NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCHOLISM
REPORT TO THE EXTRAMURAL ADVISORY BOARD

NIAAA PLAN FOR HIV-RELATED BIOMEDICAL RESEARCH:

STRENTHENING ALCOHOL AND HIV / AIDS BIOMEDICAL RESEARCH

October 4-5, 2006

BRIEFING INFORMATION

I. AIDS EPIDEMIC
OVERVIEW AND INTRODUCTION

A. Dimensions of the Epidemic

HIV and sexually transmitted diseases (STDs) are among the most common infectious diseases. There are an estimated 40,000 to 60,000 new cases of HIV infection per year in the United States. The rate of growth in new cases is particularly high among young women (who now account for 47% of new cases) and among young adults ages 13-29 (who now comprise 39% of new cases). New HIV infections also appear to be resurging among young gay and bisexual men nationwide, and may be focused within party-going and substance-using cohorts. (CDC MMWR 2004) The U.S. Centers for Disease Control and Prevention (CDC MMWR 2004) reports that an estimated 850,000–950,000 persons in the United States are living with human immunodeficiency virus (HIV), including 180,000–280,000 who do not know they are infected. To examine trends in diagnosis for 2000–2003, the CDC analyzed HIV and acquired immunodeficiency syndrome (AIDS) together as HIV/AIDS (i.e., HIV infection with or without AIDS), counted by the year of earliest reported diagnosis of HIV infection. From 2000 to 2003, in 32 states that used confidential, name-based reporting of HIV and AIDS cases for >4 years, the overall annual rate of diagnosis of HIV/AIDS remained stable. However, rates among non- Hispanic black females were 19 times higher than rates among non-Hispanic white females, underscoring the need for continued emphasis on programs targeting females in racial/ethnic minority populations, their partners, and their families to reduce the number of cases of HIV/AIDS.

GLOBAL AIDS PANDEMIC, AS OF THE END OF 2005

- More than 40 million people worldwide are living with HIV/AIDS;
- Approximately 2.3 million are children under the age of 15 years;
- About half of the infected adults are women;
- An estimated 4.9 million people (adults and children) acquired HIV in 2005;
- The global HIV/AIDS epidemic killed more than 3.1 million people in 2005; and
- More than 2.5 million people have died since the beginning of the epidemic.

Source: UNAIDS

AIDS is one of the leading causes of death among men and women between the ages of 25 and 44, many of whom became infected as young adults. Although deaths from AIDS are beginning to decline, the characteristics of these deaths are shifting in response to chronic disease management practices using highly active antiretrovirals (and their toxic interactions with alcohol and other drugs). In 2004 an estimated 14,000 deaths in the US were directly attributed to AIDS-related diseases, as compared to approximately 40,000 in 1996. However, individuals now live longer with effective treatment of AIDS, and mortality is attributed to other non-AIDS-related diseases such as liver failure and lung disease. Internationally the scope and impact of the AIDS epidemic is growing rapidly. Limited epidemiological information makes it difficult to accurately estimate incidence

and prevalence rates in developing countries. However, the overall level of infection is currently estimated at approximately 45 million and will surpass 65 million by 2010. (World Health Organization, 2004) Increased incidence rates can be found in some of the countries with the highest per capita drinking rates and in specific heavy drinking populations at risk for HIV in these countries (e.g. Russia, India, China and South Africa). [AIDS Epidemic Update December, 2004]

Drinking alcohol increases both the risk for infection with HIV and the morbidity and mortality of patients who progress to AIDS, the clinical manifestation of HIV infection. Both animal and clinical research point to intervening biological and biomedical-related processes as explanations for accelerated progression of AIDS. These underlying processes are poorly understood. Identifying these mechanisms is important to predict the course of the illness for individuals who have misused alcohol in the past and may continue to drink while infected.

It is increasingly clear that both the direct and indirect effects of alcohol misuse contribute to HIV treatment failure and lost years of life. These outcomes are important at both the individual and population levels of analysis and must be considered in the development of effective interventions. While problem drinking in the general population impairs host immune-mediated responses and causes alcohol-related tissue and organ damage, these effects may also be attributed to the toxicity of AIDS medications among AIDS patients who drink. In particular, the research presented here and work that is in progress support the conclusion that perhaps no level of alcohol consumption is "safe" once individuals are infected with HIV. However, medical care providers are often unaware of the acute and chronic interactions of alcohol with HIV/AIDS progression and treatment, and do not take this into account when making clinical care decisions.

B. Context for Research: Major Themes of the NIH Plan

(Excerpted from NIH Plan for FY2007)

The FY 2007 NIH research agenda continues the following overarching themes: a strong foundation of basic science; research to prevent and reduce HIV transmission, including **vaccines, microbicides, and behavioral interventions**; research to develop better therapies for those who are already infected; international research, particularly to address the pandemic in developing countries; and biomedical and behavioral research targeting the disproportionate impact of AIDS on minority populations in the United States. In particular, this budget request places highest priority on the discovery, development, and preclinical testing of additional HIV vaccine candidates. The evaluation of an AIDS vaccine will require extensive testing in the United States and in international settings where there is a high incidence of HIV. High priority is placed on funding to move promising vaccine candidates into large-scale clinical trials to evaluate the potential for effectiveness.

The Plan establishes the NIH AIDS research agenda in the following Scientific Areas of Emphasis: Natural History and Epidemiology; Etiology and Pathogenesis; Therapeutics; Vaccines; and Behavioral and Social Science. The Plan also addresses the cross-cutting

areas of: Microbicides; HIV Prevention Research; Racial and Ethnic Minorities; Women and Girls; International Research; Training, Infrastructure, and Capacity Building; and Information Dissemination. The key priorities for the two research areas most directly related to NIAAA biomedical research interests, Natural History and Epidemiology and Etiology and Pathogenesis, and directions for future research are summarized below.

Alcohol Consumption

Alcohol consumption likely plays a pivotal but incompletely defined role in HIV viral replication, disease progression, potentiation of comorbid conditions, and increased frequency of adverse medical events from treatment with antiretroviral regimens. These findings have clear implications for prevention and treatment research in those countries that have been regularly employing medications for the treatment of AIDS and those that are beginning to develop the capacity to distribute and use these drugs. **As has been continually emphasized by major world health organizations both in the US and abroad, improved prevention strategies should be the primary focus for stopping the spread of HIV, and effective treatment is part of a comprehensive prevention plan to reduce rates of infection.** Effective treatment for both alcohol and HIV/AIDS leads to reductions in viral load, viral mutation, and subsequent infectivity.

Table one, compiled by Braithwaite et al., (2007) presents a summary of the 1) prevalence of recent alcohol consumption; and 2) prevalence of hazardous alcohol consumption for multiple domestic HIV+ samples. Recent Drinking ranged from 37% to 68%. . Prevalence of hazardous drinking ranges from 5% to 28%. International samples from resource-poor countries have reported substantially higher rates in excess of 80%. AUDIT scores have also indicated problem drinking in as high as 60% of HIV+ individuals. This range of drinking indicates that samples of HIV+ patients vary over a wide range. Presumably, those that are receiving AIDS medications have also been warned about continued drinking. These findings indicate that drinking may be problematic for some individuals before, during, and after infection and may shape the context for treatment with medications when they are available.

C: Table 1. Prevalence of alcohol consumption among United States HIV+ samples in the era of Highly Active Antiretroviral Therapy. (From Braithwaite et al, 2007). See Recent Publications, Appendix C.

Author	N	Year	Study	Prevalence of *recent* alcohol consumption	Prevalence of *hazardous* alcohol consumption
Braithwaite (ACER05)	2,762	2005	Veterans Aging Cohort Study (VACS)	46%	9%*
Tucker (03)	1,910	2003	HIV Cost and Services Utilization Study (HCSUS)	52%	14%*
Lucas (02)	695	2002	Hopkins	NR	5%
Kleeburger (01)	539	2001	Multicenter AIDS Cohort Study (MACS)	NR	6%
Samet (03)	349	2003	Boston	43%	19%
Stein (05)	262	2005	Brown	48%†	28%†
Cook (01)	212	2001	Pennsylvania	46%	19%
Golin (02)	140	2002	Adherence and Efficacy to Protease Inhibitor Therapy (ADEPT)	37%	NR
Arnsten (01)	67	2001	Bronx HIV Epidemiologic Research on Outcomes Study (HEROS)	NR	22% ‡
Chesney (00)	65	2000	Adult AIDS Clinical Trials Group) (AACTG) (AACTG)	68%	NR

NR = Not reported

* Only considers those hazardous drinkers who are also binge drinkers (>=5 drinks in at least one day during previous month); therefore these are likely underestimates.

† All individuals had a history of alcohol problems.

‡ "Alcohol use more than several days per week" was used as proxy for hazardous alcohol consumption

II.

NIAAA ALCOHOL AND HIV/AIDS BIOMEDICAL RESEARCH

II NIAAA Alcohol and HIV/AIDS Biomedical Research

A. Introduction and Background

Research studies are beginning to elucidate the influence of alcohol use, abuse, and dependence on the systemic biological changes observed during the course of HIV infection and treatment. Data from these studies are of particular clinical significance because heavy and sustained alcohol consumption can change the physiology and biology of virtually every cell in the body, thereby modifying the function of components of the digestive tract, immune system, cardiovascular system, endocrine system, reproductive system, brain and central nervous system, and musculoskeletal system.

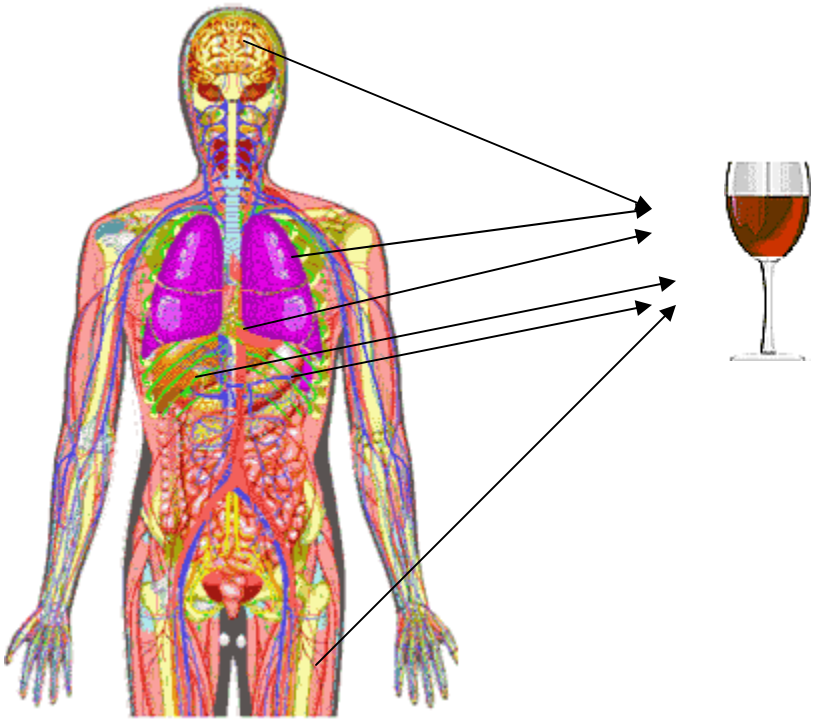


Figure 1: Multiple Impact of Alcohol on Tissue and Organ Systems

Thus, the biomedical consequences of alcohol abuse or dependence on HIV infection, transmission, pathogenesis and treatment are important research priorities. The AIDS Research Center (ARC) located in the Louisiana State University Health Sciences Center (Nelson, 2004), the Veterans Aging Cohort Study, an NIAAA Cooperative Agreement (Justice, 2004), and the other studies incorporating accurate alcohol measurement (e.g., HIVLIVE, Samet, 2004) represent a comprehensive approach to understanding these disease issues in the context of alcohol use and complex disease comorbidities. Emerging research on the neurological sequelae of AIDS infection is also garnering attention (Pfefferbaum, 2002), and new directions focusing on vaccine and microbicide use along

with already successful behavioral strategies will fully complement HIV/AIDS preventive intervention strategies.

Alcohol Use and Immune Response

Both acute and chronic alcohol use can affect the immune system at the level of innate or acquired immune responses. The humoral and cellular components of the specific immune system can be equally damaged by alcohol use, emphasizing the impact of alcohol as a modulator of host defense. Impaired immunity in patients with chronic alcohol use has long been described in the medical literature. Studies investigating lymphocytes and lymphocyte subpopulations in chronic alcoholics have consistently shown decreased lymphocytic cell numbers in the circulating blood. These lymphocytes have also been reported to respond abnormally to in vitro stimulation by mitogens or antigens, suggesting an adverse effect on their capacity to react appropriately to infection.

In addition to the alcohol-induced decreased number of lymphoid cells, impaired proliferation response has been described, suggesting that ethanol-exposed lymphocytes have a reduced capacity to undergo proliferation and differentiation in response to an antigenic challenge. Research demonstrating the adverse effects of alcohol on immune system function is consistent with clinical evidence of an increased incidence of infections among alcoholics. Chronic alcoholics are more prone to infections with a variety of pathogens and have decreased ability to fight against infections. Clinical findings support a correlation between excessive alcohol consumption and infections with certain extracellular (e.g., *Streptococcus pneumoniae*) and intracellular (e.g., *Mycobacterium tuberculosis*) bacterial infections, and with the progression of some bacterial infections to sepsis. The increased incidence of infections caused by intracellular bacteria (i.e. Mycobacterium tuberculosis [tuberculosis] and Listeria monocytogenes [meningitis]) among alcoholics is explained in part by impaired phagocytic function.

The combination of alcohol abuse and hepatitis B in a patient is more detrimental than either of the two conditions alone. About 10% to 35% of heavy drinkers develop alcoholic hepatitis. Hepatitis B-infected alcoholics have a greater risk of developing cirrhosis and hepatocellular cancer. Gender also plays a role, with women who abuse alcohol at greater risk for developing alcoholic hepatitis than men. **It is clear that effective vaccines to reduce the risk of infection in alcohol abusers are critically needed. However, it has been shown that vaccine efficacy is diminished among people who abuse alcohol.**

Viral Replication

In the past, researchers (Bagsara, 1996) demonstrated that alcohol could impair white blood cell responses to HIV. A provocative study that warrants replication found that a single drinking episode depressed certain immune responses of white blood cells taken from healthy volunteers. (Bagsara, 1996) In addition, white blood cells isolated after this drinking episode were more susceptible to HIV infection than were cells isolated from subjects who did not drink, hinting that even occasional alcohol consumption may

increase the likelihood of infection upon exposure to HIV. Subsequently, researchers have focused on understanding the role of viral replication in both laboratory cell cultures, including neural cells, and in animal models which focus on mucosal membrane response. (Bean, 2001; Liu, 2003)

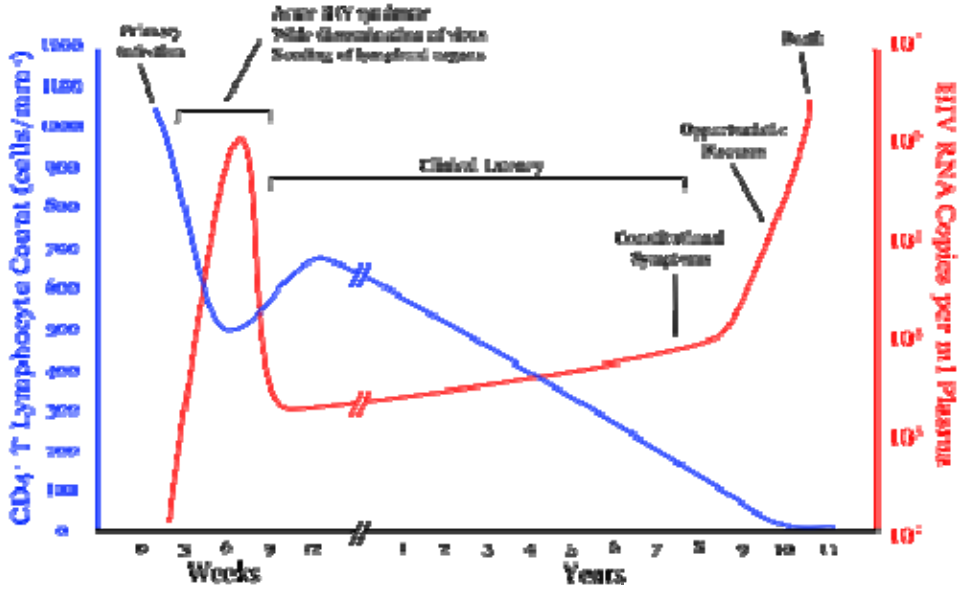


Figure 2: Viral life cycle: Early Infection to Death – Researchers are focusing on "set points" early in the life cycle of the virus. These set points may be affected by alcohol use and determine the course of disease. (Bagby et al, 2006). See Recent Publications, Appendix C.)

The pathologic consequences of HIV are a function of the completion of the life cycle of the virus. (NIAAA, 2004) HIV-1 has a complex viral life cycle that utilizes 15 distinct proteins in specific functions, some of which may interact with alcohol. (Figure 3) They are: Gag and Env structural proteins, capsid proteins, nucleocapsid, SU and transmembrane proteins, Pol enzymes, reverse transcriptase, integrase, gene regulatory proteins Tat and Rev and accessory proteins Nef, Vif, Vpr and Vpu. Elevated levels of HIV transcription are regulated, among other things, by nuclear factor kappa B, which is functionally dependent on Tat activation. Thus, cell activities unrelated to HIV infection-inducing signal transduction pathways and ultimately up-regulating transcription factors can enhance HIV transcription. Oxidative stress and certain interleukins have been demonstrated to enhance HIV replication through these pathways. A specific cell activity unrelated to HIV infection is cell activation via alcohol exposure. As observed in alcoholics, elevated levels of TNF contribute to the activation of NF-κB. (Gonzalez-Quintela, 2004; Zeldin, 1996) NF-K B, on the other hand, influences HIV promoter activity. (NIAAA, 2004).

