Heavy drinking lowers the threshold for ventricular fibrillation and elevates the plasma levels of low-density lipoproteins. Only one out of four patients with out-of-hospital ventricular fibrillation arrest will survive to hospital discharge, so any substantial reduction in the incidence of sudden cardiac death will require preventive interventions. In the setting of underlying alcohol abuse, these include (1) brief interventions to encourage and promote abstinence and/or therapy for dependence and addiction, (2) rhythm monitoring (12-lead electrocardiogram or Holter monitor) at predetermined intervals or by telemedicine transmissions of patients at risk and (3) consideration of early defibrillator implantation for otherwise relatively borderline indications in heavy alcohol abusers.

The pathophysiology of alcohol-related dysrhythmias is not well understood, but structural changes including myofibrillar necrosis, interstitial fibrosis and dysfunction of the myocyte sarcolemma and mitochondria have all been implicated. Electrolyte balances at the cellular level may also be changed by alcohol or its metabolite, acetaldehyde, and electrolyte imbalances in combination with increased catecholamine stimulation may also contribute to alcohol-mediated dysrhythmias.

Alcoholic patients show often atrial arrhythmias, and the atria are particularly sensitive to the arrhythmogenic effects of acute alcohol consumption [37], and 30–60 % of patients with atrial fibrillation have a history of alcohol consumption [38]. A variety of abnormalities may underlie this predisposition to arrhythmias. In particular, alcohol prolongs conduction times and can increase the refractory period of myocardial cells. As a result, binge drinking in chronic alcoholics often leads to even the absence of clinically apparent cardiomegaly or cardiomyopathy. Further, alcohol has been shown to have negative inotropic effects on cardiac muscle cells [39], and stimulation of the ß-adrenergic receptors does not improve alcohol-induced myocardial dysfunction under experimental conditions. Although not well understood, the well-recognized chronotropic and inotropic effects on the right atrium in alcoholics may be associated with a compromised stress response during trauma, surgery or infection.

Alcohol and Coronary Artery Disease

Although low levels of regular alcohol consumption may offer some protection against the development of coronary artery disease (CAD), heavy alcohol use has harmful effects on left ventricular structure and function [40]. The acute ingestion of alcohol in the presence of CAD may exaggerate stress- or exercise-related myocardial ischaemia and impairment, and alcohol can cause coronary vasospasm and angina. When cardiomyopathy is present, the elevation of catecholamines in combination with poor ventricular function may increase the coronary demand and cause

variant angina. Although alcohol consumption within 24 h before the onset of myocardial infarction had no influence on infarct size or risk for Q-wave infarction, chronic moderate or heavy alcohol consumption has been reported to increase the risk of cardiac complications in the presence of clinically existing CAD [41]. However, in the development of CAD and ischemic stroke a curvilinear effect is observed, in that light or moderate drinking (25–50 g/day) conferred a protective effect, whereas heavy drinking was associated with a significantly increased disease risk. Therefore, alcohol use follows the pharmacologic principle *Dosis sola venenum facit* ("the dose alone makes the poison"). The beneficial effects of moderate alcohol ingestion are thought to be mediated at least in part by increasing the circulating concentrations of high-density lipoproteins and inhibiting coagulation. In contrast, a large amount of alcohol ingested in the evening resulted in an acute inhibition on fibrinolysis that persisted the following morning [42]. Importantly, the "cardioprotective" effects of moderate alcohol ingestion observed in population studies do not necessarily apply to some subsets of less healthy individuals, and those with diabetes mellitus, hypercholesterolaemia, hypertriglyceridaemia and hypertension have a higher risk of developing CAD and in general are advised against alcohol intake. Hypertension is also associated with unsafe levels of alcohol use, and in a large study the UK women that consumed more than two drinks per day had an increased odds ratio (1.68) for the prevalence of hypertension (95 % CI 1.14–2.46) [43].

Alcoholic Cardiomyopathy

Patients with long-term heavy alcohol use (>90 g/day for ≥5 years) may develop a nonischaemic dilated cardiomyopathy, referred to as "alcoholic cardiomyopathy" [36, 44]. Alcohol causes alterations of the myocardial muscle with concentric left ventricle modelling or hypertrophy [40]. Even though they are often asymptomatic one-third develops diastolic dysfunction correlating with the alcohol consumption [44]. The pathogenic mechanism involves multiple pathways that influence the myocardium (see Fig. 14.1). Principal histological changes are myocyte and nuclear hypertrophy, interstitial fibrosis and myocyte necrosis [33, 45, 46]. Alcohol causes cardiac myocyte loss through apoptosis and inhibition of myocyte proliferation through myostatin up-regulation [47]. There is some evidence that alcohol may induce the expression of genes that are associated with cardiac hypertrophy, including atrial natriuretic peptide and p21 [48], as well as protein synthesis leading to a loss of muscle strength [49]. Alcohol also causes intracellular organelle abnormalities and, in particular, mitochondrial dysfunction [46]. The diagnosis of alcoholic cardiomyopathy is typically made on clinical grounds in the right setting, but in some cases an endomyocardial biopsy is indicated, particularly if the underlying AUD is unrecognized or to rule out other causes of cardiomyopathy. Biopsies of alcohol-induced cardiomyopathy typically show myocyte hypertrophy with enlarged nuclei and lymphocytic infiltration of the myocardium [50].

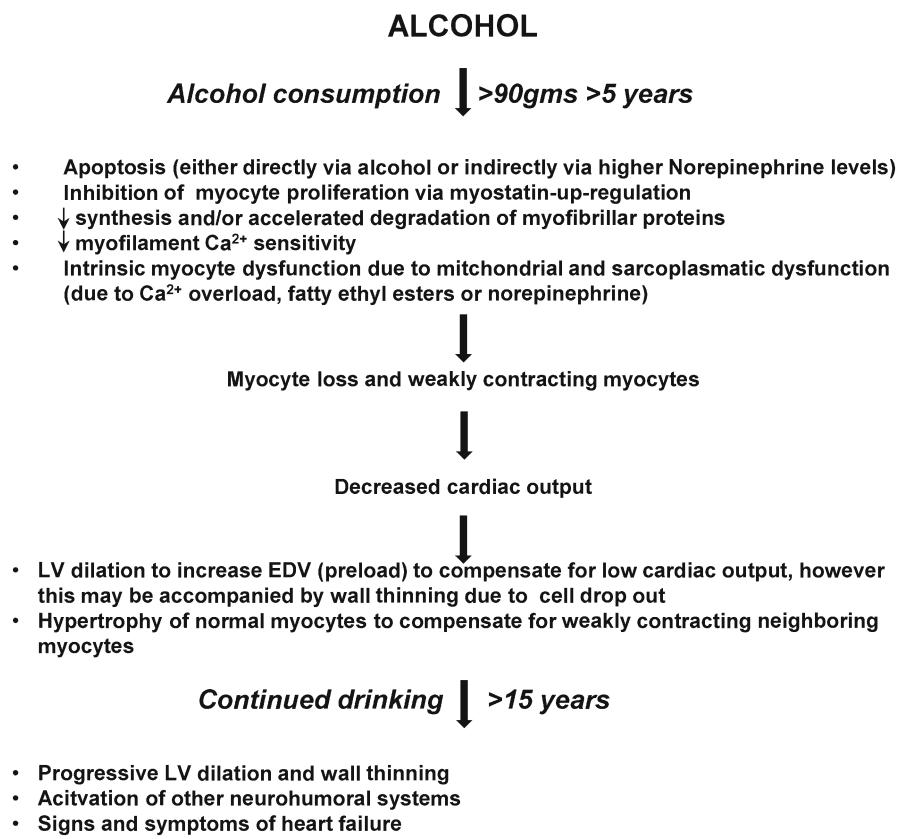

Fig. 14.1 Proposed hypothetical schema for the pathogenesis of alcoholic cardiomyopathy. *LV* left ventricular, *EDV* end-diastolic volume (adapted from Piano) [46]

However, in response to acute and critical stresses such as major surgery or sepsis, patients with chronic alcohol abuse may have limited reserves because of underlying decreased biventricular function, even if there is no clinical evidence of cardiomyopathy.

Abstinence from alcohol intake is the mainstay of treatment and is remarkably effective in that left ventricular function can improve significantly over time [51]. Unfortunately, complete abstinence is difficult for some to achieve, and a significant percentage of alcoholics are only able to reduce their intake. However, decreasing daily intake to a more moderate level such as 60 g/day can still lead to improvements in left ventricle function. Therefore, brief alcohol interventions, dietary recommendations, additional pharmacological treatment with ACE inhibitors and ß-blockers when indicated and interdisciplinary follow-up should all be used even for individuals who are not completely abstinent. Fortunately and unlike for many other types of cardiomyopathy, the prognosis even for patients with New York Heart Association Class IV heart failure caused by cardiomyopathy is good if complete

abstinence is maintained [51, 52]. However, continued smoking (common in alcoholics) and non-compliance to abstinence from alcohol dramatically increased the risk for hospital readmission among patients with heart failure [53].

Alcohol and Bleeding Complications

Chronic alcohol intake causes haemostatic dysregulation. Moderate intake of alcohol prolongs the bleeding time by interfering with platelet aggregation and increases the risk for bleeding complications. Metabolic factors or alcohol itself can alter the balance between coagulation and fibrinolysis. Further, alcohol can impair thrombopoiesis at the level of megakaryocyte maturation, leading to a reduced platelet count and mean platelet volume. Platelet aggregation triggered by agonists (collagen, adrenaline, arachidonic acid, platelet-activating factor and adenosine diphosphate) is also reduced by alcohol. In parallel, alcohol inhibits the release of thromboxane A_2 and B_2 as well as the activity of phospholipase A_2, thereby reducing the synthesis of arachidonic acid metabolites and further impairing platelet function [5].

In the later and more severe stages of alcoholic liver disease, hepatic synthesis of coagulation factors is decreased. Moderate drinking reduces the plasma levels of fibrinogen coagulation factor VII and von Willebrand factor and decreases platelet function (Table 14.3 provides a summary of the coagulopathic effects of alcohol at different levels of ingestion). One effect of lower fibrinogen levels is that alcohol ingestion can lower blood viscosity [54], and plasma fibrinogen levels have been shown to be highest in abstinent persons and lowest in persons with moderate alcohol intake. In those with significant alcohol-induced liver disease the fibrinogen levels and the levels of other coagulation factors are often quite low. Factor VII levels decline with higher alcohol intake and are an indicator of the degree of alcoholic liver damage. In parallel, severe liver disease is associated with decreased intake and/or absorption of vitamin K levels, which limits the production of clotting factors such as prothrombin.