Figure 3: HIV Virus Proteins

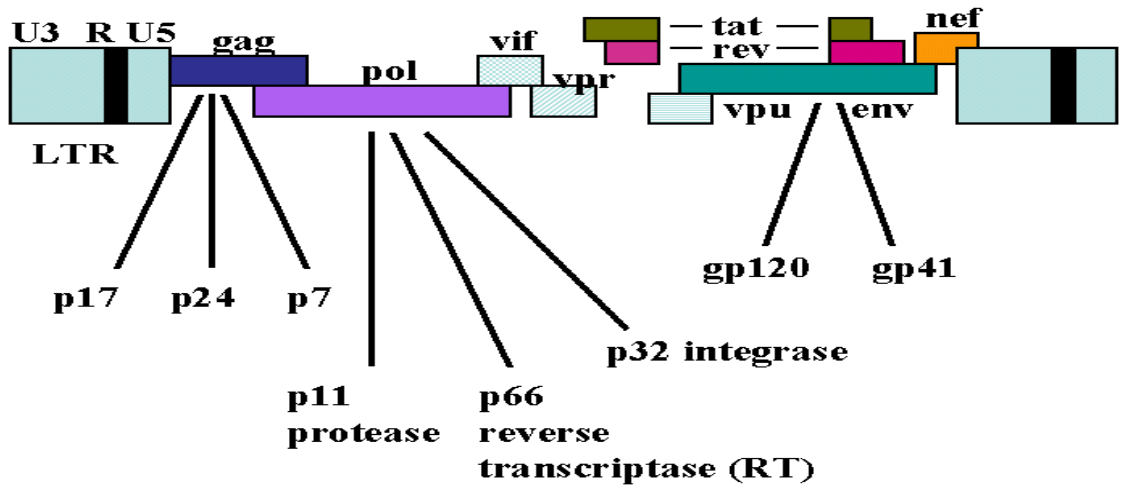


Figure 1

The key questions for alcohol/HIV researchers are, "Does alcohol modify expression of HIV proteins or the cellular biology of infected cells to promote or inhibit HIV replication via NF-K B or any other mechanism? Is alcohol a viral "adapter" that can influence and/or modify cellular functions to enhance the replicative capacity of the virus?" Conflicting results have been obtained by those studying HIV replication in isolated peripheral blood mononuclear cells (PBMC). Bagasra and colleagues reported increased HIV-1 p24 levels *in vitro* in infected peripheral blood mononuclear cells from individuals after a one-dose acute alcohol infusion or binge drinking. (Bagasra,1996) In a different experimental design , no consistent increase in HIV replication after exposure to alcohol was shown. (Fitzpatrick, 1995) Further studies have recently shown that even alcohol concentrations as low as 0.25% increase the number of peripheral blood lymphocytes expressing CXCR4, thereby increasing viral entry 5 to 10 fold. (Bean, 2001; Liu, 2003) Thus, alcohol may facilitate enhanced viral infection by increasing the availability of HIV-1 co-receptor, and the increased intracellular cAMP may also facilitate its replication. Additional studies are needed to resolve the controversy in the field. (Liu, 2003)

Differing research approaches could be used to determine whether alcohol consumption enhances HIV replication. (Pomerantz, 2004) The first could be a clinical study of alcohol consumption and viral load over time. A positive correlation or direct relationship would provide important clinical data to inform patient management. Determining the mechanism of interaction between alcohol and HIV would likely involve molecular studies of the HIV genome to determine the presence of alcohol binding and/or inducible response elements. Such genomic sequences, on exposure to ethanol or its

metabolites, could enhance viral replication. This enhancement could be through the recently described (Rampalli, 2003) NF-κβ activation/ augmentation of the HIV promoter, LTR, or other regulatory gene (s), such as Tat or Rev. A second approach to determining the role of alcohol in HIV replication is to examine the action of alcohol or its metabolites on tissue-specific cellular metabolism.

Animal Models

The animal model that most closely resembles human infection with HIV is SIV infection of rhesus monkeys. (Nelson, 2004) SIV is a lentivirus that is genetically related to HIV. SIV is T cell tropic and infects both lymphocytes and macrophages, inducing an immunodeficient state that correlates with the depletion of $CD4^+$ lymphocytes. Infection with SIV results in three stages comparable to HIV human infections (see figure 1): (a) acute infection characterized by high viremia, fever, lethargy and dermal rash; (b) asymptomatic stage with anti-SIV antibody and a decline in CD4 cell count;and (c) AIDS, characterized by substantial CD4 cell depletion and opportunistic infections. Beyond these similarities, an additional advantage of the use of the primate animal model is the ability to establish and monitor specific parameters related to alcohol consumption and HIV infection. These include time and route of infection, timing and quantity of alcohol consumption, assessment of nutritional and behavioral variables, as well as characterization of systemic and organ-specific pathogenesis. Limited data are available using this model (due to a variety of cost and care factors for monkeys; personal communication, Steve Nelson) and studies are needed to determine the interactions among alcohol, immune function, host SIV infection, and disease progression in nonhuman primate infection.

A recent study in *Alcoholism: Clinical & Experimental Research* (Bagby, 2003; in press) used simian immunodeficiency virus (SIV) infection of rhesus monkeys to examine the combined effects of chronic, binge alcohol consumption on the primary stage of SIV/HIV infection. Researchers found that alcohol consumption may increase host susceptibility to SIV/HIV infection. This study had two primary purposes: 1) to develop an animal model to study the interactive effects of alcohol on HIV disease transmission, pathogenesis, progression and anti-viral therapy; and 2) to examine the effects of alcohol consumption on what is called the 'primary stage' of infection. This stage is extremely difficult to study in humans because it is rare to be able to identify infected people this early.

The investigators adapted the primate model using SIV, which infects rhesus monkeys in the same way that HIV infects humans and produces a disease that is very similar to the human disease. Approximately one week after SIV infection, there was a 64-fold increase of the SIV virus in the blood of the alcohol-treated monkeys compared to the sucrose-treated monkeys. The researchers hypothesized that more cells are infected with virus at this early stage or that infected cells are producing more virus; i.e., that alcohol either increased the number of susceptible cells or increased the infectivity of cells. Alcohol consumption also enhanced lymphocyte turnover (as assessed by expression of the cell cycle protein marker Ki67) in SIV-infected monkeys during the early stage of infection, which may have contributed to the observed increase of virus in the blood.

Immunosuppressive Effects of Alcohol

Clinical Evidence

The physiochemical characteristics of alcohol allow it to interfere and damage most organ systems. In addition to the well-established relationship between alcohol and liver disease, alcohol may increase morbidity and mortality through its impact on immune system function. (Cook, 1998 and Watson, 1994) The clinical manifestation of immune changes caused by alcohol consumption are evident in drinkers, who exhibit increased susceptibility to infectious diseases such as respiratory infections and sepsis. (Cortese, 1992; Esposito, 1984; Jerrels, 1994; Manson, 2004) Infections that may lead to septicemia in the alcoholic include pneumonia, urinary tract infections, and bacterial peritonitis. (NIAAA, 2004) Alcoholics have twice as high a risk for pneumonia-related mortality as non-alcohol users. (Cortese, 1992; Esposito, 1984) The incidence of tuberculosis is also significantly increased by alcohol consumption. (Cook, 1998; Friedman, 1987; Manson, 2004) In animal models, alcohol-consuming mice not only had significantly higher lung organism burdens, their lymphocyte proliferation and production of gamma interferon were decreased. Researchers from Emory University (Holguin, 1998) have reported a number of abnormalities among alcohol users, including impaired alveolar type II pneumocyte function, decreased surfactant production, reduced barrier integrity, and increased apoptosis that could increase the risk of lower respiratory infections in alcohol users. (Kovacks, 2004) Thus, infections in ethanol-consuming individuals who drink large amounts of alcohol are both more frequent and more severe (Cook, 1998), in part because of ethanol-induced dysregulation of the immune system response. Further research is needed on the effects of alcohol on susceptibility to HIV infection, including the relationship of alcohol use-related host defense impairment to infection with opportunistic pathogens such as *Mycobacterium tuberculosis, Streptococcus pneumoniae, Pneumocystis carinii* and hepatitis C (HCV).

It is also important to consider frequency and duration of exposure to alcohol in relation to infection, as well as other host characteristics impacted by alcohol abuse, such as nutritional status, that can influence susceptibility to AIDS-related illnesses. Alcohol-induced malnutrition can further compromise the human immune system. (Watzl, 1993) Heavy alcohol abuse is associated with high caloric intake derived from alcohol and inadequate intake of protein, vitamins, and minerals, creating a malnutrition/wasting – type syndrome. (Watzl, 1993) Thus, alcoholics may not ingest adequate levels of vitamins and trace elements, such as zinc, iron, selenium, and magnesium, necessary for maintaining a competent immune response.

Laboratory Evidence

Chronic and even acute alcohol consumption results in significant changes in the immune system of experimental animals and humans. (Szabo, 1997; Brodey, 1994) A variety of short and long-term alcohol-induced effects on both cell mediated and humoral immune response have been described (Szabo, 1997; Miguez, 2001; Nair, 1994; Petrovick, 1996), with alcohol exposure linked to impaired host defense through a combination of cellular

defects, altered cytokine production, and oxidative stress. (Dominguez, 2001; Isaki & Kresina, 2000; Laso, 1999; Manso, 1997; Starkenburg, 2001; Wang, 1997)

Studies in alcohol users have consistently shown decreased lymphocyte numbers accompanied by impaired function. (Roselle, 1992; Jerrels and Sibley, 1996**)** Although the mechanism for an ethanol-induced decrease in lymphoid cell number is yet to be defined, reduced antigen presentation and subsequent decreased antigen-dependent T-cell proliferation may be involved. (Brodie, 1994; Szabo, 1999) In a chronic alcohol-consuming animal model, a defect in antigen presentation occurred during the cognitive phase of the immune response when antigen presenting cells (APC) engage uncommitted T helper $CD4^+$ cells. (Szabo, 1999) This defect, however, could not be reproduced when alcohol exposure occurred after the presentation and recognition steps. Specific to HIV, researchers from the University of Nebraska (Haora, 2004) recently demonstrated that elevated ROS following ethanol exposure decreased proteosome activity, and that this impairment could be restored by anti-oxidant supplementation. The data support the notion that HIV-1 infection and alcohol may work in concert to affect antigen presentation. (Haora, 2004) An additional suggested mechanism is programmed cell death, known as apoptosis. (Szabo, 1995; 1997).

Alcohol effects on T cell proliferation are dose-dependent and seem to be associated with inhibition of early signaling events of calcium mobilization and/or decreased interleukin production. (Brodie, 1994) Even acute alcohol intoxication can suppress inflammatory responses in normal subjects. (Dingle, 1997; Szabo, 1999) Recent findings indicate that the ethanol-suppressed response is mediated through Toll-like receptors (TLRs). (Dai, 2005) Current research on cytokine imbalance produced by alcohol is leading to new insights into the regulation of the immune system in alcoholics. The nature of the APC-T helper cell interaction helps to determine the effector response; i.e., cell- mediated (Th1) or humoral (Th2). Cytokine expression after exposure of normal human monocytes and murine splenic cells to alcohol is shifted toward Th2 dominance. (Laso, 1999; Wang, 1997) This polarization to Th2 is further enhanced in alcoholics, since levels of pro-oxidants such as glutathione, which plays an integral role in determining the Th1/Th2 maturational pathway of an immune response (NIAAA, 2004), are depleted in alcoholics. These experimental observations are of concern in HIV/AIDS, since high levels of TH2 interleukins have been associated with increased oxidative stress-induced damage and increased viral replication, as well as increased prevalence of opportunistic infections. (Isaki & Kresina, 2000; Laso, 1999; Szabo, 1999) In simian immunodeficiency virus (SIV)-infected animals, alcohol has been shown to suppress TNF-α and lead to increased susceptibility to secondary infections. (Stoltz, 2000)

Alcohol, HIV Infection and the Central Nervous System (CNS)

Higher rates of alcohol use are often reported among HIV+ individuals when compared to the general population. (Penkover, 1995; NIAAA, 2004) The combination of heavy alcohol use and HIV infection is also associated with increased neuropsychiatric impairment. Whether alcohol interacts with HIV neuroinvasion to increase synergistic cell death is under investigation. Neuropsychological testing indicates, however, that

there is decreased motor and visuomotor speed and generally poorer executive functioning. In addition, heavy alcohol use and poor executive functioning is associated with reduced adherence, suggesting that planning functions impacted by the synergistic interaction may mediate behaviors such as adherence.

In addition to causing major dysregulation of the immune system, HIV infection profoundly affects the CNS. Viral invasion of the brain has been documented as early as two weeks post-infection, a time well before seroconversion can be determined. (NIAAA, 2004) Autopsy reports have confirmed neuropathological abnormalities in as many as 90% of patients with AIDS. (Meyerhoff, 2002) Consistent with these observations of CNS damage, HIV-associated cognitive/motor complex, characterized by psychomotor slowing, memory deficits, and behavior changes, is thought to afflict between 15% and 40% of AIDS patients, and to be the clinical correlate of HIV encephalitis.

Despite the widespread recognition of the devastating effects of HIV on neural tissue and brain function, the mechanism(s) underlying these pathologies remain unclear. Penetration of the virus into the CNS arena appears to be critical, however, as neurobehavioral deficits correlate with viral load. Mounting evidence points to the ability of HIV or HIV-infected mononuclear cells to gain access to the CNS compartment by penetrating the blood-brain barrier (BBB). The cerebral microvessel endothelium is the major cellular element of the BBB and comprises the primary limitation to passage of substances from the blood to the brain. Brain Microvessel Endothelial Cells (BMECs) possess unique features that distinguish them from cells of peripheral endothelium, and these may significantly limit the paracellular flux through the BBB, posing a major impediment to invasion of the brain by both microorganisms and circulating leukocytes. The lack of fenestrae in BMECs, as well as the presence of specific membrane-associated transport systems, further restricts the transcellular movement of materials from blood to brain. (NIAAA, 2004) It stands to reason that modulation of any of these BBB properties could significantly impact the ability of HIV to enter the CNS and cause destruction of neural tissue.

While many factors could potentially alter BBB integrity and function and thus facilitate HIV access to the brain, particular attention should be given to alcohol. Alcohol has been linked to increases in BBB permeability to various tracers. Using proton magnetic resonance imaging (MRI), Thomsen demonstrated in humans that alcohol induces transient opening of the BBB. Thus, alcohol has the potential to heighten susceptibility to and progression of HIV-related CNS disease. Potential routes by which alcohol may facilitate HIV entry into the brain include augmented expression of pro-inflammatory cytokines, modulation of membrane permeability and inter-endothelial junctions, and stimulation of viral replication. (NIAAA, 2004) Alcohol may also act in concert with HIV-1 proteins (gpl20, Tat) and/or cytokines present in the circulation of HIV-1 infected patients to accelerate HIV-1 disease progression. In support of this concept, interaction of Tat protein with alcohol has been confirmed in an animal model. Belmadani and colleagues (2001), however, suggested that moderate ethanol consumption is neuroprotective by reducing excitotoxicity induced by Gp120. A study examining the metabolic effects of advanced HIV infection and alcohol use using breakdown products

of membrane-molecules and magnetic resonance spectroscopy studies provides evidence that chronic alcohol use may exacerbate some metabolic injury in the brains of HIV-infected individuals. (Meyerhoff, 2003) Given the widespread use of alcohol and rapidly growing HIV-infected population, there is an urgent need to delineate the role of alcohol in the development of HIV-related CNS disease.

Previous alcohol abuse may create a point of vulnerability that is exacerbated by the effects of the virus on the brain. In contrast, in the absence of HIV infection a past history of chronic alcohol abuse, combined with current abstinence from alcohol, appears to cause no significant lasting cognitive impairment (although detectable cognitive impairment may remain as a result of chronic heavy drinking). In the presence of HIV, however, several researchers have hypothesized that in some cases there may be enough impairment of cognitive function to make the brain more susceptible to the damaging impact of a second, independent process. (Green, 2004**)** The known risk of cognitive decline in HIV infection has prompted attempts to identify risk factors for this decline. Numerous studies have reported deficits in memory and learning, slower reaction times, and decreased speed in decision-making in HIV- infected patients. (Antunes, 2004; McArthur 2003; Ohio State Research, 2004) The most severe cognitive changes, which sometimes progress to the point of dementia, are almost always reported in the latest stages of the illness, but some research (Ohio State Research, 2004) has demonstrated that asymptomatic HIV-positive patients may experience subtle cognitive impairments that influence their daily activities.

AIDS Dementia/Cognitive Impairment

Several studies (Fein, 1995; Meyerhoff, 1995; Meyerhoff, 2001; Pfefferbaum, 2002, in press. See Appendix C) have raised the possibility of increased vulnerability to cognitive impairment and HIV/AIDS Dementia (HAD) development and progression in patients with HIV and alcohol co-morbidity. Durvasula and colleagues recently reported that alcohol use exacerbated adverse HIV effects on sequential reaction time. (Durvasula, 2001) Meyerhoff and colleagues add an additional piece of information by demonstrating synergistic effects of heavy alcohol use and HIV infection on both motor and visuomotor speed. Similar results were obtained by Rothlind and colleagues, with the most robust group differences observed between those with comorbidity (HIV+ heavy drinking) and the seronegative control group (light drinking). Heavy drinking showed robust effects on measures of working memory, balance, and executive function.

Neuropathological and neuropsychological studies (Durvasula, 2001; Meyerhoff, 2001) have indicated that certain brain regions are affected by both HIV infection and chronic alcohol abuse. There has been little research on how extremely heavy drinking affects the clinical outcomes related to neurological impairment and HIV disease. Initial studies (Pfefferbaum, 2002) demonstrated a potentially additive or synergistic effect of alcohol use and HIV disease on cognitive performance. The current studies focus on brain and underlying neural metabolic mechanisms which may interact to lead to cell death in specific regions of the brain. These studies **(**Durvasula, 2001; Meyerhoff, 2001) include subjects who drink heavily, on average at least 100 drinks per month over many years,

and are being treated for HIV. Magnetic resonance spectroscopy studies (Meyerhoff, 2001) of both HIV-positive and HIV-negative people who were either heavy or light drinkers found that chronic alcohol abuse exacerbates some metabolic injury in the brains of HIV-infected people, although this effect may be less pronounced in patients receiving effective antiretroviral therapy.

B. Past Biomedical Research Priorities for Alcohol and HIV/AIDS*:

NIAAA biomedical research on basic, applied, and preclinical studies to address the biological interactions between alcohol and HIV pathogenesis has focused in the past on:

- Effects of alcohol on viral burden, immune function, organ pathogenesis and neuropsychological function in HIV infected individuals.
- Effects of alcohol consumption on seroconversion and progression of disease in defined cohorts, including biological endpoints which relate to both alcohol abusing populations (e.g., MCV, CDT, liver function enzymes) and AIDS-specific measures (e.g., viral load, CD4+ and CD8+ levels).
- Mechanism(s) of enhanced progression of liver disease by alcohol consumption in individuals infected with HIV and/or co-infected with HCV/HIV.
- Drug-drug interactions between alcohol and antiretroviral drugs and altered pharmacology due to alcohol consumption.
- Alcohol use-related host defense impairment and opportunistic infection caused by pathogens such as *Mycobacterium tuberculosis, Streptococcus pneumoniae, Pneumocystis carinii* and hepatitis C (HCV).
- Interaction between alcohol and HIV proteins in neurodegeneration, organ pathogenesis and immune deficiency in animal models of HIV infection.
- Preventive interventions against tissue injury related to alcohol and HIV-related illnesses including neurological, hepatic, cardiac, and metabolic processes.

*These criteria for research were developed through a collaborative process at NIAAA in 1998 and presented in "Alcohol and AIDS: A Guide to Research Issues and Opportunities (June 2001). Contributors to these goals were Charles Flexner, M.D., Craig McClain, M.D., Joel Pachter, Ph.D., Judd Shellito, M.D., Jack Stapleton, M.D., Carl Waltenbaugh, Ph.D., Leslie Isaki, Ph.D., and Kendall Bryant, Ph.D.

C. Summary of Funded Research

The complete table of grants represents the distribution of all of NIAAA grant activities in FY2005. (This table does not reflect partial award components in multiple areas, in that grants are assigned to more than one Strategic Planning area). A total of 65 awards were active for a total dollar amount of $26,705,105. The greatest number of awards was in the area of Preventive Interventions (17). Approximately equal numbers of awards were active in Etiology and Pathogenesis (Basic Biological Science) and Basic Behavioral and Social sciences (16 and 14 respectively). Few or no awards were made in the areas of Therapeutics and Vaccine Development or Information Dissemination. Consequences of HIV, which reflect both behavioral and treatment outcomes, had 9 active awards. In the Etiology and Pathogenesis area, HIV Virology and Pathogenesis (Immunology) received the greatest dollar total, approximately 4 million, and Neurological Disease received approximately half as much, or 2 million dollars.

Support for prevention activities has strengthened and diversified into international settings as the result of both past announcements and direct support for this research by the Office of AIDS Research through targeted prevention science supplements. Epidemiological research has an increasing focus on biological outcomes in addition to behavioral risk factors. Although not directly reflected in this table, biological and behavioral research has been supported in the past through participation in networks including HIVNET and the ATN (Adolescent Trials Network) in collaboration with other NIH Institute partners. Initiation of specific activities such as the Veterans Aging Cohort Study and expansion of prevention and treatment activities in Russia are the result of direct support from the Office of AIDS Research, NIH. OAR has approved funding for continued and expanded activities in the areas of basic behavioral prevention research and neurocognitive research in its FY2007 NIH Plan for HIV Research.

D. Research Opportunities: A New Research Paradigm: Bedside to Bench and Back Again:

Populations At-Risk: Clinical Cohort Samples

The complex outcomes among HIV-infected individuals who drink may be especially salient among American veterans. This population represents the largest single population receiving care within a single system of care in the US. In preliminary work with HIV positive veterans, 33% report binge drinking, 21% report hazardous drinking, and 32% have a diagnosis of alcohol addiction or dependence. (Justice, 2004) These veterans suffer from an increase in AIDS-related illnesses, including neurological, respiratory, and metabolic disorders. A number of other clinical cohorts are under investigation. Research on some of these cohorts has carefully characterized alcohol use. For example, HIVLIVE, a predecessor to the Veterans Aging Cohort Study, and other studies (e.g., MAC, WITS) contain basic alcohol-related questions. All of these studies have identified the proximal association of levels of alcohol use and HIV disease progression rates, although the multiple pathways leading to accelerated progression are unknown. Alcohol abuse and dependence have been associated with increased risk behaviors and increased rates of infection. Several studies have demonstrated that rates of progression are more rapid for drinkers than non-drinkers, particularly in the context of antiretroviral non-adherence and treatment failure. (Samet 2002, in preparation) These well-characterized cohorts provide unique opportunities for both clinical and basic science research.

Much has been written about "Bench to Bedside Research" in which basic scientists develop new mechanistic insights, research pharmacologists turn these into therapeutic agents, and clinical investigators conduct randomized clinical trial research to determine the efficacy of these agents in human beings. This approach works well when the goal is to develop therapeutic agents targeting specific mechanisms of disease. In such circumstances the bench to bedside approach has revolutionized medical care. In the treatment of Human Immunodeficiency Virus infection, combination antiretroviral therapy (CART), which attacks multiple stages of HIV entry and replication (Fauci et al, 1996) was developed using the bench to bedside model. CART has dramatically increased HIV survival (Palella et al, 1998;2000), thereby converting a rapidly fatal disease into a complex chronic illness.

It is clear, however, that newly emerging complexities of disease management call for alternative approaches to therapeutics research and development. Complex chronic diseases (e.g., chronic HIV infection, diabetes, cirrhosis, kidney disease, heart disease, and many forms of cancer) are long-term conditions for which there is no sure cure, and in which disease progression and longevity are influenced by a complex array of interactions between the "primary" disease, the individual (host) affected, and the larger environment. Important host characteristics that moderate HIV infection include genetic susceptibilities, past and ongoing toxic exposures (e.g., alcohol, tobacco, marijuana, cocaine and, in some cases antiretroviral treatments), and comorbid diseases (e.g., viral hepatitis, diabetes, cardiovascular diseases including hypertension and atherosclerosis, obstructive pulmonary disease, etc.). Host behaviors such as adherence and risky sexual

and IV drug use behaviors can also have important effects on the progression of disease in the individual, and of course on the spread of the disease in the population.

Environmental factors influence the profile of "opportunistic" infectious agents to which the host may be susceptible (for example, in Africa, rapidly progressive malaria and tuberculosis; in the US, bacterial pneumonia and hepatitis C) and the timing and availability of antiretroviral treatment. When faced with the reality of complex chronic diseases affecting the entire human organism, the common approach is to try to unravel the complexity into single threads that may be analyzed. This approach does offer important insights, but it inherently overlooks the possibility of interactions among these "separate" threads, and may fail to identify the most important etiologies if these etiologies are based on important interactions. For example, liver failure is now a leading cause of death among those in treatment for HIV infection and in many cases is likely secondary to incompletely understood interactions between primary disease processes, antiretroviral medications, alcohol, and other substances.

This meeting seeks to explore how the process of unraveling the complexity of HIV disease can be enhanced by partnerships between basic science and clinical researchers using a bedside to bench model. In this model, large, detailed clinical databases, such as those available in electronic medical record-based healthcare systems, are used to identify potentially modifiable indices (for example, in addition to CD4 cell count, HIV-1 viral load and viral resistance; macrocytosis; anemia; transaminitis; and decreased creatinine clearance, etc.) that are strongly and independently associated with important clinical outcomes such as survival and HIV disease progression. Observational data analyses can also use nested models to determine the extent to which these indices are associated with particular aspects of "primary" disease progression. These nested models can also be used with comorbid disease (e.g., time-varying covariates) to help determine the extent of evidence of important interactions among these disease processes. Bench scientists in turn use these observations to strategically target their search for pathophysiologic mechanisms of interaction and, in time, for new treatments.

Evolving New approaches: the Next Step – Collaboration

By way of illustration of the "bedside to bench" approach to research on alcohol and HIV/AIDS, we offer some collaborative "works in progress", including 3 examples from the ongoing Veterans Aging Cohort Study (VACS).

Liver Injury in HIV (Collaboration with the University of Hawaii, Mitochondrial Medicine Laboratory; Dr Mariana Gerschenson)

With the substantially improved survival made possible by antiretroviral therapy has come a new list of clinical management concerns regarding the long-term effects of HIV infection itself, antiretroviral treatment, and aging in an immune-compromised host. While these concerns include a wide array of medical conditions (e.g., coronary artery disease, hypertension, diabetes, liver disease, and cancer, etc.), liver disease has become a central concern.

Liver disease in HIV is multifactorial and includes coinfection with viral hepatitis B and C, exposure to alcohol, diabetes, and both acute and chronic antiretroviral treatment toxicity. Initially clinicians followed alanine transaminase (ALT) because it has been established as the most sensitive and specific marker of liver injury outside HIV infection. However, a growing body of observational data suggests that aspartate transaminase (AST) may be more important than ALT among those with HIV infection (just as it is in alcoholic injury). AST is both more strongly associated with survival (?) when both markers are included in analyses and, when models are adjusted for AST, elevations in ALT are relatively protective. That is to say, individuals with isolated ALT elevations do better with respect to survival and liver fibrosis than those with isolated AST elevations or elevations in both AST and ALT.

On further analyses it became apparent that those with AST elevations had lower CD4 cell counts, longer duration of antiretroviral therapy, and were more likely to have undetectable viral loads. These observations suggested that AST may be a marker of mitochondrial injury in HIV. At this point the epidemiologic investigators in VACS consulted with an expert in liver and mitochondrial injury (Dr. Mariana Gerschenson) to ask whether basic science investigations might use VACS samples to test whether or not AST is a marker of mitochondrial injury.

What resulted has been a three year collaboration in which assays have been developed to identify the mitochondrial isoenzyme of AST (mAST) and to measure the association of this isoform with markers of mitochondrial injury and oxidative stress. This work will be presented at a major HIV liver meeting this summer and is being written up for publication.

Anemia in HIV (Collaboration with Yale University Departments of Hematology and Rheumatology, Dr. Nancy Berliner and Dr. Rich Bacala)

Anemia is independently predictive of survival with HIV infection after adjustment for CD4 cell count, HIV-1 viral load, age, and HIV risk behavior both in the pretreatment and post treatment eras. (Mocroft et al, 1999; More and Forney, 2002; Sullivan et al, 1998) It is commonly believed that the anemia seen with HIV is “anemia of chronic disease”, a microcytic or nomocytic anemia. However, none of the major descriptive analyses of anemia in HIV have characterized the anemia by cell type. Further, some have suggested that much of what is called anemia of chronic disease is either “unexplained anemia” or “anemia of chronic inflammation”.

VACS has presented analyses demonstrating that all three cell types of anemia occur among those in care for HIV infection and that the association of anemia with survival depends upon the type of anemia. (Work presented at the International HIV Database Meeting 2005; paper submitted). Further, as has been described in other diseases, the factors associated with the different cell types of anemia vary. For example, CART is protective against anemia overall and protective against microcytic and normocytic anemia. However, CART increases the probability of macrocytic anemia, which is also more strongly associated with mortality after adjustment for CD4 cell count, HIV-1 viral

load, age, race, drug use, and exposure to AZT and d4T (both known to cause anemia). Interestingly, macrocytic anemia is more strongly associated with platelet and absolute neutrophil deficiencies as well.

The epidemiologic investigators in VACS approached the chair of hematology at Harvard University School of Medicine, Dr. Nancy Berliner (a bench scientist), to discuss possible use of banked blood and DNA specimens from fully clinically characterized HIV positive and negative subjects in VACS to understand why macrocytic anemia should be more strongly associated with survival and to better understand the etiologies of anemia in HIV. Dr. Berliner is currently conducting preliminary analyses with Dr. Rich Bucala to determine the associations between anemia by cell type and markers of anemia of inflammation (serum iron and iron-binding capacity, serum ferritin), urinary hepcidin levels, and migration inhibitory factor (MIF) levels in age-stratified HIV positive and negative samples from VACS. They are also planning to study the role of genetic polymorphisms in predicting the development of anemia by class. This work is ongoing and a grant supporting further evaluation has been favorably reviewed by NHLBI and NIA.

A Project in Early Proposal Stage: Genetic Markers of Susceptibility to Addiction and Depression: The Role of Precipitating Stressors; John Crystal, MD and Arthur Simen, MD, PhD

Work by John Crystal and his team have demonstrated that genetic predisposition to behavioral problems such as alcohol addiction/abuse often requires a major stressor before it is likely to be expressed. Drs. Crystal and Simen are interested in exploring the role of HIV disease as such a stressor in the context of VACS, using its detailed clinical data and stored DNA samples. The DNA and blood samples being collected by VACS will be invaluable resources for continued bedside to bench collaboration, especially in support of large scale multivariate analyses of the associations between a given blood or DNA assay and certain clinical phenomena. Additional support is need to expand this collection to include samples on all the HIV-negative controls (currently collecting from only half of negative controls) in order to provide more highly powered analyses comparing the relationships of these biomarkers among positives and negatives with important clinical outcomes. Specific alcohol-related analyses should be targeted to the following variables:

Analyses of genetic predisposition to:

1. alcohol abuse/dependence
2. depression
3. tobacco addiction
4. narcotic addiction
5. liver injury
6. lung injury
7. bone marrow injury

Examples of particular genes that could be studied include those involved in:

1. Nicotine breakdown (i.e., CYP2B6)
2. Opioid receptor genes (OPRMI, OPRK1, OPRD1)
3. Dopamine Receptor Gene (DRD2)
4. Mitochondrial DNA
5. Macrophage migration inhibitory factor (MIF gene)

Examples of bioassays that might be studied:

1. mAST
2. TNF, IL-6, IFN gamma, MIF
3. Erythropoietin levels
4. Rates of nicotine metabolism
5. Rates of alcohol metabolism
6. Homocystine

Depending upon the results of these assays, additional specimens may need to be collectedto enable investigators to observe changes in patients over time. Urine, muscle, liver, bone marrow, and lung specimens would be useful to explore more localized effects. Additional funds would be used to support these targeted sample collections in appropriate subsamples of patients.

Collaboration

The "open platform design" of the Veteran's aging Cohort Study invites the use of this rich clinical dataset by diverse researchers in collaboration with VACS researchers. The diversity of research is represented in the special supplement to *Medical Care* on "Alcohol in HIV Infection: Insights from the Veterans Aging Cohort Study and the Veterans Affairs National Health Information System" (Justice, special editor, 2006. See Appendix C). While not represented in this publication, a wide range of virological modeling activities are also being carried out (See section entitled "Modeling Clinical Outcomes", p. 37.). As the costs and difficulty of assembling critical resources for carrying out large-scale multidisciplinary research increases, the need to provide increased use of these resources is becoming increasingly apparent. Supporting collaborative studies is consistent with the renegotiation of the AIDS Clinical Trials (NIAID) and with the guiding principles of the NIH Roadmap.

III.

NATURAL HISTORY AND EPIDEMIOLOGY

III. STRATEGIC PLAN AREA: NATURAL HISTORY AND EPIDEMIOLOGY

A. Scientific Issues

Natural history and epidemiologic research is needed to monitor epidemic trends, develop and evaluate prevention modalities, follow the changing clinical manifestations of HIV disease in different populations, and measure the effects of treatment regimens. The NIH will continue to support research to examine topics in HIV transmission, HIV/AIDS disease progression (including the occurrence of opportunistic infections [OIs]), malignancies, metabolic complications, neurological and behavioral dysfunctions, and the development of other HIV/AIDS-related conditions. Domestically, as well as internationally, the populations affected by HIV/AIDS are also those most severely affected by the spreading epidemics of sexually transmitted diseases (STDs), TB, and other comorbidities, such as hepatitis C. Researchers are studying the effects of viral, host, and other factors on transmission and disease progression. Since biological, pharmacological, psychological, and behavioral factors all potentially influence the impact of antiretroviral therapies on HIV transmission, researchers are evaluating the specific contributions of these factors and their net impact on HIV transmission. Research also is focusing on determining the biological characteristics, sociocultural factors, and health services issues that contribute to the differential dynamics of HIV transmission and disease progression in men, women, and different racial/ethnic groups. Results from these studies will provide new directions and improvements in HIV/AIDS prevention and care. The NIH will continue to emphasize the importance of epidemiologic studies to investigate the mechanisms of disease progression, the causes of death, and the impact of therapy in changing the spectrum of HIV disease. The expansion of existing study populations in the United States will allow the identification of long-term effects of HIV therapy. The assembly of new, representative cohorts, specimen repositories, and databases in developing countries will be important to study key cofactors that modify HIV disease. The NIH will foster basic and applied research to develop inexpensive virologic, immunologic, and genetic assays for use in domestic and developing country settings.

Characterizing Patient Populations with Alcohol and HIV/AIDS

A need for more complete and accurate ability to characterize patients infected with HIV and alcohol-related chronic diseases emerged from the beginning of the epidemic. However, this proved more difficult than expected given the evolving nature of the epidemic. With improved medical characterization of the disease comes the need to understand how to guide clinical decision-making for alcohol and AIDS patients, particularly in the development of ARV treatment and EMR systems. Two clinical cohorts of patients have been well-characterized and yielded useful results in identifying potential etiological factors related to alcohol misuse in these populations. The earliest cohort was HIVLIVE/HIVALC and the second was the Veterans Aging Cohort Study. These studies can also directly reflect complex systems of inter-related host/virus dynamics. Understanding emerging epidemics and their dynamics among infected and

uninfected populations requires combining knowledge of epidemiologic patterns with knowledge of viral genetics, mutation, and viral fitness in alcohol users. This integrated knowledge may then be used to develop targeted prevention activities, including vaccine effectiveness trials and trials of new therapeutics for resistant virus.

International Research

In many developing countries where the impact of AIDS is greatest, poor measurement of alcohol use and related consequences makes it difficult to accurately assess the impact of alcohol on the epidemic. Expanding our understanding of the accurate measurement of alcohol use in HIV/AIDS prevention and treatment is essential to reducing the rates of HIV infection and expanding the treatment of a range of associated alcohol problems. Preliminary work from Kenya has indicated that over 60% of patients seen in clinics receive scores on the AUDIT, a standardized internationally validated alcohol questionnaire (Babor et. al, 2001), indicating the presence of alcohol use disorders. (Shaffer, 2004) Other than alcohol misuse, little other substance abuse is found in many populations in Africa. (WHO, 2002) Similar levels of alcohol use disorders are being found in HIV+ patients in other African settings. (Seage, 2004; Bangsberg , 2004) Delivery of antiretroviral medications, which interact with alcohol, may severely compromise the effectiveness of these medications and directly lead to increased rates of disease progression, treatment failure, and mortality. (Samet, 2005; Braithwaite, in press).

New tools need to be developed to improve the diagnosis and treatment of this complex illness. Use of electronic medical record technology has made it possible to evaluate the effectiveness of clinical decision-making and to conduct research on the evolving role of evidence-based medicine. One issue that has been raised is how to begin to conceptualize the development and use of informative data for these interventions. Datasets can be conceptualized as minimal patient care datasets or as optimal research datasets. Combining data may greatly increase the prognostic value of patient biomedical information. (Siika, 2005; Tierney, 2003) Identifying the benefits and tradeoffs of this approach will allow for academically supported heath research in patients with HIV/AIDS and multiple comorbidities, including alcohol abuse and dependence, who need advanced care. Research is needed to identify additional strategies that may be used to improve care in under-resourced settings.

B. Research Priorities of the FY 2007 NIH Plan

• Sponsor domestic and international epidemiologic investigations into the interactions between HIV genetic variability, host genetics, and other factors that influence disease morbidity and mortality, with special emphasis on different routes of transmission, chronic and infectious comorbidities and malignancies, and long-term use of antiretroviral therapies.

• In order to increase the value of different sources of epidemiologic information on HIV/AIDS, develop, maintain, and effectively utilize domestic and international cohorts, repositories, and nested studies of populations

experiencing emerging and ongoing HIV epidemics, with particular emphasis on:

Assessing the short- and long-term effects of preventive and therapeutic interventions at the individual, family, and community levels;

Establishing integrated databases that allow analyses of large datasets to address new or unresolved scientific questions; and

Generating new hypotheses regarding the transmission and pathogenesis of HIV infection.

• Implement epidemiologic and simulation studies among HIV-infected individuals and appropriate controls to inform, monitor, and evaluate intervention strategies, including initiation of treatment programs, in domestic and international settings.

• Continue improving key measures to diagnose and monitor HIV/AIDS in diverse settings by encouraging development of and evaluating late-generation laboratory assays. These include accurate, reproducible, and affordable virologic, immunologic, pharmacologic, and genetic assays; measures of adherence to therapy; and markers of toxicity and comorbidity for use in domestic and international settings.

C. Special Emphasis Area: Natural History and HAART

Introduction and Background

Not enough is known about the important factors associated with morbidity and hospitalization rates in HIV-infected men and women or how they compare with rates in HIV infected subjects with similar CDC stages but without alcohol problems. In the current era of declining opportunistic infections and hospitalizations, it would be important to determine what factors, including alcohol use, are predictors of morbidity and mortality in HIV-infected subjects during the HAART era.

In a recent study of causes of mortality among AIDS patients in a heterogeneous sample of men and women, the following were identified as causes of death from death certificates. Alcohol misuse may be contributory to many of the most prevalent causes of death among AIDS patients.

Table 3: Causes of Death in CHORUS 1997-2004, limited to causes contributing to at least 3% of the deceased population.