These and other effects of alcohol likely play a major role in increasing the incidence of perioperative surgical bleeding in alcoholics fivefold, and 50 % of individuals with an AUD have a postoperative bleeding complication with the attendant higher transfusion requirements. Further, patients with underlying chronic alcohol abuse are twice as likely to need secondary surgeries [55]. Surgical interventions activate both coagulation and fibrinolysis. Fortunately, the risk of alcoholics for postoperative thromboembolic complications seems to be comparable to the risk in non-alcoholics. However, during acute alcohol withdrawal the platelet count and thromboxane formation increase, and any prolongation in the bleeding usually decreases. The clinical relevance for these potentially "prothrombotic" changes during alcohol withdrawal remains unclear, particularly in the context of the complex physiological interactions in the postoperative period.

Table 14.3 Effects of alcohol on haemostasis

Parameter	Chronic moderate alcohol consumption	Chronic high alcohol consumption	Acute alcohol consumption	Alcohol toxic liver disease
Fibrinogen	Slight reduction within normal range	Elevated	Elevated	Reduced
Factor II	?	reduced	?	Reduced
Factor V	?	?	?	Reduced
Factor VII	Slight reduction within normal range	Reduced	?	Reduced
Factor X	?	Reduced	?	Reduced
Factor VIII	?	Elevated	Elevated	Elevated
Factor IX	?	Reduced	?	reduced
Factor XI, XII, XIII	?	?	?	reduced
Von Willebrand Factor	Slight reduction within normal range	Elevated	Elevated	Elevated
Platelet count	Normal	Reduced	Normal or reduced	Reduced
Platelet function	Normal or reduced	Reduced	Initially reduced than elevated	Reduced
tPA	Elevated	Different results	Elevated	Elevated
PAI-1	Normal	Elevated	Elevated	reduced

PAI-1 Plasminogen activator inhibitor Type 1, *tPA* tissue plasminogen activator
? unknown

Hepatic Insufficiency and Coagulopathy

Patients with chronic alcohol abuse are prone to multiple coagulation abnormalities including thrombocytopenia (low platelet counts) and prolonged prothrombin time with a corresponding elevated international normalized ratio (INR) [56]. There are multiple factors in these individuals that can contribute to the development of low platelet counts including decreased production (thrombopoiesis) in the bone marrow due to decreased bioavailability of vitamin B12 and folic acid, increased sequestration in the spleen and accelerated platelet degradation [57]. When nutritional deficiency of vitamin B12 and folic acid is the primary defect, nutritional supplementation alone will enhance thrombopoiesis and there will be a rapid increase in the number of reticular platelets. However, when hypersplenism and platelet sequestration is the dominant cause then this treatment will be ineffective. In parallel to thrombocytopenia, alcohol-induced hepatic dysfunction can interfere with the synthesis of the vitamin K-dependent coagulation factors II, VII and X. In addition, although factor V synthesis is not dependent on vitamin K its production can also be impaired by alcohol and in fact can serve as an index of hepatic synthetic capacity. Among these factors, factor VII has the shortest half-life and may become

deficient relatively quickly in the setting of acute illness. In contrast, fibrinogen production is maintained even in relatively severe liver disease, and therefore a reduction of fibrinogen is a sign of advanced alcohol-mediated hepatic dysfunction, and the INR becomes prolonged when fibrinogen plasma levels fall below 100 mg/dl [58]. In addition to defects in coagulation, alcohol-mediated liver disease can lead to aberrant fibrinolysis if portal hypertension is present with an associated increase in the release of tissue plasminogen activator (tPA) and decreased hepatic production of plasminogen activator inhibitor-1 (PAI-1). Overall, the combination of thrombocytopenia, impaired synthesis of vitamin K-dependent and -independent clotting factors and the chronic low-level release of bacterial endotoxins and exotoxins into the circulation because of intestinal epithelial barrier dysfunction can lead to a chronic pattern in alcoholics that mimics disseminated intravascular coagulation [58, 59]. Fortunately, in most cases coagulation function is compensated at least at a relatively low level, and spontaneous bleeding is uncommon. However, individuals with alcohol-mediated hepatic dysfunction and/or defects in coagulation undergoing major surgery often require transfusions with coagulation factors and platelets.

Impaired Immune Function and Increased Risk of Perioperative Infection

Patients with long-term alcohol abuse have a three- to fivefold higher postoperative infection rate compared to non-alcoholic patients [5, 8, 10, 11]. According to the analysis of the "Nationwide Inpatient Sample", a large database of approx. 1,000 hospitals in the United States (2007 and 2008), the most frequent infections are urinary tract infections, surgical wound infections and nosocomial pneumonia [60]. In the intensive care unit nosocomial pneumonia occurs in 38 % of long-term alcoholics, 10 % of social drinkers and 7 % of non-alcoholic patients [8]. Long-term alcohol use causes alterations of cellular and humoral immunity leading to increased infection rates [5, 8, 10, 61].

The Preoperative Period

Even if they appear relatively healthy preoperatively, individuals with AUDs have impaired innate and adaptive immunity that renders them susceptible to perioperative infections [62, 63]; see also Fig. 14.2. Alcohol inhibits the ability of innate immune cells to respond to pathogens. For example, monocytes, tissue macrophages and dendritic cells all show signs of abnormal phagocytosis, impaired superoxide production and defects in antigen presentation. In addition, they do not activate the adaptive immune response in the necessary robust manner required to respond adequately to infection. In parallel, neutrophil recruitment and/or activation at sites of

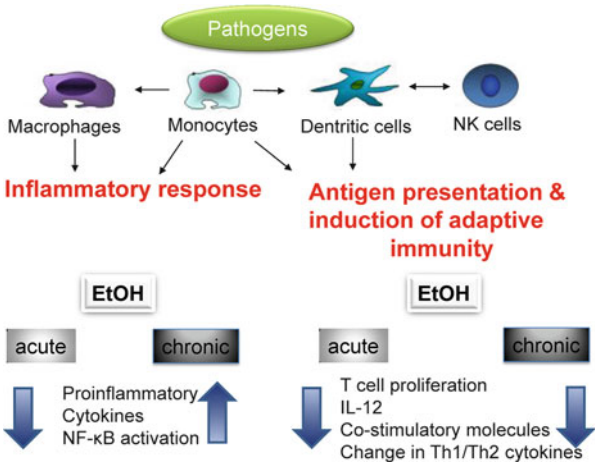

Fig. 14.2 Alcohol alters innate and adaptive immunity. Cells of the innate immune system (macrophages, monocytes, dendritic cells and NK cells) are limited in their capacity to respond to pathogens. Acute alcohol intake reduces the pro-inflammatory cytokines (IL-1, TNF-α) and inhibits NF-kB activation, while chronic alcohol consumption enhances the pro-inflammatory responses. The antigen presenting function in both acute and chronic alcohol intake contributes to impaired activation of the adaptive immune response. Both acute and chronic alcohol intake disturb the T-cell functions and IL-12 production. This leads to a reduced Th1 and Th2 cytokine production. These alcohol-induced abnormities contribute to impaired pathogen elimination and reduced adaptive immune response (adapted from Szabo) [62]

infection is dampened. Interestingly, there are some differences in immune responses from acute versus chronic alcohol ingestion. For example, in acute alcohol exposure the production of pro-inflammatory cytokines, such as IL-1 and TNF-α, can be impaired along with dampening of NFκB activation. In contrast, chronic alcohol can enhance the pro-inflammatory response in some tissues but impairs this response in some tissues including the lung. In contrast, both acute and chronic alcohol ingestion inhibits T-cell functions and IL-12 production (critical in activating the adaptive immune response) and thereby impairs Th1 and Th2 cytokine production. Therefore, the effects of excessive alcohol ingestion on the immune system may depend on the duration and intensity of the intake but may also have tissue- and/or cell-specific differences.

The proliferation of CD3+ T cells is also inhibited by alcohol, and the number of CD4+ and CD8+ T cells is reduced [64]. The preoperative ratio of T helper-1 cells to T helper-2 cells (the Th1/Th2 ratio) is significantly decreased in chronic alcoholics and correlates with a higher incidence of postoperative infections [10]. The immune system communicates via different cytokines among different cell components. Pro-inflammatory cytokines play a role in initiating an inflammatory reaction, whereas anti-inflammatory cytokines protect against an overshoot of the immune response in order to protect the host cells. Cytokines released from Th1 cells, such as IFN-γ, may be decreased in chronic alcoholics, whereas the production of cytokines from Th2 cells, such as IL-4 and IL-10, may be increased [65]. In contrast, cytotoxic

lymphocyte numbers are similar in alcoholics and non-alcoholics. Overall, the levels of pro-inflammatory and anti-inflammatory cytokines may be unbalanced in alcoholics, but this appears to depend on whether or not there is underlying hepatic dysfunction. For example, increased activation of the transcription factor NFκB has been associated with alcoholic hepatitis [66]. Overall, it is plausible to predict that a more comprehensive understanding of, and ability to detect, alcohol-mediated immune dysfunction in the preoperative setting would enable us to identify individuals who are at high risk for perioperative infections and perhaps even other complications.

The Intraoperative Period

All surgical procedures have a measurable effect on the immune system [10, 11, 67, 68]. In the healthy host a surgical stress produces an initial pro-inflammatory immune reaction followed by an anti-inflammatory immune response [69], with the degree of each depending on the extent of the surgical procedure. Typically, Th1 cells are decreased after major surgery without an alteration in the Th2 response, leading to a lower Th1/Th2 ratio. Smaller procedures, such as laparoscopy, do not change the cytokine production of IFN-γ, IL-4, and IL-10 in T cells within the first 24 h. Within 2 h after surgical incision, lower levels of the pro-inflammatory cytokine TNF-α and higher levels of the anti-inflammatory cytokine IL-10 are seen, and the ability of peripheral blood monocytes to express HLA-DR is decreased. In fact, within as few as 20 min after incision the levels of IL-6 are increased, and high intraoperative levels of IL-6 and IL-10 correlate with the risk for postoperative infection and sepsis after major non-cardiac surgery [67]. In parallel, the ability to react adequately to LPS stimulation is reduced by the acute stress of surgery but typically returns within 24 h after surgery [70]. Therefore, even in the healthy host there are significant stresses on the immune system that influence the risk of perioperative infections.

Unfortunately, there are relatively few data on intraoperative changes of the immune system in chronic alcoholics. However, there are some recognized changes in the immediate postoperative period. The Th1/Th2 and Tc1/Tc2 ratios remain low 1 day after surgery in alcoholics, and these impairments of T-cell-mediated immunity have been shown to be predictive for postoperative infection, particularly nosocomial pneumonia [10, 11]. IL-10 levels are higher in alcoholics at the time of ICU, and postoperatively ratio of IL-6 to IL-10 is decreased in chronic alcoholics, and these decreased ratios correlate with an increased postoperative infection rate [68, 71].

The Postoperative Period

Th1/Th2 and Tc1/Tc2 ratios remain depressed for up to 5 days in chronic alcoholics undergoing upper digestive tract surgery compared to non-alcoholics [10, 11], and plasma levels of IL-10 also remain elevated [10]. These derangements correlate with

suppressed function of peripheral monocytes and suppression of their expression of HLA-DR up to 7 days after surgery [72]. The ratio of LPS-stimulated IFN-γ/IL-10 in whole blood cells is decreased on the first postoperative day and remains low for 5 days in chronic alcoholics, whereas in non-alcoholics this ratio is increased [10]. This is consistent with the observation that a decreased IL-6/IL-10 ratio early after surgery is associated with increased postoperative infection rates in chronic alcoholics [68]. At the onset of infection and the early phase of septic shock, chronic alcoholics have lower plasma levels of IL-1β, IL-6, and IL-8 compared to non-alcoholics [73], although the levels of the anti-inflammatory cytokine IL-10 do not appear to be different in alcoholics.

Interventions to Improve Perioperative Immune Function

There is a wide range of possibilities to influence immune function of chronic alcoholics in the perioperative period. Preoperative abstinence from alcohol is the first approach. Remarkably, in one study alcohol abstinence for a month before elective surgery reduced the postoperative morbidity from 71 to 31 % [5], although to date there is no consensus as to whether or not this is a practical approach for elective surgeries. In the more acute or emergent setting, other strategies that have been used in an attempt to reduce the stress response in surgical patients include low-dose ethanol infusions (0.5 g/kg body weight per day), morphine infusions (15 μg/kg body weight per hour), and ketoconazole (200 mg four times daily; only as proof of concept and not recommended as standard treatment), and these strategies have been shown to decrease postoperative hypercortisolism and postoperative pneumonias as well as to shorten ICU lengths of stay in at least one study [11]. However, whether or not we can significantly enhance immune function of chronic alcoholics in the perioperative setting and thereby improve outcomes requires further investigation.

Therapy and Prevention of AUD-Related Co-morbidities

To prevent postoperative complications, alcoholic patients with organ pathology should be either abstinent before surgery or should have prophylactic treatment to reduce the risk for alcohol withdrawal depending on the needs of the patient. Short-term brief interventions can reduce alcohol intake [32], and in order to identify high-risk patients an efficient screening tool is essential. Substance-specific questionnaires such as the CAGE or the AUDIT (see Chap. 3 for details) and laboratory parameters have been proven of value [27, 74, 75]. Among the laboratory tests, an abnormality in any two within the “panel of four” that includes (1) mean corpuscular volume (MCV), (2) hepatic transaminase aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), (3) γ-glutamyl transpeptidase (GGT), and (4)

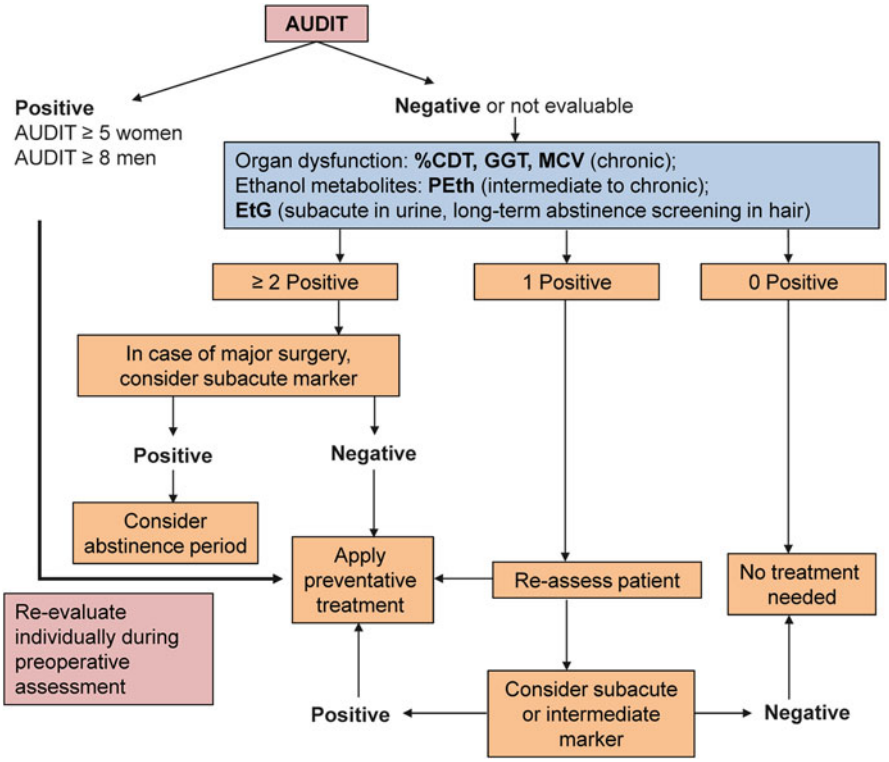

Fig. 14.3 The evidence-based algorithm for detection of harmful alcohol abuse of the Charité University—Medicine Berlin, Germany. *AUDIT* Alcohol-use disorder identification test (range 0–40), *%CDT* carbohydrate-deficient transferrin (reference range <2.6 %), *GGT* y-glutamyl transpeptidase (reference range, ≤565 U/l), *MCV* mean corpuscular volume (reference range, 81–100 fl), subacute marker: *EtG* ethylglucuronide in urine samples (reference range <100 µg/l), intermediate marker: *PEth* (a promising new marker, not yet clinical standard) [1, 78]

carbohydrate-deficient transferrin (CDT) in combination with a positive score on the AUDIT provides good sensitivity and predictive value in identifying patients who may develop alcohol withdrawal symptoms (sensitivity 70.6 %, specificity 98.8 %, positive predictive value 54.5 %, negative predictive value 99.4 %) [76]. Therefore, it is imperative that the clinician integrate the medical history, the clinical examination and an appropriate screening tool to identify individuals who are at increased risk for perioperative complications because of underlying (and often unrecognized) AUDs (Fig. 14.3).

When an individual is identified as being at increased risk for perioperative complications because of underlying alcohol use, preventative treatments should be discussed with the patient and initiated. The evaluation of laboratory parameters should be done at an early point of hospital administration. In the case of urgent/emergent

Table 14.4 Estimated recovery times for alcohol-related organ dysfunction after abstinence

Organ dysfunction	Recovery time
Immune competence	2–8 weeks
Wound healing	<2 months
Endocrine stress response	2–12 weeks
Bone healing	<6 months
Haemostasis	1–4 weeks
Cardiac function	
Asymptomatic	1 month
With severe failure	1–6 months

The data on how long the abstinence time should be before surgery is still not clear [79]

(i.e. "non-elective") surgery, the blood alcohol level and a toxicological screening should be performed. These tests and the aforementioned laboratory screening tests are especially useful when the personal history is not believable or cannot be assessed. For example, early studies showed an association of CDT with post-traumatic morbidity [74]. The measurement of ethylglucuronide (EtG), a long-acting biomarker of alcohol ingestion, can be performed in urine or hair samples to assess abstinence as levels in urine samples can detect alcohol consumption up to the last 80 h [32, 77, 78]. A recent systematic review demonstrates that total phosphatidylethanol, an abnormal phospholipid formed in the erythrocyte membrane exclusively in the presence of ethanol, exhibits high diagnostic sensitivity and specificity for detecting active chronic excessive drinking with a regular daily alcohol intake of more than 60 g [67, 68].

AUDs are treatable, and there are various therapeutic options that can be offered to the patient with the goal of avoiding or at least decreasing the need for ICU treatment. Important tools have to be put in place: efficient screening procedures, brief interventions that can motivate behavioural changes, preoperative abstinence therapy, adapted anaesthetic procedures, withdrawal prophylaxis and attention to alcohol-related co-morbidities before and after surgery.

Alcohol-induced pathophysiological changes are potentially or at least partially reversible. After 2 weeks to 2 month of abstinence, the delayed-type hypersensitivity is within normal range although alterations of the hypothalamic-pituitary-adrenal axis and the cortisol stress response may persist for 6 months or longer. Cardiac dysfunction may improve within 1 month, and even significant alcohol-mediated cardiomyopathy improves in 50 % of affected individuals after 3–6 months of abstinence [5, 10, 12, 69]. Importantly, as few as 8 weeks of alcohol abstinence may improve wound healing [5, 10, 12, 69], and this can be important particularly when planning elective surgery. In parallel, alcohol-related sleep disorders improve, and hypoxaemias are less frequent after 3–6 weeks of abstinence [5, 10, 12, 79] (Table 14.4). A small randomized trial of patients with alcohol consumptions ranging from 60 to 420 g/day undergoing colorectal surgery showed that postoperative morbidity could be decreased by a combination of preoperative alcohol abstinence and psychological support over a 4-week period, with an abstinence rate of ~90 %

in the intervention group and a significant decrease in the postoperative complication rate from 71 to 31 % [5, 79]. In addition, a placebo-controlled trial determined that pharmacological intervention with morphine, low-dose ethanol and ketoconazole prevented the prolonged cortisol response to surgical stress and lowered the postoperative infection rate [11]. It is also important to remember that adequate perioperative pain management is essential for patients with AUDs [80, 81]. Finally, there is evidence that brief interventions can reduce alcohol intake for up to 48 months. These brief interventions range from 5 to 60 min and include motivational interviews with a direct conversation style. Interestingly, individual computer-supported counselling with feedback was able to reduce alcohol consumption for up to 6 months [75]. Therefore, interventions undertaken to improve perioperative outcomes in the short term may have more lasting beneficial health effects.