Cause of Death	Immediate (n, %of total)	Underlying (n, % of total)	Total (N, % of all deaths)
Hepatitis/ cirrhosis	**50 (8.0)**	**40 (6.4)**	**90 (14.4)**
AIDS, unspecified	**30 (4.8)**	**53 (8.5)**	**83 (13.3)**
Wasting	**22 (3.5)**	**48 (7.7)**	**70 (11.2)**
Lymphoma	**48 (7.7)**	**7 (1.1)**	**55 (8.8)**
NON-AIDS Cancer	**44 (7.0)**	**3 (0.5)**	**47 (7.5)**
Pneumonia, recurrent	**33 (5.3)**	**7 (1.1)**	**40 (6.4)**
Sepsis	**31 (4.8)**	**9 (1.4)**	**40 (6.4)**
MAC, disseminated	**18 (2.9)**	**18 (2.9)**	**36 (5.8)**
CMV Disease	**13 (2.1)**	**22 (3.5)**	**35 (5.6)**
Trauma/ Suicide/ Homicide	**33 (5.3)**	**0 (0)**	**33 (5.3)**
Dementia/ Encephalopathy	**18 (2.9)**	**11 (1.8)**	**29 (4.7)**
Sudden Death	**25 (4.0)**	**0 (0)**	**25 (4.0)**
Cardiovascular Disease	**23 (3.7)**	**2 (0.3)**	**25 (4.0)**
PCP	**20 (3.2)**	**4 (0.6)**	**24 (3.8)**
KS	**4 (0.6)**	**19 (3.0)**	**23 (3.6)**
Cryptococcosis	**15 (2.4)**	**4 (0.6)**	**19 (3.0)**

Whether alcohol use influences the progression of AIDS in persons already infected with HIV has been explored in past animal and human studies. Findings from experimental animal models clearly indicate that chronic alcohol consumption exacerbates the immunosuppressive effects of the retrovirus, resulting in accelerated progression to clinical illness and decreased resistance to secondary infection in the host. The organisms used in the experiments were *Mycobacterium avium complex* (MAC), *Streptococcus pneumoniae, Cryptosporidium parvum*, and *Giardia muris,* all pathogens commonly occurring in patients with AIDS. (Dingle, 1997) Human studies, however, have yielded controversial information. During the pre-HAART era, Watson and colleagues (1990; 1994) suggest that alcohol may have accelerated the development of AIDS, and case reports have supported their hypotheses. (Fong. 1994) Nevertheless, a prospective study by Kaslow (1989) failed to demonstrate a relationship between percentage of CD4 cells, progression to AIDS, and alcohol use. Another study conducted by Chandiwana and colleagues (1999) similarly revealed no significant differences in mean CD4 counts between alcohol users and "non-users"; in fact, most of the patients with CD4 <200 cells did not use alcohol (p=0.023). Crum and colleagues (1996) confirmed that there were no significant differences in CD4 cell counts nor cell decline among drinkers in different alcohol categories during 5 years of follow-up. They demonstrated, however, that between 2 to 5 years post-seroconversion, there was a statistically significant increase in CD8 cell count among the heaviest drinkers ($\geq$21 drinks/week). Although Penkower and colleagues confirm that single measurements of alcohol intake did not predict AIDS-related symptoms and disease progression, the pattern of drinking over time was significantly associated with several AIDS- related symptoms. (Penkower, 1995)

1. Current Research

Considering that a significant proportion of HIV-infected patients and those receiving HAART develop liver toxicity, the effect of antiretroviral therapy and alcohol use on liver fibrosis in HIV-infected patients was examined by Benahmu and colleagues . Multivariate analysis identified 4 independent predictors of progression to cirrhosis: 1) absence of protease inhibitor therapy (relative risk [RR] = 4.74, 95% confidence interval [CI], 1.34-16.67); 2) heavy alcohol consumption (> or = 50 g daily) (RR = 4.71, 95% CI, 1.92-11.57)/ 3) low CD4 cell count (<200/microL) (RR = 2.74, 95% CI, 1.17-6.41); and 4) age at HCV contamination (> or = 20 years) (RR = 2.37, 95% CI, 1.04-5.38). Scientists in Miami (Miguez, 2005) have reviewed causes of hospitalization in HIV+ patients (n=538) admitted to Jackson Memorial Hospital from 9/2001 to 9/2002. Their data indicate that independent of HAART use, alcohol users had more CNS-related hospitalizations than drug users (15% vs. 4.5%, p=0.03) or controls (7.5%, p=0.027). In a 2005 retrospective analysis of medical records from the Adult/Adolescent Spectrum of HIV-Related Diseases Study of Public Health, Seattle and King County, a correlation was observed between alcohol use and Kaposi's sarcoma. Patients diagnosed with KS in the HAART era (n = 40) were significantly more likely to be diagnosed with alcohol problems (43% v 18%). (Gallafent, 2005)

HAART and Alcohol Use

Recent advances in antiviral therapies for HIV infection have raised the hope that AIDS can be treated as a chronic disease. This offers the patient a longer potential life span, fewer complications, and possibly less social stigmatization, while also reducing the demand on the health care system for "catastrophic event" interventions of a therapeutic or supportive nature. At the same time it may increase the overall burden on the health care system, including care-givers and programs, that may need to provide sophisticated and expensive therapies over a long period of time as patient life spans increase unless economies of scale and improved technologies develop in parallel. The community-at-large will also need to adapt as the previously catastrophically ill become chronically ill and need to be re-integrated into society as "more healthy" participants.

Immune reconstitution is observed at some level in patients managed with Highly Active Antiretroviral Therapy (HAART). To be successful, these HIV treatments require access and strict adherence to drug regimens. While long-term treatment of HIV with these new drug therapies has improved the quality of life and extended survival for many people with AIDS, use of these medications is particularly difficult for alcohol-abusing populations. Heavy alcohol consumption is known to limit a person's ability to adhere to HIV treatment, and non-adherence is known to lead to more rapid disease progression. (Miguez, 2001, Samet, 2004; Wagner, 2001) Further, alcohol is known to exacerbate common comorbid conditions among those with HIV infection, such as hepatitis C or chronic hepatitis B. Finally, heavy alcohol consumption may also lead to increased rates of serious toxicity from antiretroviral therapy, as both HIV and ARVS can be toxic to the liver and bone marrow. Thus, heavy alcohol consumption may lead to non-adherence and even complete cessation of antiretroviral therapy through a multitude of behavioral mechanisms.

Research published in *Addiction Biology* by the Miami group (Miguez, 2003) indicates that HIV-infected patients with a history of alcohol problems who are receiving highly active antiretroviral therapy (HAART) and are currently drinking are twice as likely as light or non-drinkers to have CD4 counts below 500, (95% CI, 1 - 5.5, p = 0.03). Moreover, it was shown that HAART-treated heavy alcohol users were four times less likely to achieve a positive virological response (95% CI, 1.2 - 17, p = 0.04). (Miguez, 2003) These findings were also confirmed by Palepu (2005).

If the main goal of physicians who treat HIV is to restore the CD4 population, it needs to be understood that alcohol use may affect thymus-induced immune repletion. Recent findings from the Miami team demonstrate that hazardous alcohol users, and particularly heavy alcohol users, undergoing 6 months of antiretroviral treatment have half of the thymus size of non-hazardous alcohol users. (Miguez, 2005) A variety of models are being developed for estimating the relative toxicity of the interactions between alcohol, HIV, and medication regimens, and the impact of non-adherence to these regimens in an observational clinical cohort study of U.S. veterans. (Braithwaite et al, 2005a, 2005b)

Highly Active Antiretroviral Therapy (HAART)

The activity level of CYP3A4 is important in the metabolism of drugs comprising HAART. Orally administered drugs comprising HAART regimens are rapidly converted to inactive metabolites via first-pass oxidative metabolism by the P450 cytochrome system, including CYP3A4. (Ast, 2004; Rodriguez-Novoa, 2005) HIV protease inhibitors amprenavir, indinavir, nelfinavir, ritonavir and saquinavir and the non-nucleoside reverse transcriptase inhibitors efavirenz, nevirapine and delavirdine are susceptible to drug-drug interactions resulting in either enhanced or decreased metabolism. Drugs that are inhibitors of specific cytochromes reduce clearance and extend the serum half-life of drugs. Inducers of cytochromes enhance oxidative metabolism and reduce serum concentrations of drugs. (Ast, 2004; Rodriguez-Novoa, 2005). Additional inducers of CYP3A4 modify (reduce) HAART pharmacokinetics. Isopentanol has been shown to be a more potent and effective inducer of CYP3A4 than ethanol in human liver cells in vitro. In animals, the administration of isopentanol/ethanol mixtures results in increases in CYP3A greater than those induced by either agent alone. An elevation in CYP3A4 activity in these individuals may result in enhanced drug metabolism and reduced therapeutic drug levels. Thus, HAART may not be as effective in some individuals who consume alcoholic beverages. Individuals consuming alcoholic beverages may place themselves at risk for developing drug resistant HIV strains due to sub-therapeutic levels of protease inhibitors as a consequence of enhanced protease inhibitor metabolism. Therapeutic drug monitoring studies, stratified by alcohol consumption levels, are needed in HIV patients who consume any alcoholic beverages to confirm this hypothesis. In addition, the success of emerging treatment strategies needs to be evaluated. New targets for interruption of viral replication need to be identified. (Ast, 2004; Rodriguez-Novoa, 2005) The HIV- type 1 (HIV-1) life-cycle involves a number of cellular cofactors. Some are essential for HIV-1 replication and thus may serve as targets for therapeutic intervention. There is growing evidence of an important role for cellular DNA repair in HIV-1 infection, which suggests

that inhibition of these repair functions may lead to suppression of viral replication. (Daniel, 2005)

2. Research Opportunities: Additional Host Factors: Pharmacogenomics and Pharmacogenetics

Two genetic characteristics are relevant when considering a patient's response to drug therapy and drug metabolism: pharmacogenomics and pharmacogenetics. Pharmacogenomics, a function of the human genome, is the emerging field that uses genetic information to predict a patient's response to a drug. (Ast, 2004; Rodriguez-Novoa, 2005) Examples of genes that influence the response of a patient to a medication are genes regulating the enzymes and proteins involved in drug(s) metabolism and transport genes that code for specific drug targets or genes that influence the expression of a disease. (NIAAA, 2004) Pharmacogenetics is the field of science that identifies and characterizes polymorphic expression of genes related to drug metabolism. Most important for alcohol detoxification are alcohol and aldehyde dehydrogenases and the cytochrome P450 systems in Phase I biotransformation, and glutathione-S-transferase for phase II biotransformation. For the metabolism of drugs used for the control of HIV infection, the cytochrome P450 system and glutathione-S-transferases are important. Polymorphisms in the genes that encode for enzymes in phase I or phase II biotransformation result in differing enzyme kinetics, with relatively slower or faster than normal rates of metabolism. (Ast, 2004; Rodriguez-Novoa, 2005) Thus, based on the expression of polymorphic genes, ethanol metabolites and toxic intermediates could build up or be quickly biotransformed based on the nature of the kinetics. This could change the bioavailability of the drug in question and present dosing difficulties for drugs with narrow therapeutic ranges. The question "How do individual patient pharmacokinetic profiles contribute to HIV treatment failure?" is now being addressed through the elucidation of an individual's pharmacogenetics. It is anticipated that pharmacogenetics research will ultimately enable physicians to design individualized drug regimens that take alcohol consumption and metabolism into account, thereby maximizing treatment success.

D. Special Emphasis Area: Biomedical Aspects of Alcohol, AIDS and Liver Disease

1. Background and Introduction

Because the life expectancy of patients infected with HIV has been prolonged, liver disease has assumed far greater importance as a cause of morbidity and mortality in these patients. The use of antiretroviral therapy (ART) by patients for extended periods of time has resulted in overt hepatotoxicity. In addition, patients who have HIV often are co-infected with hepatotropic viruses such as hepatitis C (HCV) and hepatitis B (HBV), further compounding liver injury. Chronic alcohol consumption causes injury to the liver and may result in more rapid progression to cirrhosis, end-stage liver disease, and hepatocellular carcinomas in coinfected patients. In addition, hepatic steatosis is now recognized more commonly in patients who have HIV, particularly in the setting of ART.

Steatosis is also more common and more severe in HIV/HCV coinfected patients than in those with HCV alone. Thus, understanding liver disease progression in the setting of HIV infection and alcohol abuse becomes an important focus in caring for these individuals. Furthermore, there is no information on the response of patients who have HIV and abuse alcohol to treatment of their liver disease, particularly in those coinfected with HCV and HBV.

2. Current Research

ART has been reported to cause severe liver injury requiring cessation of therapy in up to 30% of patients. Alcohol induces CYP2E1, resulting in the formation of free radicals which are also damaging to the liver. In addition, the HIV protease inhibitors (PI), such as ritonavir, are associated with severe hepatotoxicity and have differential effects on CYP isozymes. The interactions between PI and CYP2E1 are not known. The standard liver treatment for HCV is the administration of interferon and ribavirin (RBV). HIV infection is treated with the nucleoside reverse transcriptase inhibitor (NRTI) didanosine. In addition to their hepatotoxic effects, the combination of RBV and didanosine is associated with cardiomyopathies, pancreatitis, lactic acidosis, and lipodystrophy. Much of this toxicity is likely attributable to inhibition of enzymes involved in mitochondrial DNA replication by NRTIs. Chronic alcohol consumption also leads to some of the same adverse events. Therefore, the interaction between RBV and alcohol use in patients coinfected with HIV/HCV is in need of exploration.

It is known that most of the protease inhibitors (components of ART) are metabolized in the intestine and liver by CYP3A family enzymes, CYP3A4 and CYP3A5. Furthermore, CYP3A4 is the most abundant isoform of cytochrome P450 3A family in human liver and is responsible for the metabolism of many clinically used drugs. On the other hand, ethanol has been shown to induce hepatic CYP3A4 activity in vitro and vivo systems. Therefore, ethanol intake may interfere with ART therapy in AIDS patients by accelerating the metabolism of protease inhibitors via induction of CYP3A4.

NRTIs cause severe macrosteatosis in HIV patients by impairing mitochondrial β-oxidation. Chronic alcohol consumption causes steatosis, steatohepatitis, and fibrosis, and impairs fatty acid metabolism. The interactions between alcohol and NRTIs in altering lipid metabolism in HIV-infected patients that may impact the course of liver damage are in need of resolution. Designing preventive or treatment measures would be facilitated by understanding the combined role of alcohol, HIV and HCV on fatty acid metabolism.

3. Research Opportunities

- Hepatotoxic interactions between chronic alcohol consumption, alcohol metabolism, and antiretroviral therapy.

- Interactions between alcohol and medications used in the treatment of the HIV/HCV coinfected patient.

- Alcohol and HIV-induced effects on fatty acid metabolism leading to steatosis.
- Expansion of secondary analysis of multiple datasets to identify alcohol-related hepatotoxic interactions and thresholds.

E. Special Emphasis Area: Alcohol and HIV-related Anemia

Alcohol is a toxin known to cause macrocytosis and macrocytic anemia. (Montalto NJ, 2003; Neuman T, 2003) Recent research has suggested that one mechanism for these effects may be through acetaldehyde-induced changes in the red blood cell membrane. (Latvala J, 2001) Alcohol dependence may also be associated with anemia secondary to liver disease, alcohol-related GI blood loss, and nutritional deficiencies. Anemia is also common in HIV infection and has been demonstrated to predict survival independent of CD4 count and HIV viral load. (Justice AC, 1989; Moore RD, 1998; Sullivan PS, 1998; Mocroft A, 1999; Belperio PS, 2004) Prior to the introduction of highly active antiretroviral therapy (HAART), the one-year incidence of anemia was 37% in patients with clinical AIDS and 12% for patients with CD4 counts <200/mm 3**.** Causes for anemia in the setting of HIV infection include antiretroviral therapy, opportunistic infections, nutritional deficiencies, liver disease, and drug toxicity. (Bain BJ, 1999; Sullivan PS, 1998; Levine AM, 2001; Semba RD, 2002) While certain antiretroviral therapy may cause anemia, the prevalence of anemia has been declining in the era of HAART. (Semba RD, 2001; Moore RD, 2002; Buskin SE, 2004)

Given that the association of anemia with survival in HIV infection is likely to depend upon the etiology of the anemia, investigators in the Veterans Aging Cohort Study recently conducted a study involving over 20,000 veterans in care to determine if the type of anemia as characterized by MCV and degree of anemia differentially influenced survival. Of the approximately 20,000 participants in the study, normocytic anemia was most common (22% of men and women); macrocytic ((9% and 7%) and microcytic anemia (2.6% and 6.6%) were also present. (Fultz SL, 2006; *unpublished data)* Patients with any form of anemia demonstrated poorer survival than patients without anemia, with an approximately 25% increase in the hazard of mortality per one gram decrease in hemoglobin. However, survival for men with macrocytic anemia was significantly worse compared to men with microcytic anemia or men with normocytic anemia (<0.0005 for both comparisons). Among men (N = 19,843), after adjustment for hemoglobin difference, age, race, CD4 cell count, HIV viral load, viral hepatitis, alcohol abuse and dependence, diabetes, and renal insufficiency (fully adjusted model), macrocytic anemia was most associated (HR 1.48, 95% CI 1.35-1.63), normocytic anemia was modestly associated (HR 1.09, 95% CI 1.003-1.19), and microcytic anemia was not associated with mortality (HR 1.02. 95% CI 0.86-1.20). Of 391 women in the study, only 28 had macrocytic anemia, making further analyses difficult.

Having demonstrated an association between anemia type and mortality, VACS investigators also examined the association between anemia type and demographic and clinical characteristics. Men with macrocytic anemia were older (mean 48.7 years), more likely to be white (39.7%), had higher CD4 counts (median 156 cells/mm 3), and lower viral loads (median 9306 copies/ml). Men with macrocytic anemia were also more likely to have received HAART (35.9%), mono/dual therapy (36.3%), zidovudine (54.7%) or stavudine (18.8%) in the 90 days prior to baseline. On the other hand, exposure to these ARVs was protective against microcytic and normocytic anemias.

Given that antiretroviral therapy at baseline was a strong predictor of anemia type, a data sensitivity analysis was performed to determine if the relationship between macrocytic anemia and survival remained in subjects who were ARV naïve. Of subjects who were HAART naïve, macrocytic anemia was associated with a 93% increased hazard of mortality (HR 1.93, 95% CI 1.59-2.34) compared to no anemia in the fully adjusted model. This contrasts with no additional hazard for microcytic anemia (HR 1.01 95% CI 0.81-1.27) and a 15% increased hazard to normocytic anemia (HR 1.15, 95% CI 1.01-1.21). Severity of anemia remained independently significant with a 24% increased hazard for each gram/dl change (HR 1.24, 95% CI 1.20-1.29). The impact of age, race, CD4, viral load, and comorbidities were similar to those in the full sample of men.

3. **Research Opportunities**

The relationship between HIV infection, macrocytosis, macrocytic anemia, and antiretroviral therapy is likely to be complex, and cannot be unraveled by a simple cross-sectional analysis. Simply adjusting for potential confounders does not adequately examine this problem, since both HIV disease severity and anemia many influence the decision to start ARV therapy as well as the agents chosen, and ARV therapy can influence CD4 cell count, viral load, and anemia. Future research will need to employ advanced statistical techniques such as marginal structural modeling to examine possible additional confounders in the causal pathway. There is also a need for further research examining the impact of treating anemia with antiretroviral therapy, transfusions, or erythropoietin therapy and examining the occurrence and course of anemia in HIV-infected women.

F. Special Emphasis Area: Modeling Alcohol-related Outcomes

One key objective for Natural History and Epidemiological Research is "to develop and evaluate methods and resources for HIV/AIDS epidemiological and clinical studies that use culturally appropriate approaches; incorporate new laboratory, sampling, and statistical methods with information systems; and better integrate research findings into clinical practices and regional, national, and international policy." To address this objective there is a clear need for multiple cohort samples to study host by virus interactions in the prediction of alcohol and alcohol-related organ injury.