Summary

The increased morbidity and mortality associated with AUDs in the critically ill surgical patient (and in other contexts) are treatable. Based on adequate diagnostic procedures and early diagnosis the treatment should be effective, preventative and symptom oriented. This concept can be summarized in the acronym "FRAMES" and should be used from the first interaction with the patient through the end of therapy to build a successful working alliance that can improve outcomes. Specifically, patients should be informed (*F*eedback) and be integrated in the treatment plan (*R*esponsibility) with clearly defined aims (*A*ims). It is important to define modifications of behaviour (*M*enu of behavioural change) with an objective and empathic communication style (*E*mpathy) emphasizing the self-confidence (*Self-efficacy*) of the patient.

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Chapter 15
Alcohol and HIV: Experimental and Clinical Evidence of Combined Impact on the Lung

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Abstract Despite antiretroviral therapy, lung disease is a leading cause of death in individuals infected with human immunodeficiency virus type 1 (HIV). Individuals infected with HIV are susceptible to serious bacterial and viral infections, such as pneumococcus and influenza, which are particularly problematic for lung health, resulting in lung injury. Additionally, HIV-infected individuals are susceptible to a number of pulmonary diseases for unknown reasons. Alcohol, the most commonly abused drug in the world, continues to exact an enormous toll on morbidity and mortality in individuals living with HIV. Chronic alcohol abuse has been shown to affect lung immunity, resulting in significant lung injury. There is a paucity of literature on the additive effects of HIV and alcohol, two diseases of immune senescence, in the lung. This chapter begins by discussing the latest literature evaluating the epidemiology of HIV, alcohol use, and lung health focusing on two prevalent infections, tuberculosis and pneumococcal pneumonia. In parallel, we discuss the interactions of alcohol and HIV on the risk for acute lung injury and subsequent morbidity and mortality. We then discuss the pathophysiology of how these two diseases of immune dysfunction affect the lung, with a focus on the oxidative stress, alveolar macrophage host immune capacity, and immunomodulatory role of zinc in the airway. Finally, we review the latest literature on how HIV and alcohol affect other pulmonary disorders including chronic obstructive pulmonary disease, pulmonary hypertension, and lung cancer.

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Overview

HIV/AIDS is a disease that continues to be of paramount global importance. The Centers for Disease Control (CDC) estimate that approximately one million people are living with HIV in the United States and approximately 20 % are unaware that they are infected [1]. Additionally and Prevention, in the past decade the number of people living with HIV has increased considerably. Prior to the widespread use of antiretroviral therapy (ART), pulmonary diseases, especially lung infections, were among the most frequent complications in individuals with HIV and were associated with significant morbidity and mortality [2]. However, even in the era of ART, individuals living with HIV continue to suffer from pulmonary diseases including bacterial pneumonia, chronic obstructive pulmonary disease (COPD), and pulmonary hypertension. Alcohol is the most widely used drug in the world. Chronic alcohol abuse continues to result in increased morbidity and mortality, exacting a tremendous burden on society. Chronic alcohol abuse can affect a number of organ systems including the lung resulting in increased susceptibility to infection and injury. However, there is a paucity of literature examining the compound effects of alcohol and HIV, two diseases of immune senescence, in the lung. This chapter presents epidemiology and pathophysiology of how HIV infection and alcohol use affect lung immunity and discusses other clinical syndromes that are also affected by the interactions of alcohol and HIV.

Part 1: Epidemiology of HIV, Alcohol Abuse, and Lung Health

HIV, Alcohol, and Lung Infection: Tuberculosis

Worldwide, tuberculosis remains one of the most important causes of death from an infectious disease [3]. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis has complicated the efforts at control for this disease. The HIV epidemic has led to an increase in the incidence of tuberculosis globally. At least one-third of HIV-infected persons worldwide are infected with *Mycobacterium tuberculosis*, and 8–10 % of them develop active disease annually [4]. In the United States, there has been a progressive decrease in rates of active tuberculosis and only 10 % of patients are coinfected with HIV [5]. Coinfection results in difficult issues in diagnosis and

treatment, especially because of drug interactions and complications related to immune reconstitution [6, 7]. Numerous studies have documented the increased risk of TB disease among HIV-infected compared to non-infected persons. Among the HIV-infected population, low CD4 counts and high viral loads have been found to be further risk factors for disease, while treatment with effective ART reduces risk.

The association of alcoholism and TB has been known for decades, but only one systematic review has examined this association [8]. In the 21 studies identified, all forms of TB disease were about three times higher, and pulmonary TB was four times more frequent, in heavy drinkers (>40 g of alcohol per day) than in controls. It is likely that adverse effects of alcohol on the immune system and increased patterns of transmission because of social interactions are both important in this increase. Further, excessive alcohol use complicates (and often decreases) adherence to the TB treatment regimen [9].

Alcohol-use disorders have been related to the acquisition and progression of HIV disease as well as to the adherence and immunologic response to ART [10–14]. A systematic review and meta-analysis of African studies documented a similar association between HIV and alcohol use [15]. Lower CD4 counts have been associated with alcohol consumption, especially in patients not on ART [16]. In addition, a recent model demonstrated that alcohol is a modifiable risk factor for poor survival among individuals with HIV [17]. These effects can be attributed biologically to the alcohol itself and not simply to related factors such as malnutrition, as chronic binge alcohol consumption accelerates progression of simian immunodeficiency virus disease in a primate model [18].

The risks of bacterial pneumonia and COPD are elevated among those with HIV infection compared to uninfected individuals, even after adjustment for smoking exposure [19, 20]. Alcohol impacts both innate and adaptive immune responses leading to immunosuppression [21], so it would be likely that the association of alcohol and HIV would increase the risk of tuberculosis.

Limited clinical data are available to define the interactions of alcohol, HIV, and tuberculosis. Patients diagnosed with TB have higher alcohol intake than persons without TB [8, 22–24]. HIV-infected patients have higher alcohol consumption than non-HIV-infected patients, and patients coinfected with TB and HIV have higher alcohol consumption than either TB- or HIV-infected patients [25–27]. A study in Botswana found that patients with MDR-TB had high rates of alcohol use and abuse. Further, among TB patients alcohol abuse was associated with a diagnosis of MDR-TB [28]. Recently, alcohol abuse was found to be an independent risk factor for poor outcomes in patients treated for MDR-TB [29].

Overall, there is convincing evidence that HIV and alcohol independently affect lung function and response to infection. These interactions appear to be complex and have yet to be sorted out completely, but it is clear that they contribute to an increased incidence of TB infection, a decreased response to therapy, and, as a consequence, an apparent increase in TB mortality.

HIV, Alcohol, and Lung Infection: Streptococcus Pneumoniae (Pneumoccocus)

Community-acquired pneumonia (CAP) is a leading cause of overall death worldwide [30–34]. *Streptococcus pneumoniae* (*pneumococcus*) is associated with more severe forms of pneumonia and is one of the main organisms leading to hospitalization in all groups [35]. However, pneumococcal disease has been shown to be higher in persons with underlying medical conditions (such as HIV), with low socioeconomic status, or who engage in high-risk behaviors such as cigarette smoking, intravenous drug use, and alcohol abuse [36–40]. In fact, recurrent bacterial pneumonia has been considered an AIDS-defining illness since the expanded European definition was adopted in 1993 [41].

In the VACS-3 (Veterans Aging 3 Site Cohort Study), a large study of 881 HIV-infected veterans enrolled between 1999 and 2000, there was a significant association between obstructive lung disease and bacterial pneumonia, and these two diseases demonstrated a linear association with the level of alcohol use (OR 1.4 for bacterial pneumonia, $p<0.001$ for level of alcohol use). In this cohort, approximately 67 % were current users and of these, 41 % drank in moderation, 23 % drank hazardously, and 35 % carried a diagnosis of abuse or dependence. The degree of alcohol use varied by demographic and HIV-related factors, and antiviral therapy (ART) did not vary significantly by alcohol use. When considering timing of alcohol consumption, past consumption demonstrated an equivalent or a higher prevalence of disease than did current consumption, so the authors concluded that past consumers were somehow different than lifetime abstainers. Associations of medical disease with alcohol use were independent of age, CD4 cell count, viral load, intravenous drug use, smoking, and exercise. Consequently, the authors concluded that there was no evidence of a "safe" level of consumption of alcohol among those with HIV infection [20]. Using 1999 and 2000 data from Active Bacterial Core surveillance and the National Health Interview Survey, Kyaw et al. determined the rates of invasive pneumococcal disease in healthy adults and in adults with high-risk conditions and found that, compared with healthy adults, the risk of invasive pneumococcal disease was 11-fold higher for those that abused alcohol and 23–48-fold higher in adults with HIV/AIDS [42]. The risk also increased with the number of conditions present, suggesting that the combination of HIV/AIDS and alcohol had compounding effects on the risk of pneumonia [42]. Murdoch et al. showed in an observational cohort of 300 HIV-infected individuals receiving care between 1996 and 2005 that a high proportion (60 %) reported prior and/or current alcohol abuse [43]. Multivariate analyses showed that younger age, alcohol use, lack of ART use, lower CD4 counts, and higher HIV RNA were independent predictors of pneumonia. Most recently, Crothers et al. analyzed data from the VACS virtual cohort of 33,420 HIV-infected veterans and 66,840 age-, sex-, race-, and site-matched uninfected control subjects to determine the incidence of pulmonary diseases in HIV-infected persons compared with non-HIV persons. They found that, even in the era of ART, HIV infection increased the risk of bacterial pneumonia ~7-fold (7.5 % vs.

1.1 %, $p<0.001$) and alcohol disorders were significantly more common among HIV-infected individuals compared to the control subjects ($p<0.001$) [44].