Multiple cohort samples can provide the basis for studying liver, lung, and brain injury in the widest range of drinkers and allow for cross-comparisons between samples to evaluate the effects of race, gender, age and a variety of other demographic variables. These comparisons not only allow us to identify and characterize the highest risk individuals but also provide a basis for targeted interventions and modeling disease course. Preventive interventions can be put into place in systems where compound indices of risk can be developed and this information can be made available to health care providers. In summary, multi-cohort samples may 1) characterize association of alcohol and other host factors with survival among those with and without HIV infection; 2) characterize variation in survival by alcohol and HIV status, and other related comorbidities and organ injury (e.g., liver, lung, brain, etc.); 3) determine the benefits and harm of ARV's by varying mixtures of acute and/or chronic alcohol use and alcohol-related organ injury; and 4) determine if patient pretreatment alcohol use characteristics justify altering timing of ARV treatment.

Analytic Techniques

New methodologies allow the application of innovative analytic techniques with informative data sources such as multicohort samples with appropriate statistical precautions. Using multiple pooled datasets will allow, through increased power and multivariate estimation procedures, accurate characterization of the role of alcohol in clinical outcomes and HIV, aging, HCV, and other comorbid disease. Clinically-based cohorts of both men and women with well-characterized drinking patterns, HIV status, and detailed medical records will allow for analysis of biological and interactive behavioral risk factors over time. Demographically similar HIV-negative controls with varying degrees and patterns of alcohol use can be compared to HIV+ patients with similar stratification on alcohol use characteristics across data sets.

These multiple cohorts provide a basis for pooling data and using conventional analytic techniques (survival analysis, longitudinal models, etc), newer approaches (marginal structural equations models) and computer modeling and simulation via Markov models and Active Agent models. Collaboration with biostatistical experts is necessary to implement both current models of HIV infection and ones under development that take into account multiple temporally dispersed biological markers. Pooled data sources may

be used to test a wide range of hypotheses that take into account the limitations of the data and statistical problems such as over-fitting.

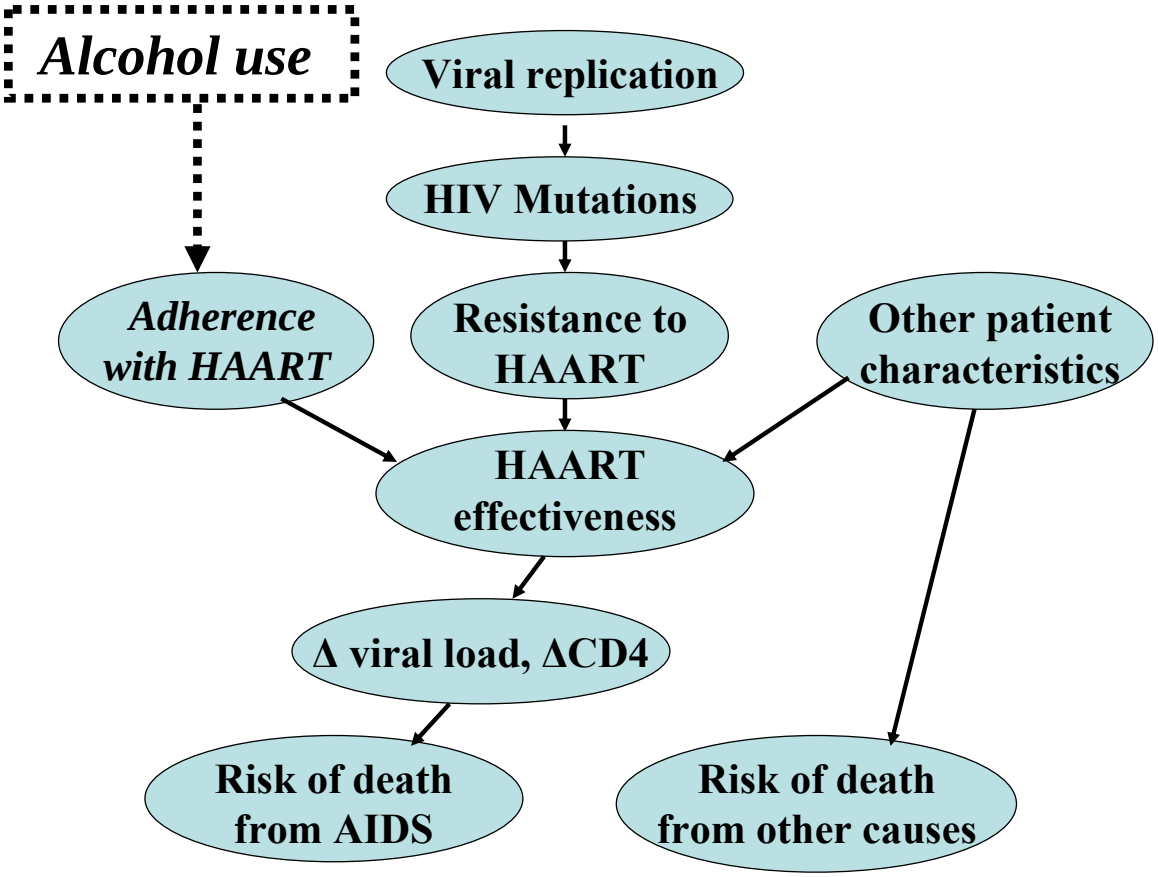
Identifying Viral Set Points and Viral Fitness: Opportunities for Analysis

Of particular importance is the understanding of the early trajectory of HIV disease, viral characteristics such as mutation rates, and if alcohol use impacts viral set points or viral fitness. As new tests for HIV infection are developed, it is expected that these tests will be able to more rapidly identify an individual's HIV status closer to the infection event. This will allow the biological and behavioral role of alcohol to be more clearly understood. (Bagby et al, in press) The development of new tests will also provide an opportunity to evaluate the role of viral set points on disease course among HIV-infected individuals. In addition, investigators will be able to measure multiple dimensions of viral activity, including mutation accumulation, to track changes in the rate of viral proliferation and other important biological processes.

Expansion of Integrative Biomedical Research Paradigm

In the area of therapeutics research, current drug regimens have resulted in extended survival and improved quality of life for many HIV-infected individuals in the U.S. and Western Europe. However, a growing proportion of patients receiving therapy are demonstrating treatment failure, experiencing serious drug toxicities and side effects, and developing drug resistance. The increasing incidence of metabolic disorders, cardiovascular complications, major organ dysfunction, and physical changes associated with current antiretroviral drugs underscores the critical need for new and better treatment regimens. Improved regimens also are needed to treat HIV coinfections such as hepatitis B and C, as well as other opportunistic infections, and to reduce drug interactions and problems with adherence to complicated treatment regimens. The goal of this research is to develop new, safe, less toxic, less expensive, and more effective therapeutic agents and regimens. Research in this area will require the development and collection of new datasets to implement both prevention and treatment strategy trials. These types of trials are described in the recent IOM report on poverty and starvation (IOM, Global Strategies for Intervention **(**2005**)** and are contrasted with standard Randomized Controlled Clinical Trials (RCT). The goal of these trials is to meet the immediate need to address critical disease issues in developing countries in a timely and cost-effective way using appropriate research methods which meet ethical considerations.

Figure 4. (From Braithwaite et al, 2007)



An example of this simulation approach is given in a recent poster presented at the 2006 Research Society on Alcoholism Meeting (Braithwaite, submitted for publication) in which investigators estimate the proportion of patients infected with HIV who will die of comorbid illness and alcohol. More specifically, time to failure of medication regimens and risk of death are modeled using non-hazardous and hazardous levels of alcohol use. The direct effects of alcohol on adherence as a mediator of multiple biological parameters (e.g., viral resistance, viral mutation, etc.) are used to predict treatment failure and death. In this model (based on the CHORUS sample) alcohol use is linearly related to medication failure and lost years of life. (See figure below.)

Graph 1. (From Braithwaite et al, 2007)

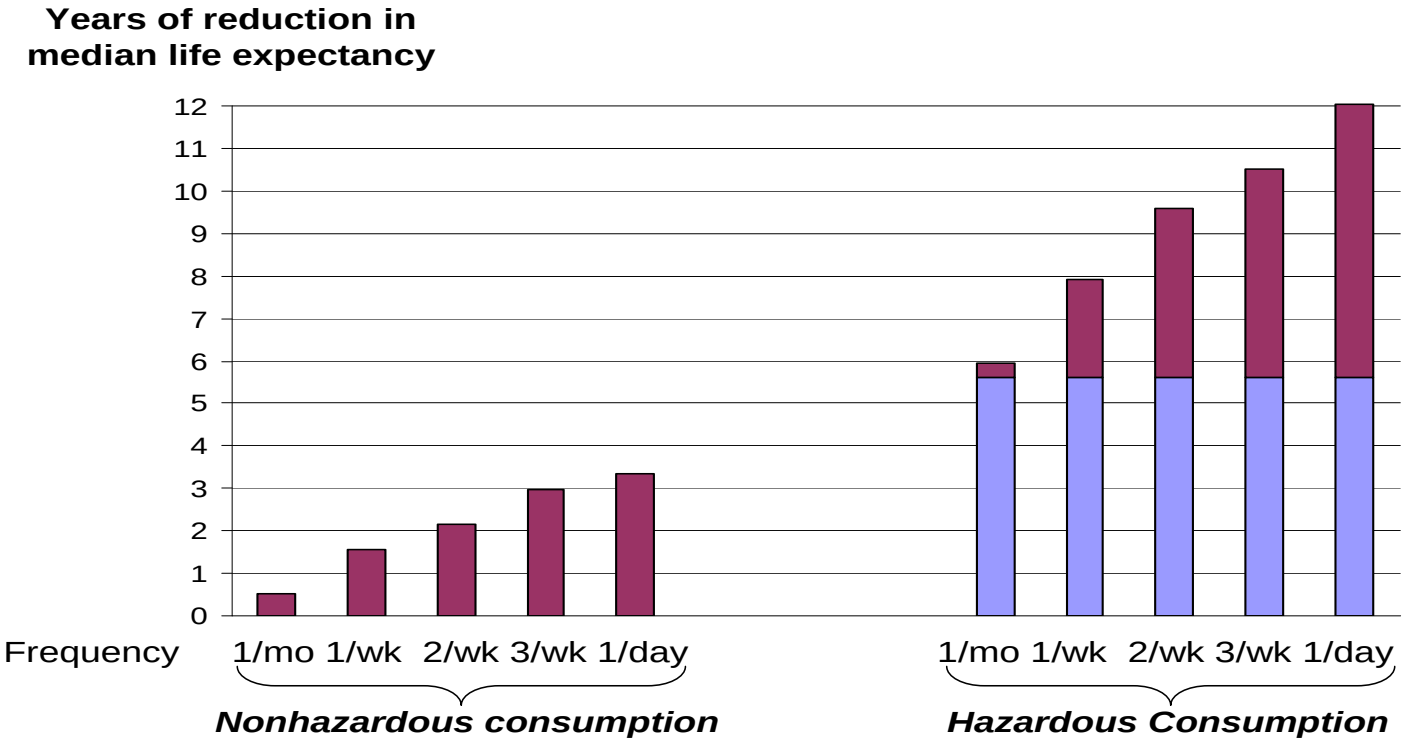


Figure 3. Premature death from alcohol consumption (Braithwaite et al, 2005)

Red shaded regions denote the effect that is attributable to alcohol consumption itself, and blue shaded regions denote the effect that is attributable to confounding factors rather than to alcohol consumption. Decreased time to treatment failure among drinkers leads to decrements in survival time. While a substantial proportion of the decrement in survival time experienced by hazardous drinkers arises from confounding factors rather than from the hazardous drinking itself, the decrement attributable to hazardous drinking is greater than the decrement attributable to nonhazardous drinking at each frequency.

Opportunities for Research

- Characterize association of alcohol and other host factors with survival among those with and without HIV infection.
- Characterize variation in survival by alcohol and HIV status, and other related comorbidities and organ injury (e.g., liver, lung, brain, etc.).
- Determine the benefits and harm of ARV's by varying mixtures of acute and/or chronic alcohol use and alcohol-related organ injury.
- Determine if patient pretreatment alcohol use characteristics justify altering timing of ARV treatment.

IV.

ETIOLOGY AND PATHOGENESIS

IV. STRATEGIC PLAN AREA: ETIOLOGY AND PATHOGENESIS

A. Scientific Issues

Tremendous progress has been made in understanding the fundamental steps in the life cycle of HIV, the host-virus relationship, and the clinical manifestations associated with HIV infection and AIDS. Groundbreaking research on basic HIV biology and AIDS pathogenesis has revolutionized the design of drugs, the methodologies for diagnosis, and the monitoring for efficacy of antiviral therapies. Maintaining a strong commitment to basic research is of paramount importance in our fight against HIV/AIDS. This research is focused on gaining a better understanding in two areas: (1) how HIV infection is established and maintained, and (2) what causes the profound immune deficiency and severe clinical complications that accompany this infection.

Critical questions in this area are: What role do specific HIV proteins play in the viral life cycle in individual cells and within the bodies of infected individuals? What are the primary modes of HIV transmission between cells and between individuals? What contribution does the immune system make to controlling the infection and to the disease process? What mechanisms are involved in cell injury and death in the immune, nervous, and other organ systems affected by HIV? What host factors and cofactors influence primary infection and the course and outcome of HIV infection? What is the relationship of HIV infection to the associated malignancies, OIs and coinfections, neurological impairments, and metabolic disturbances that characterize AIDS? This basic knowledge is critical for our efforts to prevent and control HIV infection and disease progression.

Research is focusing on the different mechanisms of viral persistence to understand the reasons for drug failure, to design rational approaches for virus eradication, and to better assess the impact of persistence on HIV transmission and its implications for HIV prevention. HIV can persist in a latent reservoir of resting memory CD4 T cells that is established very early after infection and by continuously replicating, albeit at very low levels, even in the presence of antiretroviral therapies that can drive viral load below the limits of detection.

Understanding the normal development and functioning of the human immune system is crucial to our ability to understand the effects of HIV on the immune system and the pathogenesis of AIDS. This understanding also holds the key to designing rational immune reconstitution approaches in persons undergoing antiretroviral treatment and identifying the characteristics of the immune response that are needed for a protective vaccine.

The basic science underlying HIV etiology and pathogenesis research is generally gender neutral. Basic mechanisms of viral replication and pathogenesis are not expected to differ in women and men. However, there are differences in the way HIV infection is transmitted and how the disease is manifested in women and men. Studies have been designed to elucidate the pathogenic mechanisms more commonly observed in women, children, and adolescents infected with HIV. Transmission of HIV from a mother to her infant may occur in utero through transplacental passage of virus, during delivery, or

after birth through breastfeeding. Many basic research questions associated with maternal-fetal transmission remain unclear and are actively under investigation.

AIDS is associated with a broad spectrum of cancers and tumors. As HIV causes immunosuppression and most AIDS-associated malignancies are strongly associated with viruses, HIV infection provides a unique model to study the interplay of viruses, a dysfunctional immune system, and the development of cancers. Elucidation of the interactive factors involved in the pathogenesis of AIDS-associated malignancies will possibly translate into the identification of new targets for prevention and treatment.

HIV infection results in the progressive damage of the immune systems of infected individuals and makes them susceptible to a diverse collection of bacteria, viruses, fungi, and protozoa that represent the major causes of suffering and death for HIV-infected individuals. Opportunistic infections remain one of the most important complications of HIV infection and the principal cause of death in AIDS patients. Understanding the fundamental biology and pathogenesis of these organisms, their interaction with the host immune system, and the effect of therapy-associated immune reconstitution on the clinical course and manifestations of OIs will translate into new or more rational approaches to the prevention and treatment of OIs in patients on ART, as well as in patients who lack access to or who are not responding to ART. HIV infection is also associated with multiple endocrine alterations, probably reflecting critical interaction between the endocrine and the immune systems. HIV-associated hematologic, pulmonary, cardiac and vascular, renal, mucocutaneous, bone, and liver complications also represent causes of morbidity in infected subjects. Some of these complications are disproportionately affecting certain racial groups. The pathogenic mechanisms involved in all these AIDS manifestations are not well understood and are under investigation. Their definition will permit a more rational approach to both preventive and therapeutic interventions.

B. NIH Research Priorities of the FY 2007 Plan

- **Facilitate the translation of new insights into HIV biology to develop novel interventions for the prevention and treatment of HIV infection. Identify and validate cofactors for viral genes as new targets capitalizing on novel technologies including RNA interference and genomic screening.**
- **Elucidate the biologic determinants of HIV transmission between individuals, and define the mechanisms by which host factors, viral factors, and cofactors may influence the process of HIV transmission and dissemination.**
- **Understand the dynamics of virus-host interaction through the course of HIV infection.**
- **Investigate the mechanisms of persistence of HIV infection.**
- **Develop innovative technologies in human and nonhuman primate immunology to guide HIV prevention and immune reconstitution efforts in HIV-at-risk/infected individuals.**

- **Advance the understanding of the mechanisms responsible for the toxicities and long-term complications of ART and the factors that underlie changes in the causes of morbidity and mortality in HIV-infected patients in an era of increasingly effective therapies.**

C. Special Emphasis Area: Alcohol and HIV-Induced Immune Modulation

1. Introduction and Background

Studies conducted over the past 20 years have extensively documented the immunosuppressive effects of alcohol. Excessive alcohol consumption has clearly been shown to be associated with an increased risk of infection with human immunodeficiency virus (HIV) and microorganisms. Both alcohol and HIV modulate innate and adaptive immunity by disrupting cytokine and chemokine signaling, depleting crucial immune cell populations, inhibiting maturation of key immune effectors such as dendritic cells, and impairing CD4+ T helper function, and antiviral cytotoxic T cell activity. Viral products, as well as alcohol, inhibit antigen presentation, a key step in the initiation of the adaptive immune response. Alcohol consumption has been shown to increase HIV replication. In these and other ways, such as increasing oxidative stress, alcohol and HIV negatively impact on immune processes, thereby increasing susceptibility to co-infection with other viral pathogens in the liver and viral and bacterial pneumonias within the lungs. Alcohols' effects on immune function are evident in the systemic circulation and more recently, its effects on organ-specific immunity, particularly in the liver, lung, and brain, have begun to be appreciated. To date, most work has been done in the liver. Chronic ethanol consumption impairs lymphoproliferative responses in the liver during viral infection. Lymphocyte trafficking to the liver and NK and NKT cell frequency in the liver are likewise impaired in alcoholics, resulting in increased incidence of coinfection with HBV and HCV. HIV and HCV viral replication is enhanced by alcohol in vitro and in peripheral blood mononuclear cells (PBMCs) obtained from alcoholics. Furthermore, alcohol decreases viral clearance, thus facilitating the establishment of viral persistence and leading in some cases to chronic disease. By compromising innate immunity in particular, alcohol consumption functions as a co-factor in the immunopathogenesis of HIV disease.

Acute and chronic alcohol abuse have been implicated as cofactors in the immunopathogenesis of HIV disease. HIV strains vary in their susceptibility to alcohol. HIV R5 preferentially targets macrophages. Infectivity with the HIV R5 strain, but not other strains, is enhanced by alcohol. Exposure to ethanol in cell culture facilitated entry of HIV R5 into macrophages by up-regulating the receptor on the macrophage membrane that mediates HIV R5 entry. Furthermore, alcohol substantially diminished the production of signaling molecules by macrophages, further compromising the ability of these key players to initiate and integrate innate immunity, the first line of defense against invading pathogens. These studies reveal several ways in which alcohol facilitates viral entry and persistence. A recent report demonstrates that naltrexone, an

opioid receptor antagonist widely used in the treatment of alcoholism, inhibits alcohol-mediated enhancement of HIV infection of T cells. Alcohol was shown to enhance HIV infection of peripheral blood lymphocytes (PBL) and a human lymphoid cell line, an effect that was abolished upon the addition of naltrexone. Investigation of the underlying mechanism demonstrated that alcohol activated endogenous opioid peptides resulting in the induction of opioid receptors. These findings suggest a biological mechanism for the potential therapeutic benefit of naltrexone in treating HIV-infected alcoholics.