These studies confirm that although ART has substantially decreased opportunistic infections associated with HIV infection, pneumonias from routine pathogens such as *pneumococcus* continue to be prevalent in these susceptible individuals, and alcohol abuse may have a compounding effect. Additionally, bacterial pneumonia can result in worse outcomes in HIV-infected individuals who abuse alcohol. Large multicenter observational studies have shown that HIV-infected individuals hospitalized with pneumococcal pneumonia have significantly higher 14-day mortality, with a trend for the highest mortality in those with lower CD4 counts, compared to uninfected individuals after controlling for age and severity of illness [45]. Additionally, HIV-infected individuals with cirrhosis and a history of heavy alcohol abuse have increased mortality and increased hospitalizations from community-acquired pneumonia compared to HIV-infected individuals without cirrhosis. In this setting, excessive alcohol intake and hepatitis C coinfection are the most important causes of chronic liver disease in persons living with HIV [46]. These studies suggest that alcohol-use disorders are common in HIV-infected individuals and that the two diseases affect lung function and immunity, resulting in varied responses to infection and therapy.

HIV, Alcohol, and Lung Injury

In the last 10 years, evidence has shown that alcohol abuse significantly increases the risk of a serious life-threatening illness known as acute respiratory distress syndrome (ARDS). Characterized by alveolar cell barrier dysfunction and inflammation, ARDS can cause profound derangements in gas exchange with subsequent severe hypoxemia, respiratory failure, and death. Our group at Emory University first identified an independent association between alcohol abuse and ARDS [47]. Further preclinical studies have confirmed that chronic alcohol ingestion renders the lung susceptible to injury [48, 49]. Critical care outcomes have improved among HIV-infected persons due to advances in HIV therapy. ART has decreased morbidity and mortality [50–52], and overall ICU and hospital mortality associated with critical illness in HIV-infected individuals has decreased [53–57]. However, given the high prevalence of alcohol-use disorders in this population and the likelihood that these individuals will not adhere to ART and other HIV therapies, there is an increased likelihood of worsened outcomes in HIV-infected persons who abuse alcohol. Palepu et al. prospectively analyzed a database of 7,015 index ICU admissions at two teaching hospitals in Canada, of which 4.4 % where HIV infected and 56 % of these patients had a history of drug and alcohol dependence. In contrast, only 7.4 % of the HIV-negative patients had a history of drug and alcohol dependence. Using multivariate regression, the authors found that a history of alcohol dependence, regardless of HIV status, was not associated with hospital mortality after adjusting for age, sex, APACHE II scores (an index of acute and chronic health stresses), and acute overdose diagnosis. In contrast, HIV infection was

strongly associated with increased hospital mortality [58]. Further, other studies have shown that HIV infection independently increased mortality in patients with acute lung injury [59, 60].

HIV, Alcohol, and Outcomes

Alcohol use is well known to have comorbid effects on multiple medical diseases. Since many people living with HIV drink alcohol, it is likely that many comorbid diseases would be more common or worse in patients with both conditions. The Veterans Aging Cohort Study (VACS) has focused on the importance of alcohol use among persons with HIV infection. An early study of 881 HIV-infected subjects with strict definitions of alcohol use documented that hepatitis C, hypertension, diabetes, COPD, candidiasis, and bacterial pneumonia were all associated with alcohol use, and several of these diseases demonstrated a linear association with the degree of alcohol ingestion [20]. In an echocardiographic study of 196 asymptomatic HIV-infected individuals, alcohol use was found to be an independent risk factor of left ventricular diastolic function [61]. In a VACS study examining the incidence and severity of COPD in HIV-infected subjects, alcohol abuse as defined by ICD-9 codes, had an independent elevated odds ratio (1.46), although this did not reach statistical significance.

The rates and causes of mortality among HIV-infected individuals have evolved over the years. Although there are controversies about defining causes of death in HIV-infected persons [62], overall mortality rates have decreased markedly since 1996 with the advent of ART [52], and deaths specifically due to AIDS-defining illnesses and tumors have also declined [63, 64].

The precise influence of alcohol use on these trends in comorbidities and mortality is difficult to quantify. Relatively few studies have examined the independent effect of alcohol use on mortality, but it is likely that excessive alcohol use would affect deaths from liver disease, certain malignancies, pulmonary disease, and violence. An analysis of deaths in the CDC HIV Outpatient Study (HOPS) found a univariate hazard ratio of 1.20 (not statistically significant) for a history of all substance abuse, including alcohol [65]. An early study from VACS found that current or former alcohol use was not associated with increased mortality [66]. In contrast, excess mortality (HR 1.65) was described among HIV-infected patients diagnosed with substance abuse disorders, including alcohol dependence/abuse only, in a large study of deaths in the Kaiser Permanente Northern California health plan [67]. Consistent with those observations, an analysis of deaths in HIV-infected persons in France in 2005 found that alcohol consumption >50 g/day was independently associated with mortality [68].

Several studies from the aforementioned VACS have evaluated the "VACS Index" as a predictor of mortality. The original validation of the index included alcohol use, but this variable did not contribute to discriminatory value. This index now uses HIV biomarkers (CD4, HIV viral load, and AIDS opportunistic infections) and

non-HIV biomarkers (hemoglobin, transaminases, platelets, creatinine, and hepatitis C serology). It is likely that alcohol use is nevertheless a factor, especially in the non-HIV biomarkers, but as currently applied alcohol use does not independently predict mortality in this index [69].

A different approach to assessing causes of premature death is the calculation of expected years of life lost. This was assessed in San Francisco using death registry data and population estimates in 2003–2004. Using this method, the leading causes of premature death among men were HIV/AIDS and alcohol-use disorders [70].

Part 2: Pathophysiology of How HIV and Alcohol Affect Lung Immunity

Oxidative Stress

Oxidative stress refers to a disruption in the oxidant/antioxidant balance and is an important component of cell injury in both HIV- and alcohol-related disorders. Oxidative stress can be generated from a number of different mechanisms including relative oxidation of extracellular thiol disulfide pairs such as glutathione/glutathione disulfide (GSH/GSSG) and cysteine/cystine (Cys/CySS) and the generation of reactive oxygen species by enzymes such as NADPH oxidase [71, 72]. Chronic HIV infection alone has been known to cause significant oxidative stress systemically, both in preclinical [73–75] and clinical studies [76–78]. GSH levels were found to be decreased greater than 90 % in HIV transgenic rats compared to wild-type rats, and the GSSG/GSH ratios were increased threefold [75]. In response to endotoxemia induced by intraperitoneal lipopolysaccharide instillation, HIV-infected animals also have decreased GSH, increased nitric oxide metabolites, and increased superoxide anion production [73]. Clinically, plasma cysteine levels were significantly lower in HIV-infected subjects compared to control subjects [78], and several studies have shown that HIV-infected individuals have disturbances in GSH redox balance [79–81]. GSH deficiency has even been associated with impaired survival in HIV-infected subjects [82]. Chronic alcohol consumption is known to cause deleterious effects on host defense and immune responses. Alcohol toxicity has also been associated with oxidative stress and free radical-mediated injury [83, 84] and lipid peroxidation [85]. Additionally, alcoholics have also been shown to have considerably lower levels of other antioxidants, including vitamin E and selenium [86].

The compound effects of HIV infection and alcohol use with respect to oxidant/antioxidant balance have been noted in some studies. Bautista et al. demonstrated in a simian model of simian immunodeficiency virus (SIV) and alcohol intoxication enhanced reactive oxygen species formation by Kupffer cells and endothelial cells [87]. The liver is a major organ for clearance of microbial particles because of the preponderance of tissue Kupffer cells or macrophages. During HIV infection or prolonged alcohol use, Kupffer cells can produce a wide array of inflammatory

substances while also serving as scavenger cells. Alcohol can enhance the body's susceptibility to retrovirus infection, thus increasing the progression to AIDS [88]. Alcohol-induced alterations in signal transduction may also lead to host immune dysregulation. Helper T (Th) cells produce a number of cytokines that modulate the immune system. Retrovirus infection has been known to cause abnormal cytokine production, such as increases in IL-4 and IL-5, which results in a switch from a Th1 to a Th2 response. These changes have resulted in a progression to AIDS, defined by a decline in T-cell production [89]. In a murine model of AIDS, Wang et al. found that elevated levels of Th2 cytokines were further increased by alcohol consumption [90]. Additionally, release of IL-2, normally suppressed in murine AIDS, was further suppressed with alcohol [91]. These results suggest that chronic alcohol consumption could exacerbate the Th1/Th2 imbalance seen in AIDS. The role of antioxidant defense mechanisms to protect against oxidative stress is well characterized in the liver, and the effects of alcohol in altering oxidant/antioxidant systems in the liver are also well known. Although the exact effects of HIV on this system are unknown, there are several antioxidant defense systems that could be altered by both alcohol and HIV, including superoxide dismutase, catalase, and glutathione peroxidase. Chen et al. investigated the effects of chronic alcohol ingestion on antioxidant defenses in mice infected with retrovirus, which causes an AIDS-like disease [92]. They found that alcohol and murine AIDS alone caused specific effects on antioxidant mechanisms, but the combination led to more severe effects with respect to liver GSH levels and superoxide dismutase. The mechanism of this observed synergistic effect of alcohol and HIV infection is unclear at this time. It is possible that the virus leads to higher alcohol concentrations, as was seen in a previous animal study [92], and thereby potentiates alcohol toxicity or that HIV inhibits pathways of alcohol metabolism.

Specifically with respect to the lung, HIV infection and chronic alcohol abuse have been individually associated with oxidative stress. Our research group at Emory University has been instrumental in many studies examining the effects of alcohol abuse on lung immune function [48, 93–95]. Specifically, we have shown that chronic alcohol ingestion causes oxidative stress as reflected by GSH deficiency and epithelial barrier dysfunction in both preclinical and clinical studies. With respect to HIV infection, our group identified that chronic HIV transgene expression in animal models causes significant alveolar oxidative stress, as reflected by a greater than 90 % decrease in GSH levels, and a threefold increase in GSSG/GSH ratios [74]. Others have also shown similar results [73, 96]. In clinical studies, the results have been somewhat less conclusive. Pacht et al. reported that the concentration of GSH in the epithelial lining fluid was similar between HIV-infected and non-infected subjects [97]; however, these same authors demonstrated in a small study of 33 HIV-infected subjects that GSH levels in the epithelial lining fluid were significantly decreased over time [98]. Others have also shown that HIV-infected subjects had a deficiency of GSH in the lung [99, 100], but these studies have involved small number of subjects. Our group determined that chronic alcohol ingestion exacerbated defects in alveolar epithelial permeability and lung water clearance in HIV-1 transgenic rats [101]. Additionally, cultured alveolar epithelial cells from alcohol-fed HIV-1 transgenic rats had increased paracellular permeability

to sucrose. This dysfunction correlated with alterations in the expression of tight junction proteins. These effects appeared to be mediated by oxidative stress as alveolar epithelial barrier function and tight junction protein localization were restored by supplementation with procysteine, a GSH precursor.