The host immune response to viral infection involves distinct mechanisms chiefly dependent upon recognition of viral nucleic acids. Hepatotropic viruses, such as HBV and HCV, are detected primarily by the toll-like receptor (TLR) viral pattern recognition pathway. Not surprisingly, viruses have evolved multiple immune evasion strategies at both the innate and adaptive levels. Research has demonstrated that certain viral proteins specifically target discrete host defense reactions. To cite examples, HBV core protein inhibits transcription of IFN-β, the body's natural antiviral substance. The HCV envelope protein, env2, blocks NK cell activation, cytokine production, and cytotoxic granule release. HCV core protein has also been studied extensively. Among its effects, HCV core expression produces changes in gene transcription, signal transduction, antigen presentation, cell cycle regulation and apoptosis. HCV core expression has a direct effect on mitochondrial function, resulting in decreased mitochondrial glutathione (GSH) levels and oxidative stress. Introduction of a second environmental stressor such as alcohol has significant additional functional consequences. Exposure of HCV core-expressing liver cells to 25nM ethanol for 24 hours resulted in exacerbation of mitochondrial ROS production and GSH depletion, enhancement of mitochondrial depolarization, and an increase in oxidant-induced cell death. Similarly, alcohol feeding of transgenic mice expressing HCV core proteins resulted in a greater degree of steatosis and lipid peroxidation and a greater degree of mitochondrial GSH depletion. In addition, alcohol potentiates NF-κB activation by HCV core protein in primary hepatocytes. Thus, HCV core protein and alcohol, separately and in combination, induce a state of mitochondrial oxidative stress. There is an energetic effort to determine structure/function relationships for viral proteins, especially HIV, HCV and HBV proteins, because of their potential as therapeutic targets.

Alcohol Use and the HIV/HCV Co-Infected Patient

Viral hepatitis coinfection and alcohol abuse are the major risk factors for progression of liver disease to end stage liver disease and death in the HIV-infected patient. Steatosis, a harbinger of fibrosis progression, is more common and more severe in HIV/HCV coinfected patients than in those with HCV monoinfection. Mechanisms currently invoked to explain these observations include a greater level of immunosuppression and oxidative stress in HIV infected patients, younger age at time of infection with HCV, and alcohol-related enhanced viral replication. Antiretroviral therapy (ART) appears to be fairly well tolerated in HIV/HCV coinfected patients, however continued long-term use is beginning to reveal various associated toxicities. Anti-HIV drugs have been shown to alter mitochondrial (mt) function by inhibiting the activity of the enzyme responsible for replication of mtDNA and affecting the electron transport chain. Furthermore, treatment

with protease inhibitors (PI) induces severe liver damage. The combination of drugs used to treat the coinfected patient induces severe mitochondrial toxicity which is exacerbated by alcohol consumption. A paradoxical and unanticipated outcome of ART is the worsening of liver fibrosis/cirrhosis as a result of improvements in the immune system – referred to as immune reconstitution hepatitis - which further complicates the medical management of the coinfected patient.

2. Current Research

Much early NIAAA funded research focused on understanding the role of alcohol in impaired immune function and the need to determine whether alcohol was a significant contributor to disease progression of HIV to AIDS. Program announcements and targeted RFA's have been developed over the last 15 years in this line research. These announcements have focused on invitro studies of ETOH consumption in the etiology and pathogenesis of AIDS and form the basis for a new understanding of the role of alcohol in innate and acquired host defenses. As a result of this research we are continuing to develop our understanding of the role of alcohol over time as a "host" factor (among multiple host factors) in promoting the susceptibility to infection with HIV, the progression of the illness to AIDS in the context of continued drinking, and the resulting tissue and organ damage which impacts the effective treatment of AIDS. This evolving research includes our better understanding of the role of suppressed immunological function, viral replication and disease course, cytokine expression in inflammatory responses, and ultimately cell death and organ injury. The potential identification of primary mechanisms of action is also discussed, as is the process of establishing appropriate measures for each of these mechanisms and relating them to clinical outcomes.

3. Research Opportunities

Genomics and proteomics

- Gene expression profiling of liver biopsies from HCV and HCV/HIV-infected patients in conjunction with examination of alcohol consumption quantity/frequency patterns.

- Alcohol and virally-induced changes in the hepatocyte nuclear and mitochondrial proteome.

- Alcohol effects on genes regulating interferon production. Identification of signaling pathways involved in interferon production and alcohol-induced changes using proteomic approaches.

- Intrahepatic genomic host response to HCV viral entry, spread, and clearance in alcohol-consuming and abstaining individuals.

- Global gene expression patterns associated with alcohol consumption in human liver from AIDS patients coinfected with HCV.

- Anti-HCV and anti-HIV immune response expression profiles using proteomic approaches; Effects of alcohol on viral quasispecies.

- HIV/HCV associated perturbations in lipid metabolism in animal models of alcoholic liver disease.

- Identification of biomarkers for liver disease progression as well as novel targets for antiviral treatment.

- Drug-alcohol interactions in the coinfected alcoholic.

- Effect of alcohol on viral vaccine efficacy.

D. Special Emphasis Area: Mucosal Immunity

1. Introduction and Background

The first few weeks following infection with HIV appear to be a critical time period in which the stage is set for immune system failure, the hallmark of HIV disease. The focus of HIV-associated immune dysfunction has shifted from central and peripheral lymphoid organs to extra-lymphoid tissue at sites located in proximity to vulnerable ports of entry, such as the mucosal surfaces of the lung and gastrointestinal tract. It is here that primary infection, predominantly of CD4+ effector memory T cells and viral amplification occurs, followed by dissemination via dendritic cells to draining lymph nodes. Alcohol consumption has been shown to increase HIV replication and alter the cytokine milieu. In an animal model of HIV infection, SIV-infected Rhesus macaques, viral loads are significantly higher in the plasmas of animals consuming alcohol. Until recently, the mechanism of HIV replication and CD4+T cell turnover in response to alcohol in extra-lymphoid tissue had not been studied. New studies conducted in the SIV animal model have begun to decipher the effects of alcohol on SIV pathogenesis. These studies have demonstrated an alcohol-induced expansion of the CD4+T cell pool, the primary target of HIV/SIV, in mucosally inoculated macaques. Thus, changes in the mucosal immune compartment in response to alcohol are likely the major reason behind the higher replication of SIV observed in these animals.

2. Current Research: Alcohol-induced Effects on Membrane Permeability and Mucosal Barrier Function

Alcohol's effect on HIV/SIV pathogenesis may also be related to its effect on membrane permeability and epithelial barrier function. Alcohol use causes marked changes in gastrointestinal structure and function. Alcohol is known to increase intestinal permeability to endotoxin resulting in increased transfer of endotoxin from the intestine to the portal vein and general circulation. Persuasive evidence indicates that endotoxin plays a central role in the initiation of alcoholic liver injury via activation of Kupffer cells, resulting in up-regulation of inflammatory mediators such as cytokines, chemokines, and adhesion molecules. Endotoxin may also contribute to the development of other alcohol-associated tissue/organ injuries such as pancreatitis, acute respiratory distress syndrome (ARDS), and brain injury. Thus, restoring intestinal barrier integrity by blocking the transfer of endotoxin from intestine to the systemic circulation may mitigate alcohol-associated tissue/organ injuries. Researchers are trying to understand the underlying molecular mechanisms by which alcohol increases intestinal permeability to endotoxin. Currently, two mechanisms are receiving scrutiny: 1) acetaldehyde-induced tyrosine phosphorylation of intestinal mucosal tight junction and adherence junction proteins; and 2) alcohol-induced upregulation of nitric oxide in the intestine. Thus, it would be informative to clarify the relationship between endotoxin release from the intestine and changes in lymphocyte subsets/function in extra-hepatic tissues. Conceivably alcohol-induced changes in intestinal permeability and subsequent translocation and endotoxin release may initiate immune-mediated events at distant organ

sites in addition to the liver. Changes in mucosal surfaces and protective barrier function in chronic and acute alcohol abuse is an under-explored area.

3. Research Opportunities

- Effect of alcohol on extra-lymphoid immune effector cell populations, (lymphocyte subset destruction, regenerative and proliferative capacity, maturation, and migration) particularly those found in tissues at mucosal surfaces, i.e., lung and gastrointestinal tract in HIV-infected patients and animal models of HIV infection.

- Effect of alcohol on HIV co-receptor (CCR5) expression by extra-lymphoid immune effector cell populations.

- Viral replication in extra-lymphoid immune effector cells following alcohol consumption.

- Viral translocation across mucosal surfaces as a function of alcohol-induced changes in mucosal barrier function.

- Molecular mechanisms by which alcohol disrupts membrane permeability and barrier function at vulnerable ports of entry such as the mucosal surfaces of the lungs and gastrointestinal tract.

E. Special Emphasis Area: Alcohol and Host Defense: A New Role for Mitochondria in Coordinating the Anti-Viral Immune Response

1. Introduction and Background

The innate immune response serves as the first line of defense against invading microorganisms. Recently a spate of new discoveries has uncovered an unexpected role for mitochondria in coordinating the innate immune response. The earliest phase of the host immune response is initiated through members of the Toll-like receptor (TLR) family, which recognize specific topographical features of invading microorganisms. TLR-3, TLR-7, and TLR-9 signaling is involved in antiviral recognition whereas other members of the TLR family are involved in bacterial recognition. TLR act through recruitment of adaptor proteins to form the multiprotein complex known as an enhanceo,some which is involved transcription factor regulation. For example, TLR-3 signaling results in the activation and translocation of NF-κB and interferon regulatory factor (IRF-3) to the nucleus. NF-κB translocation activates several hundred proinflammatory genes. Likewise, IRF-3 translocation activates a large set of antiviral genes whose products limit infection by suppressing viral replication and modulating adaptive immunity. Both NF-κB and IRF-3 are required for production of the type I interferons, IFN-α and IFN-β. However, TLR-3 signaling is not the only pathway by which viral pathogens are recognized. Inside the cell, the presence of viral nuclei acids (dsRNA) is detected by an entirely different sensing mechanism involving the recently described retinoic acid inducible gene 1 (RIG-1) pathway. While the TLR-3 and RIG-1 signal through separate transduction pathways, both activate NF-κB and IRF-3, and ultimately result in the production of proinflammatory and antiviral products. There appears to be consensus that the RIG-1 pathway, rather than TLR-3, is the predominant pathway by which interferon production is mediated. The molecular components in these signaling pathways - receptor, effector, and adaptor proteins, and their function and regulation in different cell types in response to various microbial pathogens need to be more fully illuminated.

2. Current Research: Mitochondrial Anti-viral Signaling Pathways

Several groups of investigators have begun to identify the molecular participants in the mitochondrial antiviral pathway and to describe specific features of viral attack upon them. RIG-1 and MDA5 (melanoma differentiation-associated gene 5) are cytosolic RNA helicases that sense viral presence by recognizing the early-replication intermediate, intracellular dsRNA. RIG-1 and MDA5 contain caspase recruitment domains (CARD) which permit interaction with other proteins containing homotypic CARD motifs. The newly identified RIG-1 adaptor protein located on the outer mitochondrial membrane has a CARD motif though which it binds to RIG-1. This adaptor protein (termed mitochondrial antiviral signaling proteins, MAVS; interferon promoter stimulator, IPS; virus-induced signaling adaptor, VISA; and caspase recruitment domain - interferon, Cardif by four independent groups) connects RIG-1 to

downstream signaling and gene activation. Signaling through the IKKε complex, RIG-1 ultimately activates the transcription factors, NF-κB and IRF-3, resulting in enhanced transcription of type I interferon. The presence of MAVS/IPS-1/VISA/Cardif was inferred because NS3/4A, the major serine protease expressed by HCV, did not bind to or inactivate RIG-1. Subsequently, it was shown that the adaptor protein (MAVS, IPS, VISA, Cardif) was the NS3/4A binding target. NS3/4A binding cleaved the adaptor from the outer mitochondrial membrane releasing it into the cytosol thereby truncating the signaling between RIG-1 and NF-κB /IRF-3 and suppressing the induction of type I interferon. NS3/4A also disables TLR-3 signaling in response to extracellular dsRNA by cleavage of a critical adaptor protein in this pathway. The essential nature of this adaptor in mitochondrial antiviral signaling has been demonstrated using various technologies including siRNA and knockdown methods. Recently it has been demonstrated that inhibition of the HCV protease NS3/4A restores RIG-1 signaling and prevents the accumulation of viral proteins in infected cells. Together these findings demonstrate a heretofore unrecognized role for mitochondria in innate immunity as well as revealing new viral evasion strategies. A better understanding of alcohol's effects on mitochondrial coordination of the host immune response pathways will help to identify new targets for future research and novel therapeutic approaches.

3. Research Opportunities

- The role of mitochondria in coordinating signaling pathways in innate and adaptive host defense and alcohol/HIV effects that disrupt these pathways.

- The effect of alcohol/HIV on TLR dependent and TLR independent antiviral host defense pathways.

- Alcohol's effects as a positive or negative regulator of shared adaptors, receptors, and effectors that comprise the complex signaling networks regulated by mitochondria in antiviral host defense.

- Effect of alcohol on innate cellular antiviral defenses and how that influences mitochondrial sensing and initiation of protective responses against HIV infection.

- Effect of alcohol/HIV on cellular oxidative stress and the role of oxidative stress in relation to viral immune evasion strategies.

- Effect of alcohol on the key steps by which HCV/HBV evades the host immune response particularly those functions mediated through mitochondrial signaling.

- Effect of alcohol on anti-viral vaccine efficacy.

F. Special Emphasis Area: Alcohol, HIV Infection and the Nervous System (CNS)

1. Introduction and Background

Hazardous alcohol consumption, defined as large intake that increases risk of health and social problems (Saitz, 2005), is a common problem in HIV patients whether they are being treated in private clinics in the US, veterans within the VA Healthcare system, or Africans receiving care in rural and hospital based clinics in West Kenya (Justice, et al., 2006). Hazardous and binge drinking were prevalent in all three of these cohorts, overall and among subsets of men, women, over and under 50, and of black, Hispanic and white race. The rates of self-reported hazardous drinking were not stable, but increased from 24.6% at baseline to 34.3% at one or more of three serial time points. Given that HIV infection and chronic alcohol use independently affect the central nervous system in relatively high proportions (see e.g., Evert, et al, 1995; McArthur, et al., 2003), these high rates of hazardous drinking in HIV infected individuals have implications for the potential interaction of alcohol and HIV on central nervous system (CNS) morbidity, including the decline of cognitive abilities and brain structure and function. Hazardous or chronic heavy drinking may put individuals at risk for the combined effects of alcohol and HIV infection on the CNS in the following ways (see e.g., Meyerhoff, 2001; Pfefferbaum, et al., 2002): 1) suppression of normal immune responses to initial HIV exposure or enhancement of immunosuppression in HIV-infected individuals; 2) interference with treatment seeking and treatment adherence in HIV-infected people; 3) possible disruption of bioavailability of antiretrovial medications in those with HIV infection; and 4) impairment of behavioral inhibition and promotion of risky behaviors such as unprotected sex which can lead to HIV exposure or re-exposure.

Despite the advent of highly active antiretroviral therapy (HAART) in the mid 1990's and a reduction in AIDS-related CNS opportunistic infections, there as been a recent resurgence in the frequency of HIV encephalitis, HIV leukoenceophalopathy, and cognitive impairment in persons on stable antiretroviral therapy (Langford, et al., 2003; Navia and Rostasy, 2005; Sacktor, 2002). The emergence and progression of HIV-associated brain damage and cognitive dysfunction in patients on long-term antiretroviral regimens may result from the additive and/or interactive effects of multiple factors including the development of drug resistance, hepatitis C, nutritional factors, alcohol and/or drug abuse, chronic retroviral treatment, and aging (Langford, et al., 2003; Navia and Rostasy, 2005). Therefore, because chronic alcohol use may be an important contributor to CNS disease in HIV infected individuals, it is important that we study the additive and/or interactive effects of both conditions on brain structure and function as well as the specific biological and psychosocial mechanisms for these effects.

Neuropathological changes in alcoholism and HIV infection

HIV infection and chronic alcohol use each independently produce neuropathological changes in the brain. Direct HIV associated neuropathologies (and not those due to opportunistic infections) include formation of multinucleated giant cells in the

parenchyma and perivascular areas, myelin pallor, astrogliosis, microgliosis, and microglial nodule formation (See Everall, et al., 2005; Langford, et al., 2003; Meyerhoff, 2001; Navia and Rostasy, 2005; Pfefferbaum, et al., 2002 for reviews). White matter damage typically consists of focal demyelination, astrogliosis, mild infiltration by macrophages, and axonal degeneration. Neuronal loss, injury to the synaptic dendritic tree, and apoptosis of neurons and non-neuronal cells have also been observed. Most lesions are found in the basal ganglia, frontal cortex and white matter, but the hippocampus, thalamus, brainstem, cerebellum, and corpus callosum are also involved. Evidence on the neuropathogenesis of HIV induced brain injury suggests that the HIV virus does not affect neurons directly, but that the HIV-infected monocytes/macrophages cross the blood brain barrier, and then activate other neuron-inflammatory cells such as microglia and astrocytes. These cells produce chemokines, cytokines and other neurotoxic products (gp120, gp41, nitric oxide, excessive glutamate) that lead to neuronal death possibly via apoptosis (see Everall, et al., 2005; Gonzalez-Scarano and Martin-Garcia, 2005; Kaul, et al., 2005; Lawrence and Major, 2002; Rumbaugh and Nath, 2006, for reviews).

Neuropathological changes associated with chronic alcoholism (see Harper, 1998; Pfefferbaum, et al., 2002 for reviews) are reductions in gray and white matter in the cerebral cortex, with notable neuronal loss in the superior frontal cortex. Alcohol-related neuronal loss and/or white matter loss has been found in subcortical regions including the thalamus, mammillary bodies, hippocampus, cerebellar vermis, and corpus callosum. Dendritic and synaptic changes have also been observed in alcoholics (Harper and Corbett, 1990). In addition to volume reduction, white matter changes include demyelination, loss of myelinated fibers, and axonal deletion (Sullivan and Pfefferbaum, 2005). Thiamine deficiency and liver disease may also complicate the picture of alcohol-induced neuropathology because these conditions produce additional neuropathological changes in additional structures (basal ganglia, basal forebrain, and brainstem). The mechanisms for alcohol, nutritional, and/or-liver induced brain injury may overlap to a certain extent with the mechanisms of HIV neuropathogenesis; ie., oxidative stress, inflammatory cytokines, and excitoxicity (Butterworth, 1994; Crews, et al., 2004; Martin, et al., 2003). Therefore, alcohol may interact with HIV in infected individuals to increase CNS morbidity.