Taken together, there is now considerable experimental evidence and corresponding circumstantial clinical evidence that dietary alcohol consumption after or prior to HIV infection exacerbates the immune dysfunction already seen in AIDS. Further investigation is necessary to evaluate these effects, particularly within the lung.

The Alveolar Macrophage

As mentioned previously, chronic alcohol abuse often coexists with HIV disease, and therefore, it is important to understand the interaction of these two immunosuppressing conditions. The host immune defense system in the lung against infection and other toxins involves both innate and adaptive immune responses. Our group at Emory University has studied macrophage function in both preclinical and clinical models of HIV and chronic alcohol abuse. We found that both transgenic expression of HIV-related proteins and chronic alcohol ingestion decreased granulocyte/macrophage colony-stimulating factor (GM-CSF) receptor membrane expression, a necessary factor for macrophage function [102, 103]. Further, treatment with recombinant GM-CSF can restore functions of both alveolar macrophages and the epithelium [104]. Alveolar macrophage phagocytosis of microbial pathogens is also decreased by HIV infection [105, 106].

Few studies have evaluated the additive effects of chronic alcohol ingestion and HIV on alveolar macrophage function. Tumor necrosis factor-alpha (TNF-α) serves as an important mediator in the pro-inflammatory response of the host to an invading pathogen [107]. Numerous studies have shown that neutralization of TNF-α impairs clearance of a variety of microorganisms including *S. pneumoniae* and *M. tuberculosis* [108, 109]. Stoltz et al. conducted a study to determine the effects of alcohol and SIV on alveolar macrophage TNF-α production and found that alveolar macrophages from SIV-infected nonhuman primates had a depressed response and that alcohol ingestion further suppressed the TNF-α response by approximately 50 % [110]. Because of the vital role of TNF-α in generating an effective immune response, these results suggest that alcohol abuse may further impair host immune defense in HIV-infected individuals. Others have also reported decreased TNF-α production by alveolar macrophages from subjects with HIV [111] and alcohol [112]. Nelson et al. utilized the macaque SIV infection model to examine the effect of chronic alcohol feeding on SIV burden during the course of *S. pneumoniae* infection and found that the chronic alcohol-fed macaques showed a prolonged increase in SIV RNA in their lungs [113]. Additionally, alveolar macrophages from these alcohol-fed animals had greater nuclear factor kappa beta (NF-KB) activation. This study suggests that chronic alcohol abuse results in increased SIV replication within the lung and it is possible that increased NF-KB activation is part of the mechanism.

Zinc

Zinc is an essential micronutrient that plays an important role in numerous biological processes and is the focus of an earlier chapter in this book. Specifically, zinc is crucial for immune function and the catalytic functions of ~300 enzymes, and its deficiency is an important driver of oxidative stress [114]. Studies have shown that alcoholism causes systemic alterations in zinc metabolism [115, 116]. Further, there is evidence that chronic alcoholism alters zinc bioavailability in the lung and may be an important mechanism by which chronic alcohol exposure predisposes individuals to pulmonary infection and acute lung injury [117, 118]. Recent experimental evidence has demonstrated that the HIV phenotype is characterized by a similar state of zinc deficiency and immune dysfunction within the alveolar space [119, 120]. This zinc-depleted state contributes to further immune dysfunction in a host that is already compromised by HIV. While there have been no studies to determine the combined effect of alcohol and HIV, it stands to reason that zinc deficiency and its consequences would be much more severe among HIV-infected individuals who suffer from alcohol-use disorders.

Part 3: HIV and Other Pulmonary Syndromes

Chronic Obstructive Pulmonary Disease

COPD is one of the most prevalent comorbid diseases in HIV-infected individuals and is diagnosed in 12–15 % under medical care [20]. As mentioned previously, COPD is more common in HIV-infected compared to non-infected persons, and this association is linearly associated with the degree of alcohol use. COPD presents at an earlier age, with fewer pack-years of smoking, and is more prevalent in HIV-infected individuals than in uninfected individuals [19]. In addition, a high HIV viral load and a low CD4 cell count were associated with an increased prevalence of spirometry-defined obstructive lung disease in a cohort at risk for COPD and HIV infection [121]. Unfortunately, no assessment of alcohol use was reported in this study. In parallel, other studies have suggested that HIV may also be associated with an increased risk for several different manifestations of airway and obstructive lung disease, including features of emphysema [122, 123], chronic bronchitis [124], nonspecific airway disease or bronchial hyper-responsiveness (such as is seen in asthma) [125, 126], and bronchiectasis [127]; nonspecific focal air trapping with decreased expiratory flow rates [128] and bronchial dilatation [129] have also been described. Taken together, the evidence suggests that HIV exacerbates the effects of smoking on the development of COPD, but at present there is no evidence that alcohol abuse adds to this risk.

Pulmonary Hypertension

Primary pulmonary hypertension was first described in HIV-infected persons in 1990 [130], and it has been found to be more common in HIV-infected compared to uninfected persons [131]. Improved hemodynamics and survival from pulmonary hypertension have been described in patients on ART [132]. In a cross-sectional study of 116 HIV-infected outpatients, echocardiographic manifestations of pulmonary hypertension were common and associated with respiratory symptoms, more advanced HIV disease, airway obstruction, abnormal diffusion capacity, and systemic and pulmonary inflammation [133].

Lung Cancer

Compared with the general population, lung cancer risk was found to be elevated in a cohort of almost 400,000 persons with HIV from 11 US regions [134]. This increased risk was found to be independent of smoking [135]. In a large VA study comparing HIV-infected to uninfected veterans, HIV infection was found to be an independent risk factor for lung cancer after controlling for potential confounders including smoking [136]. In this study, alcohol abuse, defined by ICD9 codes, was not independently associated with lung cancer. Outcomes for patients with lung cancer were initially found to be poor and related to low CD4 counts [137]. In the large Swiss cohort study, lung cancer was not clearly associated with immunodeficiency but was attributable mainly to heavy smoking [138]. A recent report found no significant difference in clinical outcome between patients with HIV and uninfected controls with non-small-cell lung cancer, including those with curative surgical resection in early-stage disease [139]. These data suggested that HIV status should not affect therapeutic decision making.

Summary

Alcohol abuse is a common problem among individuals living with HIV, and there is considerable experimental and clinical evidence showing that chronic alcohol use accelerates HIV progression and increases the risk of opportunistic and non-opportunistic infections. Alcohol abuse also decreases adherence to medical regimens including the consistent use of ART, which only further exacerbates the HIV infection. Alcohol abuse and HIV each imposes oxidative stress and zinc deficiency within the lung and together cause severe lung epithelial and macrophage dysfunction. Therefore, it is critically important that alcohol-use disorders be identified and treated whenever possible in the care of an individual living with HIV.

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Chapter 16
Maternal Alcohol Use and the Neonate

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Abstract Maternal alcohol use and abuse can have devastating consequences to fetal development and outcomes. Although there is widespread societal pressure, particularly within developed countries, against the ingestion of any alcohol during pregnancy, many women may drink heavily before they recognize they are pregnant or may continue to do so despite their awareness. Much of the focus among healthcare professionals and biomedical investigators has been on the fetal alcohol syndrome (FAS), more commonly now referred to as the fetal alcohol spectrum disorder (FASD), which in its most advanced form is manifested by craniofacial abnormalities and severe neurocognitive deficits. However, FAS and FASD are disorders in term infants, and it is now being recognized that maternal alcohol ingestion appears to impact the risk for both premature delivery as well as medical complications associated with neonatal prematurity. In particular, experimental and clinical evidence is beginning to elucidate the mechanisms by which maternal alcohol ingestion can increase the already significant oxidative stress within the neonatal lung and impair host immune functions. As a consequence, the premature neonate with significant exposure to alcohol in utero appears to be at an even greater risk of developing serious infectious complications. Further, experimental models suggest that maternal alcohol ingestion can impair prenatal lung development and, if these findings translate to the human condition, could thereby render the premature infant at increased risk for adverse complications including bronchopulmonary dysplasia and late-onset sepsis. This chapter provides a brief overview of the

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epidemiology of maternal alcohol use during pregnancy and highlights some of the experimental and clinical evidence that show that such use can have devastating effects on neonatal outcomes.

Keywords Fetal alcohol spectrum disorder • Pregnancy • Reactive oxygen species • Immunity • Lung • Infection

Maternal Alcohol Use During Pregnancy

Over the past several decades there has been a dramatic change in societal views regarding alcohol use during pregnancy. Specifically, public information campaigns by the federal government and its various healthcare agencies as well as by countless non-governmental groups have educated the American public about the serious implications of alcohol use during pregnancy. As a result, the vast majority of individuals in our society are now at least aware of the health concerns regarding maternal alcohol consumption. However, a variety of studies continue to show alarming evidence that the use of alcohol during pregnancy remains relatively common. For example, a large lifestyle survey across four states that evaluated women with a wide range of socioeconomic status determined that ~35 % of newborns were exposed to alcohol and that ~10 % had been exposed to cocaine [1]. In a subset analysis of a larger study designed to assess infant language development, we assessed maternal alcohol use during pregnancy via healthcare questionnaires in women at two large hospitals in metropolitan Atlanta, Georgia. We determined that 25 % (83/321) admitted to consuming alcohol during their pregnancy, with 20 % of those who did drink reporting consuming >3 drinks/occasion and 10 % reporting consuming >5 drinks/occasion [2]. Of note, the average socioeconomic status of those mothers who did report alcohol use during pregnancy was relatively higher than those mothers who did not report alcohol use during pregnancy, which contradicts the stereotype that alcohol use during pregnancy is more common among the social economically disadvantaged. Such increased alcohol use in older, more affluent pregnant women has been reported by other investigators [3]. These and other studies illustrate that maternal alcohol use remains common in our society despite the widespread recognition of the potential harmful effects on fetal outcomes. Further, due to the significant societal stigma associated with alcohol use during pregnancy these studies which relied on self-reporting of alcohol use likely underestimated the true extent of maternal alcohol use during pregnancy.