HIV-associated Sensory Neuropathies

HIV-1 infection and treatment: Although the introduction of HAART has resulted in a decline in the incidence of HAD, the incidence and prevalence of peripheral nervous system complications resulting from HIV-1 infection has remained high. It is thought that the incidence of HIV-1 associated peripheral neuropathies is 30-40%, and the prevalence of neuropathy or PNS deficits as determined at autopsy is close to 100%. Many types of peripheral neuropathies are specific to HIV-1 infection, and the occurrence of each appears to depend on the stage of infection (Hoke and Cornblath, 2004). This section will focus exclusively on sensory polyneuropathies that result from both HIV-1 infection and anti-retroviral therapy. HIV sensory polyneuropathies usually develop in the later stages of the disease (advanced-symptomatic HIV infection).

The two common forms of sensory polyneuropathies are distal symmetric polyneuropathy (DSP) and antiretroviral toxic neuropathy (ATN). DSP occurs with advanced HIV-1 infection. ATN is a form of DSP that results from neurotoxicity caused by antiretroviral therapy. The clinical features of both DSP and ATN are so similar that the primary way to distinguish between each is to consider the recent history of nucleoside analogue reverse transcriptase inhibitor (NRTI) use. In both DSP and ATN, the initial symptoms are pain and burning sensations that begin in the toes and feet, and progress up the legs. The disease rarely extends to the hands. Examination is used to confirm the distal loss of sensory function.

The prominent pathological feature of DSP and ATN is primarily distal axonal degeneration of unmyelinated fibers, although myelinated fibers are also affected. Demyelinated and remyelination rarely occurs. Degeneration is largely limited to distal axons, as minimal degeneration of dorsal root ganglia (DRG) neurons is observed.

The mechanisms of HIV-1-associated DSP are unknown. One hypothesis is that neurotoxicity is caused by the actions of viral proteins, such as gp120. Evidence supports both indirect and direct actions on sensory neurons for gp120-induced neurotoxicity. It has been shown that the binding of gp120 to the CXCR4 chemokine receptor of Schwann cells causes the release of a CCR5 ligand, RANTES. This ligand in turn binds to CCR5 receptors of sensory neurons causing TNF-α mediated neuritic degeneration and apoptotic cell death (Keswani et. al., 2003a). Additionally, it has been shown that gp120 can bind directly to chemokine receptors on sensory neurons thereby triggering intracellular calcium signaling that result in neurotoxicity (Oh et. al., 2001). The mechanism underlying ATN appears to be through the ability of antiretroviral agents to cause mitochondrial dysfunction and energy failure (Keswani et. al., 2003b).

Alcohol Polyneuropathy

Polyneuropathy is also a frequent complication of chronic alcoholism. Reports vary as to the exact incidence of alcoholic polyneuropathy, which may be approximately 50%. For example, Zambelis et. al. (2005) reported that polyneuropathy was diagnosed in 58.2% of the study's subjects, and Vittadini et. al. (2001) reported that 48.6% of the subjects had some degree of alcoholic polyneuropathy. The total lifetime amount of alcohol consumption and the duration of excessive alcohol use are associated with the clinical expression of alcoholic polyneuropathy.

The clinical symptoms of alcoholic polyneuropathy are similar to those shown for HIV-associated sensory neuropathies (Vittadini et. al., 2001). When presented, the initial symptoms are distal pain and burning sensations of the feet. Sensory symptoms may progress up the legs. In severe cases, there may be sensory loss and tingling in the hands. Furthermore, and in keeping with its similarity to DSP and ATN, the main pathologic feature of alcoholic neuropathy is distal axonal degeneration involving both myelinated and unmyelinated fibers (Koike et. al., 2001). Indeed, alcohol abuse and dependence is a

significant risk factor in the development of sensory neuropathy in HIV-infected individuals (Lopez et. al., 2004).

The pathogenesis of alcoholic polyneuropathy is little understood, but may result from a complex series of interactions that include direct ethanol neurotoxicity, nutritional deficiencies resulting from chronic, excessive alcohol abuse, and perhaps genetic susceptibility. The role of nutritional deficiencies that result from chronic alcohol abuse, especially the role of thiamine deficiency, in the development of alcoholic peripheral neuropathy has been difficult to separate from the study of direct neurotoxic actions of alcohol because of the strong association of nutritional deficiencies in chronic alcoholics. However, a study of alcoholic polyneuropathy in patients with normal thiamine status found that sensory-dominant involvement with prominent neuropathic pain was characteristic of alcoholic neuropathy in the absence of thiamine deficiency (Koike et. al., 2001). This study supports a role of a direct neurotoxic effect by alcohol or its metabolites in the development of alcoholic polyneuropathy.

The biological mechanisms for the direct effect of alcohol neurotoxicity in causing alcoholic polyneuropathy are not known and need to be fully explored. Given that excessive alcohol exposure can produce oxidative stress and the generation of reactive oxygen species similar to the consequences of exposure to antiretroviral agents, it is tempting to postulate that ethanol-induced mitochondrial dysfunction may serve a role in the development of alcoholic polyneuropathy. Supporting a role for both genetic predisposition and in particular the accumulation of the ethanol metabolite acetaldehyde, Masaki et. al. (2004) have found that individuals carrying a specific polymorphism of the aldehyde dehydrogenase-2 gene that has reduced ability to metabolize acetaldehyde may be at increased risk for developing alcoholic polyneuropathy. Employing an animal model to study nociception due to alcoholic neuropathy, Dina et. al. (2000) have reported that protein kinase C signaling pathways mediate the pain response of alcohol-induced hyperalgesia.

The need for further research on the biological mechanisms of alcohol-related polyneuropathy is even more compelling in the face of polyneuropathies associated with HIV-1 infection and antiretroviral therapies. The similarities in clinical presentations and neuropathologies suggest that there may be a common, perhaps synergistic mechanism underlying the development of sensory polyneuropathy. However, research on HIV-1 associated DSP also suggests that other independent mechanisms may underlie the development of sensory polyneuropathy. Elucidating the independent and possibly synergistic mechanisms by which alcohol, HIV, and antiretroviral medications damage peripheral nerves will set the stage for the development of new therapeutics and significant improvements in quality of life for affected individuals.

2. Current Research

Interactive effects of alcohol and HIV infection on the CNS: Evidence from neuroimaging

There is now a relatively large literature using multiple imaging modalities to define the separate structural and functional consequences of HIV infection or chronic alcohol consumption on the brain. HIV infected individuals have been found to have abnormalities in the basal ganglia, frontal cortex and white matter, as well as other cortical and subcortical regions using magnetic resonance imaging (MRI), proton magnetic resonance spectroscopy (MRS), positron emission tomography (PET), diffusion tensor imaging (DTI), and functional magnetic resonance imaging (see e.g., Meyerhoff, 2001; Navia and Rostasy, 2005; Pfefferbaum, et al., 2002; Thompson, et al., 2005, 2006; Tucker, et al., 2004).

More specifically, MRI detected ventricular enlargement, global atrophy, frontal white matter and caudate volume loss, and cortical and callosal thinning (see Pfefferbaum, et al, 2002 for review; Thompson, et al., 2005, 2006) in symptomatic HIV cases. MRS, which measures brain chemistry by analyzing metabolite concentrations and ratios, has proven to be particularly sensitive to HIV progression. Studies have shown reductions in N-acetyl-aspartate (NAA), a marker of neuronal injury, and increases in glial markers of inflammation and membrane damage, i.e., myo-inositol (MI) and choline (Cho/creatine (Cr) ratios, which correlate with increasing dementia (Meyerhoff, 2001; Navia and Rostasy, 2005; Tucker, et al., 2004). These changes were most prominent in the basal ganglia, frontal cortex white matter, and thalamus. DTI, a relatively new method that examines the integrity of white matter bundles and microstructure by measuring anisotropic diffusion of water molecules, detected subtle white matter changes in tissue of HIV seropositve patients that appeared normal with conventional MRI (see Navia and Rostasy, 2005; Pfefferbaum, et al., 2002; Sullivan and Pfefferbaum, 2003 Tucker, et al., 2004 for reviews). Reductions in white matter anisotropy were found in periventricular white matter, corpus callosum, frontal and parietal white matter, and internal capsule. Furthermore, measures of white matter microstructure correlate well with CD4 counts and viral load, and have been used to identify structural changes in neurologically intact patients (Navia and Rostasy, 2005). FMRI, which has the advantage of simultaneously linking cognitive function to increases or decreases in brain activation, identified increased activation in the parietal and frontal cortices during a working memory task in asymptomatic and symptomatic HIV positive patients (Tucker, et al, 2004). Thus, MRS, DTI, and fMRI may be particularly important for exposing early neurological involvement.

Brain changes following chronic alcohol consumption have also been observed using multiple neuroimaging methods. MRI demonstrated reduced gray and/or white matter volume in the frontal cortex, corpus callosum, temporal lobes, hippocampus, pons, cerebellum, mammillary bodies, caudate, putamen, and n. accumbens (Cardenas, et al., 2005; see Pfefferbaum, et al., 2002; Sullivan, 2000; Sullivan, et al., 2005; Sullivan and Pfefferbaum, 2005 for reviews). As mentioned above, nutritional deficiency has been

implicated in the subcortical changes in the thalamus, mammillary bodies and hippocampus. Cross-sectional MRS studies in chronic heavy drinkers and recently detoxified alcohol-dependent individuals show reduced NAA and/or choline levels in the frontal cortex gray and white matter, thalamus, and cerebellum with increases in myo-inositol in the thalamus, brainstem, anterior cingulate, and frontal and parietal regions (Durazzo, et al., 2004, 2006; Meyerhoff, et al, 2004; Pfefferbaum, et al., 2002; Schweinsburg, et al., 2000, 2001). Continued heavy drinking over two years is associated with longitudinal decreases in NAA, myo-inositol, and choline in white matter, consistent with ongoing axonal injury and demyelination. Recovery from alcoholism produces restoration of brain volume and increases in NAA, myo-inositol, and choline-containing metabolites (Mason, et al., 2005; Sullivan and Pfefferbaum, 2005). Deficits in anisotropy in the corpus callosum of alcoholic men and women were detected by DTI, suggesting disruption of cytoskeletal integrity (see Pfefferbaum, et al., 2002; Sullivan and Pfefferbaum, 2003, 2005 for reviews). FMRI uncovered differences in functional activation of frontal, parietal, and frontocerebellar regions in alcoholics compared to controls during working memory (Desmond, et al., 2003; Pfefferbaum, et al., 2001; Tapert, et al., 2001) or proactive interference tasks (De Rosa, et al., 2004) and inefficiency in cortical cerebellar circuits on a motor task (Parks, et al., 2004).

Taken together, the neuropathological and in vivo neuroimaging data suggest that HIV infection and chronic alcohol abuse affect many of the same brain regions, particularly the frontal lobes, corpus callosum, basal ganglia, brainstem, and cerebellum (see Meyerhoff, 2001; Pfefferbaum, et al, 2002; Sullivan, et al., 2005). However, despite these overlapping areas of damage, to date, only a few studies have used imaging technologies to examine the additive or interactive effects of these two diseases on brain structure and function. A phosphorous MRS study found a cumulative, but not interactive effect of HIV and alcoholism as measured by a stepwise progression of decreased white matter phosphodiester and phosphocreatine (from mild to severe: alcoholism alone, HIV alone, alcoholism + HIV;(Meyerhoff, et al., 1995). An interactive effect of HIV and alcoholism was found using MRSI to assess the integrity of the superior parietal-occipital cortex (Pfefferbaum, et al., 2005). Only the HIV-infected individuals with comorbid alcoholism had significant decreases in NAA and Cr peaks relative to controls, whereas neither alcoholism nor HIV alone resulted in parietal-occipital metabolite abnormalities. Another study (Pfefferbaum, et al., in press) found an interactive effect of HIV and alcoholism on ventricular enlargement and callosal thinning that was dependent on HIV disease progression. In individuals with an AIDS-defining event or low CD4 counts ($<200mm^3$),alcoholism comorbidity significantly exacerbated volume deficits in the corpus callosum and frontal and body regions of the ventricles compared to HIV-infected individuals without AIDS or alcohol abuse. Thus, while there is some evidence that alcohol and HIV produce synergistic effects on brain morbidity, more research is needed to identify the targets of combined vs. separate effects of these diseases on different brain regions in order to have a better understanding of cellular and molecular mechanisms of these interactive effects.

Interactive effects of alcohol and HIV infection on the CNS: Evidence from neuropsychological studies

Neuropsychological deficits have been measured in HIV-infected patients since the early onset of the epidemic when the progressive brain disorder referred to as the AIDS dementia complex (ADC) or HIV-associated dementia (HAD), first emerged. These neuropsychological deficits involve motor functioning, psychomotor skills, information processing speed, sustained attention, verbal and nonverbal learning and memory, executive functions, abstract reasoning, verbal fluency and visuoconstructive skills (Lojek and Bornstein, 2005; see Meyerhoff, 2001; Navia and Rostasy, 2005; Pfefferbaum, et al., 2002 for reviews). Attempts have been made to classify subtypes of HIV-related cognitive functioning based on either "neuropathological" systems, e.g., frontal-subcortical circuitry, or on the severity of cognitive deficits and their interference with daily functioning (Lojek and Bornstein, 2005; Navia and Rostasy, 2005). These latter classification systems, based on a continuum of severity, arose in the era of HAART, which had the effect of producing milder forms of cognitive impairment in which the memory loss, motor processing deficits, and decreases in other functions were less pronounced. However, recent evidence found that cognitive impairment is present in 30% of HIV-infected individuals on stable antiretroviral therapies (see Navia and Rostasy, 2005), suggesting that multiple factors such as aging and alcohol/substance abuse could exacerbate the cognitive impairments in long-time HIV survivors.

There is a long history of research on neuropsychological deficits associated with chronic alcoholism (see e.g., Oscar-Berman, 2000; Parsons, et al., 1987; Pfefferbaum, et al., 2002; Sullivan, 2000 for reviews). These studies have shown that recently detoxified alcoholics have significant deficits on measures of executive functions, short-term memory, visuospatial functions, and gait and balance. Although there is some recovery between one week and one month of sobriety (Durazzo, et al., 2006), deficits persist, particularly in problem solving, short-term memory, visuospatial abilities and perceptual and motor skills (Sullivan, et al., 2000a; 2002) Recovery over longer periods results in continued improvement of certain functions, but in women, the gait and balance problems remain even after several years (Rosenbloom, et al., 2004; Sullivan, et al., 2000b). Chronic heavy drinkers who have never been in treatment also show significant impairment on measures of working memory, processing speed, attention, executive function, and balance (Meyerhoff, et al., 2004).

Research on the combined effects of HIV infection and chronic alcohol abuse on cognition is limited, but the available studies are beginning to more consistently show additive and/or interactive effects of HIV and alcohol on neuropsychological performance. An early study by Bornstein and colleagues (1993) found independent effects of alcohol abuse and HIV infection on neuropsychological performance. However, in a later study, this same group of investigators found that a previous history of alcohol abuse in currently abstinent HIV infected individuals had interactive effects on verbal reasoning, auditory processing, and reaction time (Green, et al., 2004). Electrophysiological measures demonstrated an additive effect of HIV infection and alcohol abuse on the latency of the auditory P3A evoked potential, which is a marker of

frontal lobe function (Fein, et al., 1995, 1998). More recent studies of neuropsychological function found compounded effects of HIV and alcoholism in clinically nondemented individuals on attentional allocation, visuospatial construction, visual immediate memory, and finger dexterity (fine finger movement, Digit Symbol Substitution test; (Eisen, et al., 2004; Sassoon, et al., 2004; Schulte, et al., 2005). In a large sample of African American men (310 HIV-, 187 HIV+) divided into light, moderate, and heavy drinkers, HIV+ heavy drinkers performed more poorly on tests of psychomotor speed, reaction time, and motor speed than the other HIV positive groups (Durvasala, et al., 2006). Finally, in a study of currently heavy and light drinking individuals with either HIV+ or HIV- serostatus (Rothlind, et al., 2005), synergistic effects were seen in the HIV+ heavy drinking group on several measures of visual attention, motor dexterity, and speed (Trail Making Test A, Grooved Pegboard). Additional analyses showed better neuropsychological performance in HIV+ groups with antiretroviral treatment and viral suppression (defined as viral load below 400 copies/ml), across all levels of alcohol consumption. Heavy alcohol use and executive functioning difficulties were associated with decreased medication adherence in the HIV+ group. However, the relationship between alcohol-related adherence problems and progression of cognitive decline was not addressed in this study. Thus, a growing body of literature supports an additive and/or synergistic effect of alcohol consumption and HIV infection on neuropsychological performance.

3. Research Opportunities

Based on the prior review, it is becoming clearer that alcohol and HIV infection produce deleterious interactive effects on neurocognitive function and brain structural and functional integrity. The mechanisms for these synergistic effects, however, are unknown. Several areas of future research using neurocognitive and neuroimaging techniques could lead to a better understanding of the progression of brain morbidity associated with these two combined diseases, as well as the mechanisms for these brain and cognitive changes. First, neuroimaging technologies could be used to identify in vivo the separate and or combined pathological mechanisms of HIV infection and alcohol neurotoxicity. Heavy alcohol consumption generates its own mechanisms of brain damage (e.g., excitotoxicity, oxidative stress, immune responses) some of which are similar to those hypothesized for HIV-induced neuropathogenesis. When the two diseases are combined, the degenerative process in the brain may be augmented. Neuroimaging technologies such as MRI and DTI could be used to devise models/markers of brain injury (e.g., inflammation, neuronal injury, white matter integrity, cellular/membrane function) that can be correlated with neuropsychological function to identify the interactive patterns and mechanisms of neurodegenerative and cognitive decline in HIV-infected heavy alcohol users.

A second area of research is the relationship among alcohol's role in nonadherence to antiretroviral therapy, its potential interference with bioavailability of the antiretroviral medications systemically and in the brain, and the subsequent development of drug resistant HIV. As mentioned earlier, the introduction of HAART therapy has reduced the occurrence of HIV-related opportunistic infections and encephalitis in the CNS. Despite

the increased survival rates, HIV neuropathogenesis and cognitive impairment continue to surface due to several drug-related factors, including toxicity, poor CNS penetrance, and the evolution of drug resistant strains of HIV virus (Langford, et al., 2003). Chronic alcohol drinking may contribute to these drug-related pressures by preventing medication compliance and/or interfering with bioavailability of the drug in the CNS. Thus, drug resistant HIV infection combined with alcohol's direct neurotoxicity would be another potential mechanism of the negative combined effects of these two diseases on brain morbidity in HIV-infected alcoholics or heavy drinkers. Biomarkers of viral burden, immunosupression, and immune activation (see e.g, Langford, et al., 2003; Navia and Rostasy, 2005) could be combined with neuroimaging and neuropsychological measures to determine the relationship between alcohol-related nonadherence, CSF or plasma biomarkers of HIV activation, and decline in brain and cognitive functioning in HIV-infected heavy drinkers. Studies of alcohol's effects on bioavailability of the drug, particularly how it affects pharmacokinetics and entry into the brain, are also needed.

A third line of research is the interaction of aging, HIV, and alcohol consumption on progressive neurological injury in chronically HIV-infected persons. Since the advent of HAART therapy, many HIV infected individuals will reach a normal life expectancy. As a result, aging, which has been a significant interactive factor in alcohol-induced brain damage (Pfefferbaum, et al., 1992), has also been shown to impact the progression of HIV-related CNS disease (Chang, et al., 2004; Navia and Rostasy, 2005). In a recent review, Pfefferbaum, et al. (2002) pointed out that several brain regions, including the frontal lobes, corpus callosum, basal ganglia, pons, and cerebellum, are affected by aging, HIV, and alcoholism. Therefore, it may be important to study the combined and/or additive effects of HIV infection, chronic alcohol consumption, and aging on the progression of cerebral dysfunction using neuroimaging and neuropsychological techniques.