Although this chapter focuses on the neonatal effects of maternal alcohol use during pregnancy, continued investigations are warranted to further identify the detrimental effects of alcohol use on maternal reproductive health. This is highlighted by a recent evaluation by de Wit et al., who demonstrated an twofold increased risk of hospital-acquired infection, which included surgical site infection, endometritis,

urinary tract infection, sepsis, and pneumonia after cesarean delivery in women identified with alcohol-use disorders [4].

Identification of the alcohol-exposed pregnancy and its offspring continues to be problematic. Unlike biomarkers used in adult populations, there is no clear biomarker of alcohol exposure that can reliably identify the alcohol-exposed newborn [5, 6]. In nonpregnant adults, identification of patients who abuse alcohol has been standardized using the Alcohol Use Disorders Identification Test (AUDIT) [7] and the Short Michigan Alcoholism Screening Test (SMAST) [8]. Studies have assessed other biochemical blood markers such as carbohydrate-deficient transferrin and γ-glutamyl transferase in the context of pregnancy. Unfortunately the specificity and sensitivity of these routine biological tests remain less than optimal [9–11]. Therefore, the identification of the alcohol-exposed pregnancy and newborn often relies on maternal self-reporting of alcohol use during the pregnancy. Such reporting is fraught with uncertainty, particularly given the social stigma associated with alcohol consumption during pregnancy [12, 13]. Ultimately, maternal self-reporting of alcohol consumption during pregnancy continues to underestimate the scope of exposure, allowing alcohol-exposed pregnancies and exposed newborns to remain clinically undetected.

In this context, investigators have searched for objective and accurate biomarkers of maternal alcohol use [5, 14] including the determination of fatty acid ethyl esters (FAEE), a stable by-product of alcohol metabolism, in the meconium of neonates. Alcohol is metabolized by both oxidative and non-oxidative pathways [5], and FAEE are the product of the non-oxidative pathway where ethanol conjugates to free fatty acids [15]. With a prolonged half-life, FAEE have the potential to accumulate in body fluids and tissue for extended periods of time [16, 17] and as such have been described as a biomarker of alcohol abuse in adults [18]. The sensitivity and specificity of FAEE as a potential biomarker specifically for fetal alcohol exposure in newborn infants remain under study. In investigations pioneered by Bearer as well as Koren, significantly different levels of FAEE were found in the meconium of term infants exposed to alcohol in utero versus those not exposed [14, 19, 20]. In adults that abused alcohol, FAEE accumulated in the extractable hair lipids originating mainly from sebum and from the structural lipids of cell membranes [21, 22] when compared to controls. Evaluation of neonatal hair has yielded similar accumulations in alcohol-exposed newborns [23] outside of the newborn nursery [24]. This is particularly important given that accumulation of FAEE correlated with alcohol-induced neurodevelopmental disabilities seen at an older age in the exposed offspring [25].

Phosphatidylethanol (PEth), another potential biomarker of alcohol exposure, is a non-oxidative product of alcohol metabolism formed in lipid membranes via the action of phospholipase D. The phospholipid PEth holds great promise as a useful biomarker of alcohol exposure since it has been demonstrated to persist in blood samples of pregnant women 4–6 weeks after alcohol intake [26]. Furthermore, using blood spots obtained from neonatal heel sticks, measurements of PEth hold promise as a neonatal screen for in utero alcohol exposure [27].

Maternal Alcohol Ingestion and the Term Infant

Both the premature and the term infant are at risk for serious complications of alcohol exposure in utero. The best known and most extensively studied consequence of prenatal exposure to heavy alcohol use is the fetal alcohol syndrome (FAS). FAS is one of the most common forms of preventable neurocognitive development and function [28] and is characterized by distinctive craniofacial abnormalities (small head circumference, small eye openings, smooth philtrum, thin upper lip), growth retardation, and central nervous system damage [29]. However, these full manifestations are not evident in the majority of alcohol-exposed newborns [30, 31], and the diagnosis of FAS was unrecognized in 100 % of term newborns who were subsequently identified later in childhood as having neurodevelopmental deficits due to fetal alcohol exposure [30, 31]. As the breadth of the syndrome became progressively recognized, the problems associated with alcohol exposure in utero have been grouped and re-labeled as fetal alcohol spectrum disorders (FASD) or alcohol-related neurodevelopmental disorders (ARND).

Given the difficulty in identifying the alcohol-exposed pregnancy, the impacts of maternal alcohol use during pregnancy on other neonatal morbidities continue to be investigated. In a recent review of population-based health and mortality data, O'Leary and colleagues demonstrated an alarming sevenfold increase in sudden infant death syndrome (SIDS) and a doubling of non-SIDS infant death in infants whose mothers were identified as having an alcohol-use disorder during the pregnancy [32]. Unfortunately, our evolving ability to recognize the spectrum of problems that result from alcohol exposure to the fetus continues to outpace our ability to prevent and/or modify these consequences.

In addition to the effects on neurodevelopment, maternal alcohol exposure appears to have untoward effects on the risk of neonatal infections even in the full-term infant. For example, we evaluated neonatal infections in 872 singleton newborns (i.e., no multiple births) with gestational age $\geq$ 36 weeks and determined that infants whose mothers reported alcohol use, excessive drinking, or smoking in pregnancy were more likely to have an infection than infants whose mothers reported that they abstained from alcohol ingestion or cigarette smoking [2]. When we controlled for race and smoking, those infants that were small for gestational age and whose mothers used any alcohol had a 2.5-fold increased risk of infection and excessive alcohol use by the mother in these smaller infants increased the risk of infection three- to fourfold. In a multivariable logistic regression analysis controlling for low maternal income, smoking, and having a baby that was small for gestational age, we identified that excessive alcohol use during the second trimester increased the risk of newborn infection with an odds ratio of 3.7. Therefore, maternal alcohol ingestion may increase the risk of other potentially serious acute health problems in the postnatal period, even in full-term infants. These risks are far greater in the premature neonate however, and the rest of this chapter focuses on this uniquely vulnerable population.

Maternal Alcohol Ingestion and the Risk of Premature Delivery

With much focus on FAS and ARND in newborns born at term gestation, it is important to highlight the significance of maternal alcohol use on the premature newborn. Limited research has focused on the effects of alcohol exposure on the infant born prematurely with very limited knowledge as to the consequences of similar exposure on the vulnerable and developing preterm newborn. The need for such research was highlighted by recent studies which demonstrated a dramatic 35-fold increased risk of extreme premature delivery in mothers who drank alcohol during pregnancy [33].

Maternal alcohol use has been described to increase multiple risk factors associated with premature delivery. Chorioamnionitis confers a significant risk for preterm labor and premature delivery and also increases the risk of multiple adverse outcomes for premature newborns [34]. In multiple reviews, maternal alcohol use significantly increased the risk of chorioamnionitis, with odds ratios ranging from near 5 [35] to over 7 [36]. Placental abruption also increases the risk of premature delivery [37], and a large review of risk factors for placental abruption suggested that maternal alcohol ingestion increased the risk of placental abruption in adjusted analyses by over two-fold [38]. Finally, in several large database analyses, the risk of preterm delivery was significantly increased in women with a history of prior induced abortion, with a stronger association seen in women with a prior history of multiple induced abortions [39, 40]. When evaluated in one recent Canadian study, women undergoing induced abortion were more likely to consume alcohol [39]. Therefore, maternal alcohol use remains a risk factor for premature delivery; however, the alcohol-exposed premature newborn remains clinically undetected in the newborn intensive care units. Given this, the need for validated biomarkers of alcohol exposure in an already at-risk premature population is paramount. Without such biomarkers, our understanding of alcohol's effects on adverse outcomes suffered by the premature newborn population remains limited. Continued investigations are under way to delineate whether FAEE will be similarly useful and reliable as biomarkers of prenatal alcohol exposure in the premature population, as in term newborns and adults. We have yet to understand the full spectrum of the effects of prenatal alcohol exposure on common morbidities faced in the premature population such as infection with late-onset sepsis, bronchopulmonary dysplasia, necrotizing enterocolitis, and neurodevelopmental delays.

Maternal Alcohol Ingestion and the Risk of Infection in the Neonate

Infections and infection-mediated morbidity and mortality continue to cause significant health burden to the newborn infant particularly if the child was born prematurely [41–44]. Despite antibiotic therapy and modern neonatal intensive care, the

risk of bacterial infections remains disproportionately elevated in premature newborns and those born within minority groups [45, 46]. Bacterial infection in the premature population increases the risk of a variety of complications of prematurity including patent ductus arteriosus, necrotizing enterocolitis, bronchopulmonary dysplasia [45], and developmental delay [47, 48]. The growing premature newborn also remains at increased risk for significant morbidities from viral respiratory infections. Although immunization strategies such as Palivizumab target premature newborns and at-risk newborns with significant lung disease, the growing premature newborn remains at an increased risk for severe and often life-threatening respiratory syncytial virus (RSV) infection, particularly in the lower respiratory tract of the lung [49, 50]. Furthermore, the burden of severe influenza infections is unfortunately carried by former premature newborns and adversely affects their long-term prognosis [51, 52].

Although clinical research continues, data remains limited regarding the risk of infection in infants and children who have been exposed to alcohol in utero. Studies suggest that fetal alcohol exposure increases the risk of neonatal bacterial infection in the exposed offspring. In a small study of children diagnosed with FAS, lymphocyte abnormalities and increased bacterial infections such as meningitis, pneumonia, and otitis were described [53]. In our review of a term newborn population, excessive maternal alcohol use of greater than seven drinks per week significantly increased the risk of neonatal infection in the newborn nursery over threefold. This effect was most significant if the alcohol use occurred in the second trimester of pregnancy, suggesting that alcohol exposure adversely affected the developing neonatal immune system [2]. Drugs including alcohol also potentially increase the risk of maternal to fetal HIV transmission where there is a well-described association between alcohol abuse, use of other drugs of abuse, and acquisition and progression of HIV/AIDS among women [54].

Given the increased risk of prematurity with maternal alcohol use during pregnancy superimposed on the increased risk of infection in this population, further clinical data is desperately needed to fully determine the consequences of in utero alcohol on neonatal infections. We performed a small case–control analysis of very-low-birth-weight premature newborns (birth weight < 1,500 g) with maternal alcohol use identified via social work interviews. Maternal alcohol exposure significantly increased the risk of early-onset bacterial sepsis 15-fold compared to matched premature newborns without in utero alcohol exposure. Logistic regression analysis demonstrated a significant independent risk of early-onset bacterial sepsis with alcohol exposure, when controlling for chorioamnionitis and premature prolonged rupture of membranes [55]. Therefore, some evidence suggests that maternal alcohol increases the risk of infection in the newborn, but much investigation is still necessary to fully define the impact of maternal alcohol use on neonatal infection. Such investigations rest on the need for validated biomarkers of alcohol exposure in an already at-risk premature population. However, advances in animal models of fetal ethanol exposure support these clinical findings and suggest that in utero exposure alters multiple arms of innate immunity in the developing fetal lung.

Maternal Alcohol Ingestion and Lung Immunity

Understanding in utero ethanol's effects on the immune response of the developing neonatal lung has direct implications not only for infection risk but also for the relationship between the inflammatory state of the lung and long-term sequelae for the premature newborn such as bronchopulmonary dysplasia. Bronchopulmonary dysplasia has strongly been associated with a persistent influx of inflammatory cells and an elevated and sustained pro-inflammatory environment in the developing lung [56–60]. A failure to resolve this altered environment in the neonatal lung hallmarks this disease.

Viral mediated respiratory infections cause substantial injury to the former premature newborn population, particularly RSV and influenza. Emerging data from the developing animal exposed in utero to ethanol suggest that ethanol induced a persistent immune dysfunction that persisted into adulthood. Specifically, adult animals demonstrated impaired adaptive immunity and altered B cell responses resulting in increased risk and severity of influenza infection after in utero ethanol exposure [61]. These effects were exacerbated by additional alcohol exposure. Indeed, ethanol in utero may alter the hypothalamic-pituitary-adrenal axis resulting in augmented stress-induced immunosuppression and increased vulnerability to subsequent infectious illness—a form of fetal programming [62]. For the premature newborn and growing former preterm infant, in utero alcohol-induced alterations in endocrine function and immune competence remain to be defined.

Growing evidence suggests that in utero ethanol exposure deranges multiple arms of innate immunity in the developing lung. In sheep models, in utero ethanol exposure decreases the surfactant proteins (collectins) SP-A and SP-D [63, 64] which facilitate phagocytosis by alveolar macrophage and modulate dendritic and T cell immunity [65].

As the resident inflammatory cell in the lung, the alveolar macrophage provides the initial defenses against foreign and infectious particles and orchestrates the inflammatory process within the lung [66, 67], but these immune functions are impaired in the premature newborn [68, 69]. Alveolar macrophages are derived from peripheral circulating blood monocytes which constitutively move into the interstitial space of the lung and are also recruited in response to pro-inflammatory stimuli. These monocytic cells differentiate into mature alveolar macrophages in the alveolar space [66, 70] in a granulocyte–macrophage colony-stimulating factor (GMCSF)-dependent process [71]. Modulation of innate immune responses of the fetal monocyte by systemic conditions or exposures during pregnancy may subsequently affect the resident alveolar macrophage population and the inflammatory environment within the newborn lung [72, 73].

Zinc is the most widely studied microelement in infant feeding because it is an essential cofactor in approximately 300 enzyme-dependent processes involved in immunity, growth, cell differentiation, and metabolism [74, 75]. In studies of global disease burden for 2010, one of the key risk factors for death in early infancy was serious bacterial infections with links to zinc insufficiency [76–78]. Zinc is essential for

innate and adaptive immune responses [79, 80], and suboptimal concentrations result in an increased susceptibility to infection as well as exacerbation of existing infections [81]. For the newborn, there is an increased risk for suboptimal zinc concentrations if there are suboptimal maternal zinc pools. Alcohol abuse during pregnancy can initiate zinc sequestration by the maternal liver and result in low zinc pools at the time of birth [82, 83]. The resulting decreases in zinc are a relative risk factor for FASD, and zinc supplements may protect against some of the adverse effects of prenatal alcohol exposure [82, 83]. Once pregnancy is confirmed, abstinence from alcohol use may occur, but ~50 % of pregnancies are unintended [84] resulting in significant fetal alcohol exposure and risk of suboptimal zinc pools in the newborn. Relevant to respiratory immunity in the newborn, hospital stays during the first year of life are ~3 times longer for FAS infants than their controls (12.1 days vs. 3.9 days, respectively) with pneumonia being one of the main reasons for hospitalization [85]. In an adult model, chronic ethanol ingestion decreased the zinc levels in AMs due to decreased expression of zinc transporters [86, 87]. Equally important, dietary zinc restored zinc pools in the AM as well as phagocytosis. Whether suboptimal zinc pools in the newborn because of fetal alcohol exposure suppress the immune functions of the newborn or increase the risk of pneumonia remains to be determined.

Fetal Alcohol Exposure and Oxidative Stress

While most ethanol metabolism occurs through alcohol dehydrogenase in the liver, it can also be metabolized by mixed function oxidases such as cytochrome P450s which generate reactive oxygen species (ROS) [88]. Both pathways generate acetaldehyde as a by-product of ethanol metabolism. Although cytochrome P450 may minimally contribute to ethanol metabolism at baseline, chronic in utero alcohol exposure increases the activity of cytochrome P450 2E1 (CYP2E1) and its generation of ROS. With polymorphisms in alcohol dehydrogenase and P450s, there is considerable tissue variability in acetaldehyde generation and injury during fetal alcohol exposure [89, 90]. Other variables include the amount of alcohol and the period of gestation for which alcohol exposure occurs. For example, CYP2E1 activity significantly increases between gestational days 45 and 53 as organogenesis begins [91]. Acetaldehyde can also be generated in the placenta where CYP2E1 expression is related to alcohol consumption [92].

During development, alcohol-induced oxidative stress is sentinel in tissue injury in the placenta and multiple fetal tissues [93–102], including the lung [64, 103, 104]. As a result of ROS, cellular functions are altered because of oxidation of fetal proteins, lipids, and DNA [94, 105, 106]. Although normally neutralized by antioxidants, an imbalance between ROS generation and antioxidant detoxification amplifies the ROS burden and cell injury. In the developing newborn, the potential for excessive ROS generation is exacerbated because of a poorly developed antioxidant system representing less than 5 % of maternal activity [107, 108]. Therefore, the developing infant is particularly vulnerable to the adverse effects of the ROS

generated during fetal alcohol exposure. In proteins, the amino acids cysteine and methionine contain sulfur groups that routinely undergo multiple reversible oxidations [109, 110]. However, acetaldehyde or lipid peroxidation products can also react with accessible cysteine sites in a myriad of proteins and affect multiple cellular functions if the cysteine is located at a site critical for protein function [111]. For example, alcohol can inhibit neuronal cell adhesion by promoting oxidation of critical cysteine sites in the L1 neural cell adhesion molecule and blocking interaction within critical domains [112].

One major source of ROS comes during mitochondrial respiration where ROS are continuously released during oxygen consumption. With increased mitochondrial ROS generation, the antioxidant glutathione becomes depleted, thereby increasing mitochondrial derived ROS, oxidizing proteins essential for ATP synthesis, and increasing cell death through apoptosis, critical events for developing tissues [93, 98, 105]. Other sources of ROS include the multi-subunit complex NADPH oxidases (NOXs) [113] where alcohol exposure up regulates the different NOX isoforms as well as their regulatory subunits that control activity [105, 114]. NOX expression can be further amplified by ROS such as that derived from the mitochondria [115]. With inhibition of NOX activity, apoptosis in the ethanol-exposed embryos is attenuated suggesting that NOXs are a critical source of ROS and play an important role in ethanol-induced pathogenesis [114].

Availability of the antioxidant glutathione also plays a critical role in detoxifying alcohol-induced ROS, but extended exposure to alcohol and acetaldehyde depletes the cytosolic and mitochondrial pools of glutathione. This becomes particularly relevant to the developing infant where antioxidant systems are limited. In animal models of fetal ethanol exposure, glutathione levels [116] and enzymes that use glutathione for detoxification are attenuated [117]. However, there is heterogeneity in the distribution of glutathione, so not all cell types are equally susceptible to alcohol-induced ROS [106]. Heterogeneity in expression or polymorphisms in alcohol dehydrogenase, CYP2E1, and enzymes that use glutathione for detoxification would further contribute to variability in susceptibility to alcohol-induced acetaldehyde generation, glutathione depletion, capacity to remove toxic metabolites, and, ultimately, tissue injury in response to fetal alcohol exposure [90].

In animal models, fetal alcohol exposure decreases glutathione in the fluid lining the alveolar space and in the alveolar macrophages that reside within that fluid [103]. The physiological consequence of impaired capacity for alveolar macrophage phagocytosis is an increased risk of experimentally induced pneumonia in the newborn pup [104, 118]. However, dietary supplements of *S*-adenosylmethionine to the pregnant mouse prevented glutathione depletion, increased ROS, impaired phagocytosis by alveolar macrophages, and increase the risk of pneumonia. A central role for ethanol-induced glutathione depletion was further demonstrated by the ability of intranasal glutathione treatments to the newborn pup to improve macrophage phagocytosis as well as attenuate lung infections and dissemination of experimentally induced *Group B Streptococcus pneumonia* [104]. Thus, glutathione depletion in the lung caused by fetal ethanol exposure is central to the inability of the fetal lung to clear infectious particles and the increased risk of respiratory infections.

Summary

Despite the widely recognized risks of alcohol exposure in utero on the developing fetus, maternal alcohol consumption in our society remains relatively common, particularly before pregnancy is recognized. This is especially problematic for the premature neonate, as maternal alcohol use may increase the risk of premature delivery by as much as 35-fold. The premature neonate is already at high risk of serious complications including sepsis and bronchopulmonary dysplasia. Experimental models have identified that alcohol exposure in utero likely exacerbates these conditions. In addition to maintaining a strong public health campaign to educate women (and men) of the dangers of alcohol use during pregnancy, we must intensify our efforts to identify those women who are drinking when they are pregnant and provide nonjudgmental and supportive assistance in helping them maintain abstinence for the sake of their health and their baby's. In parallel, we need to refine our ability to use biomarkers to detect significant alcohol exposure in utero in neonates and develop therapies that can decrease their risks of serious complications.

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