Finally, recent epidemiological evidence revealed that at the end of 2003, 7074 adolescents and young adults, aged 13 to 24 at the time of diagnosis, were living with AIDS in the United States (Rangel, et al., 2006). Furthermore, of this group, the burden of HIV and AIDS fell most heavily on black and Hispanic youth. Although these data indicate that the number of infected youth is small compared to the adult HIV- infected population, the interaction of alcohol consumption and HIV infection on cognitive and neurological impairment in adolescents is an understudied area that may be worthy of consideration for future research. It is well known that alcohol consumption and binge drinking is common among teens. Recent cross-sectional neuroimaging studies have found reduced hippocampal and frontal cortex volumes (DeBellis, et al., 2000, 2005; Nagel, et al., 2005), as well as increased brain activation on a spatial working memory task (Tapert, et al., 2004) in alcohol abusing youths. In infants, children, and adolescents infected perinatally or early in postnatal life, the central nervous system manifestation of HIV is referred to as HIV-1 associated progressive encephalopathy (PE). Neuropathological, neurocognitive and neuroimaging studies of PE, conducted largely in children between 2 months and 8 years of age, indicate that the clinical presentation and course of PE may be very different from adult HAD/ADC(Mintz, 1994; States, et al., 1997). It has been suggested that these differences may be due the effect of HIV on the

developing nervous system, or the manner in which the immature immune system responds to the infection or treatment (Sanchez-Ramon, et al, 2002; Schwartz and Major, 2006; States, et al., 1997). As with adults, the introduction of antiretroviral therapy has improved neurocognitive function in children who acquired HIV perinatally. However, a significant percentage of children receiving HAART still display neurocognitive decline (Shanbhag, et al., 2005). Since brain development continues through adolescence, a time of risky behaviors including drinking and sex, it may be important to explore the combined or additive effects of alcohol and HIV infection on brain morbidity in HIV-infected youth who are drinking heavily. Furthermore, mechanisms of the combined effect of alcohol and HIV on brain dysfunction in youth may be different and should be explored.

Key Issues and Opportunities:

- Longitudinal studies using neuroimaging measures as biomarkers of neuropathological decline to disassociate the separate and combined effects of alcohol neurotoxicity and HIV infection on neuropathogenesis and cognitive decline in HIV infected heavy drinkers.

- Longitudinal studies combining neuroimaging and neuropsychological measures to determine the relationship between alcohol-related nonadherence, CSF or plasma biomarkers of HIV activation, and decline in brain and cognitive functioning in HIV infected heavy drinkers.

- Studies on the interaction of aging, HIV, and alcohol consumption on progressive neurological injury in chronically HIV-infected persons.

- Studies on the combined or additive effects of alcohol and HIV infection on brain morbidity in HIV-infected youth.

- Research on possible synergistic effects of alcohol and HIV-1 infection on metabolism and other neurobiological processes within the brain and their role in the development of HIV-associated dementia (HAD).

- Animal research to explore the independent, additive, and possible synergistic effects of alcohol, HIV, and antiretroviral medications on peripheral nerves.

- Studies examining the role of mitochondrial dysfunction in the development of sensory polyneuropathy, including the roles of oxidative stress, energy failure, apoptotic events, and calcium signaling.

- Research on the role of chemokine receptors and cytokine signaling in the development of sensory polyneuropathy.

G. Special Emphasis Area: Alcohol and HIV-Induced Effects on the Microvascular Endothelium

1. Introduction and Background

The existence of the blood-brain barrier (BBB) has been known for over 100 years, yet until recently the molecular and cellular biology of the BBB has not received a great deal of attention. With the recognition that BBB dysfunction is associated with HIV neuropathogenesis, the brain microvascular endothelium has become a focal point for research on HIV/AIDS-associated encephalitis and dementia (HIVE/HAD). Alcohol abuse is common among HIV-infected individuals and over 20% develop HIVE/HAD. Alcohol and HIV independently and together have profound effects on the CNS. Several studies have demonstrated that exposure to ethanol may both increase susceptibility to HIV infection and stimulate HIV replication in infected cells. Ethanol also contributes to impaired oxidative metabolism in the brains of AIDS patients. Although leakage has been reported, macroscopically very little change is apparent in BBB of HIV-infected patients. Certain HIV-specific proteins (nef) have been shown to induce apoptosis in the microvascular endothelial cells of the BBB. Nevertheless, as research described below demonstrates, seemingly small changes in structure have pronounced downstream effects on all aspects of BBB function. The BBB is composed of specialized endothelial cells surrounded by astrocytes and a basement membrane. Pericytes and neurons are in close proximity. The term 'neurovascular unit' aptly describes this unique structure. Microvascular endothelial cells of the BBB are distinguished from those in non-neural vascular tissue by the presence of tight junctions which hold them together and limit the flow of molecules from the blood to the brain. Alcohol partitions easily across the BBB and in doing so, gains access to the CNS. Alcohol activates the microvascular endothelium of the BBB and interferes with signaling and transport functions. By disrupting BBB function, alcohol may facilitate HIV access to the CNS and contribute to neuronal damage.

2. Current Research: RFA: Effects of Alcohol on HIV Invasion Across the Blood Brain Barrier (BBB) or Placental Barrier (PB)

Alcohol use is common among HIV-infected individuals. Because the effects of ethanol exposure on HIV translocation across the BBB and PB were not known, NIAAA released an RFA in October 2001 to encourage research leading to the development of new approaches, technologies, and methods to examine the effects of ethanol on HIV invasion across the blood brain barrier. Research proposals designed to increase understanding of how ethanol consumption altered the physical and immunological properties of the blood-brain or placental barriers, with special attention to how anti-retroviral therapies for HIV could be affected, were encouraged. The development of in vitro models was seen as a prerequisite for understanding neurodegenerative pathology associated with alcohol and HIV synergism or the prevention of the perinatal transmission of HIV. Since

alcohol is a commonly used drug in HIV-infected persons, evaluation of the exacerbating effects of ethanol on HIV neuroinvasion is important and necessary before an appropriate therapeutic intervention strategy can be designed. This RFA attempted to stimulate research which focused more specifically on learning about how alcohol use might alter the nature of the BBB and promote development of HIVE/HAD.

Suggested areas of focus were the following:

- The combined effects of alcohol and HIV on microvascular structure/function
- The impact of alcohol's immunosuppressive effects on BBB structure/function
- The effect of alcohol on T-cell maturation
- Isolation, characterization and maintenance of brain microvascular endothelial cells (BMVEC)
- Use of BMVEC from gene modified or knockout animals
- Culturing astrocytes and other relevant cells lines
- Use of support cell lines from gene modified or knockout animals
- Performance of perfusion devices and transwell devices
- Development of scaffolding materials and biocompatible substrates
- Improvement of standardization and quality control
- Comparison with in vivo models
- Application of analytical techniques

A total of 27 applications were submitted in response to the RFA. Of these, 11 (10 R21s, 1 R01) applications were funded. The majority dealt with alcohol and HIV-induced effects on the BBB; one application focused on the placental barrier. The following is a summary of significant research findings from investigators funded under the RFA.

Appreciation for the complexity of both the structure and function of the BBB has grown substantially since the RFA was promulgated. Once considered a largely impenetrable barrier separating the CNS from blood, the BBB is now viewed as a regulatory interface through which immune cells, drugs, cytokines, nutrients and viruses and their products pass into and out of the CNS. Interestingly, the BBB is permeable to acetaldehyde, the primary metabolite of ethanol. And while aldehyde dehydrogenase is present in microvascular endothelial cells, this metabolic barrier is quickly saturated resulting in significantly elevated brain acetaldehyde levels. The BBB performs a secretory function as well, producing neuroactive and immunoreactive substances. Whereas it had been thought that HIV access to the CNS occurred via infected immune cells (primarily macrophages), free virus and viral products such as gp120 and tat cross the BBB. Many of the transport processes used in other cells are operative in microvascular endothelial cells, including saturable transport systems, membrane diffusion, diapedesis, and endocytosis. As pointed out in a recent review by Banks, et al., the BBB is minimally disrupted in neuroAIDS; however nearly every functional aspect is involved or affected by HIV. Clearly there are multiple mechanisms whereby infected immune cells, free virus and viral proteins gain access to the CNS, raising the question as to the whether prior or concurrent alcohol use independently affects one or more of these pathways, thereby facilitating infectivity.

New insight into BBB integrity has been forthcoming as the result of the work of Persidsky et al. Using in vitro cultures of brain microvascular endothelial cells (BMVECs), they demonstrated disruption of tight junctions (TJ) as indicated by a down-regulation of TJ proteins, claudin-5 and occludin. Because small G proteins (such as Rho) can play a role in BMVEC TJ assembly, an artificial BBB system explored the relationship among TJs, Rho/Rho kinase (RhoK) activation, and transendothelial monocyte migration. Co-culture of monocytes with endothelial cells led to Rho activation and phosphorylation of TJ proteins. Inhibition of Rho blocked transendothelial passage of infected and uninfected monocytes and restored the integrity of BMVEC monolayers. These results were confirmed using a variety of BMVEC transfection systems. Thus, loss of TJ integrity was associated with Rho activation caused by monocyte migration, suggesting that Rho/ RhoK activation in BMVECs could be an underlying cause of BBB impairment in HIVE. Alcohol has been shown to alter the expression of genes involved in cell adhesion in the frontal cortex. Whether or not adhesive proteins forming TJ are similarly downregulated by alcohol is not known. These findings suggest gene expression profiling might provide further insight into the molecular pathology of alcohol effects on the BBB. Related research from Dr. Persidsky's group has shown that products of ethanol metabolism are involved in BBB perturbation. Acetaldehyde and the resulting generation of ROS activated myosin light chain kinase, thereby decreasing TJ tightness. This paralleled an increase in monocyte migration across BMVECs. This and other studies have shown that oxidative stress also leads to BBB compromise.

The BBB has recently been implicated in the physiological response to narcotics. Investigators have identified a gene (*moody*) required for the formation and maintenance of the BBB in Drosophila. *Moody* encodes a G-protein-coupled receptor (GPCR) involved in paracellular junction formation between the microvascular endothelial cells comprising the BBB. *Moody* mutants produce BBB insulation defects. Interestingly *moody* fly mutants demonstrate an increased sensitivity to cocaine and nicotine and a decreased sensitivity to ethanol. *Moody* encodes two GPCRs that regulate cocaine behaviors and blood-brain barrier permeability in Drosophila. Discovery of genes involved in maintenance of the BBB that also reflect changes in sensitivity to cocaine offers a new opportunity to study whether alcohol has similar effects on mammalian homologs of BBB structural integrity. These studies raise the possibility that ethanol-responsive genes within the BBB modulate ethanol sensitivity and/or permeability.

3. Research Opportunities

Role of innate and adaptive immunity in alcohol-induced CNS damage

Very little is known about whether either innate or adaptive immunity is involved in alcohol-related injury in organs other than the liver. Analogues of the Kupffer cell are resident within other organs, such as microglial cells in the brain and alveolar macrophages in the lung. Astrocytes possess toll-like receptors (TLR) and respond to their ligation with cytokine production. Astrocytes are also responsive to cytokines of the adaptive immune system; they apparently serve an antigen presenting function. The immunologically privileged status of the CNS presents many challenges to investigating its response to infectious challenge and how alcohol may enhance susceptibility. The

neuroimmune and neuroinflammatory cells and their associated mediators involved in alcohol-induced neuronal cell death have yet to be identified. Oxidative stress plays a role in neuronal cell damage and death. For example, ethanol administration plus injection of the HIV protein tat into the hippocampus synergistically induced the expression of genes encoding inflammatory proteins and increased the production of ROS. The effects of alcohol on immune cell recruitment, maturation and migration, immunosuppression, inflammation and oxidative stress in relation to HIV encephalitis require clarification. The role of innate and adaptive immunity in alcohol-induced damage in the CNS is an area of opportunity.

Alcohol-Induced Endothelial Cell Dysfunction

Long-term alcohol consumption plays a major role in the development of organ damage (brain, heart, liver, lung, pancreas and kidneys). While the organs themselves have usually been the targets of research on alcohol-induced injury, insults to the microvascular endothelium as illustrated above might well be a penultimate cause of damage to these vital organs. Thus additional research is needed to define the biological mechanisms that underlie alcohol-induced damage within the vascular beds that ultimately results in irreversible injury to the organs they sustain. The goals of such research would be to first identify the ways in which alcohol disrupts the endothelial cell biology unique to each vascular bed resulting in a dysfunctional phenotype and compromised functional integrity and secondly, to causally relate alcohol-induced vascular injury to end-organ damage.

Alcohol is known to increase intestinal permeability to endotoxin (LPS) resulting in increased transfer from the intestine to the portal vein and general circulation. Increasing evidence suggests that endotoxin plays a central role in the initiation of alcoholic liver injury via activation of Kupffer cells and up-regulation of inflammatory mediators such as cytokines, chemokines and adhesion molecules. In this way, endotoxin may also contribute to the development of other alcohol-associated tissue/organ damage such as pancreatitis, acute respiratory distress syndrome (ARDS), and brain injury. Thus restoring intestinal barrier integrity and blocking the transfer of endotoxin from intestine to the systemic circulation may mitigate alcohol-associated tissue/organ injuries. As the lining of the microvasculature, the endothelium plays a pivotal role as a sensor, transducer, and integrator of signaling processes regulating vascular homeostasis. Alcohol as a confounding factor in endothelial dysfunction has not been sufficiently explored; yet the endothelium is a primary target for harmful substances such as reactive oxygen species, oxidized lipoproteins, and advanced glycation end products as well as viruses and viral products.

Recent findings indicate that alcohol directly regulates endothelial cell genes involved in inflammation, in maintaining vessel patency, and in cell adhesion. Technology such as confocal microscopy and laser capture microscopy would permit analysis at the level of a single endothelial cell from human tissue specimens (such as the BBB) at various stages of disease and the confounding effects of alcohol on disease progression. High throughput arrays would enable the visualization of patterns of gene expression within

various vascular beds and their differential response to alcohol. Employing newly developed biomarkers of endothelial dysfunction would facilitate studies of alcohol's effects and link disrupted gene expression to altered function. Such research is expected to provide new insight into alcohol-induced endothelial dysfunction and the development of neuroAIDS.

- Alcohol effects on virus-mononuclear phagocyte interactions, infectivity (BBB transmigration) and dissemination within the CNS

- Identification of ethanol-responsive genes within the BBB that modulate ethanol sensitivity and/or permeability; Mammalian homologs of *moody*

- Alcohol-induced cellular oxidative stress, BBB integrity and translocation of HIV and/or neurotoxic HIV proteins such as gp120 and tat to the CNS

- Viral persistence/reservoirs and reinfection of peripheral tissues; Effect of alcohol on viral efflux via the microvascular endothelium

- Mechanisms of BBB disruption by alcohol and HIV and the temporal sequence of events leading to a functionally compromised BBB in neuroAIDS patients

- Alcohol's effects on the molecular transport systems within the BBB

- Global genomic and proteomic analyses to characterize the brain microvascular endothelium

- Genomic and proteomic profiles of HIV-infected BMVEC exposed to alcohol

- Antiretroviral drug delivery to the CNS: Modulation of drug transporters in response to alcohol

- Alcohol effects on neurotoxin production by HIV-infected BMVEC (glutamate, pro-inflammatory cytokines, chemokines, nitric oxide, ROS)

- The effects of alcohol on immune cell recruitment, maturation and migration, immunosuppression, inflammation and oxidative stress in HIV encephalitis

- Modulation of on HIV-1 co-receptor expression in human brain microvascular endothelial cells by alcohol and/or its metabolites

H. Special Emphasis Area: Alcohol Effects on Pulmonary Function in the HIV-Infected Patient

1. Introduction and Background

While the incidence of illnesses associated with HIV has decreased with the advent of ART, the frequency of pulmonary and other diseases not directly related to underlying HIV, has paradoxically begun to rise. The incidence of opportunistic infections and severe respiratory disease remains high in poorer segments of the HIV-infected population who are not receiving or fully complying with ART. Alcohol consumption is disproportionately high in the HIV-infected population, substantially worsening respiratory function and increasing the risk for infection with pneumocystis pneumonia, bacterial pneumonia and tuberculosis. Moreover asthma, emphysema and acute respiratory disease syndrome (ARDS) are increasingly common in HIV-infected individuals. These patients are at high risk for developing ARDS, a life-threatening form of respiratory failure frequently requiring mechanical ventilation. Furthermore, both alcohol and HIV independently increase systemic and pulmonary oxidative stress.

2. Current Research: Alcohol Abuse and Lung Injury

Alcohol use is an independent risk factor for lung disease. The observations by Moss et al. and the Emory group that alcohol abuse renders the lung susceptible to acute respiratory distress syndrome (ARDS) has given rise to the concept of the alcoholic lung. Approximately 15-25,000 people die of alcohol-related ARDS each year, a number comparable to liver cirrhosis-related mortality. While chronic alcohol abuse produces no overt pathology, its use is associated with various subclinical events, particularly oxidative stress, that ultimately erode protective defenses within the lung. These include increased apoptotic and necrotic cell death, decreased surfactant production, glutathione depletion and epithelial barrier dysfunction. The ability of alveolar macrophages to clear infectious organisms via phagocytosis is compromised in the alcoholic patient. Likewise, phagocytosis and other innate immune functions are impaired in macrophages from HIV-infected individuals. Because the effect of alcohol abuse on lung injury in HIV-infected patients is a relatively unexplored area of research, clinicians confronted with treating these critically ill patients, have few treatment options available. Antioxidant therapy, GM-CSF, and glutathione supplementation are some approaches that could be explored in attempts to restore the protective barrier function of the epithelial airways. Alcohol effects on pulmonary function in the HIV-infected patient is an area of opportunity.

3. Research Opportunities

- Effect of alcohol on extra-lymphoid immune effector cell populations, (lymphocyte subset destruction, regeneration, proliferation, maturation, and migration) particularly those found in association with the lungs in HIV-infected patients and transgenic animal models of HIV infection.

- Mechanism whereby ethanol and HIV induce oxidative stress within the lung and how this disrupts alveolar macrophage innate immune function.

- New therapeutic strategies that decrease oxidative stress and enhance lung function in HIV infected individuals.

V.

PREVENTION SCIENCE: VACCINES

V. PREVENTION SCIENCE

Overview

The NIH/NIAAA HIV/AIDS Research Plan is focused on developing and implementing effective prevention strategies. These strategies include both behavioral prevention (reduction in risk behavior and increase in protective health behaviors) and biomedical prevention strategies (vaccines, microbicides, and therapeutics). Developing these prevention strategies requires a foundation of basic behavioral and biomedical research which results in an understanding of specific cultural or environmental factors that will influence the implementation of interventions. Research needs to be extended into high-risk populations, particularly those with limited prevention and treatment resources. NIAAA has recently expanded its research in response to the changing characteristics of the epidemic to increase focus on women, international settings, and biological and behavioral aspects of adherence to antiretrovirals. The Institute will continue to adjust its priorities in response to newly emerging trends in the epidemic.

A. Alcohol Use and Vaccine Response
Adapted from presentation to AIDS Clinical Trials Network (NIAID, 2005)

Introduction

HIV infected subjects, as well as hazardous alcohol users, are at high risk of infections and co-morbidity with hepatitis, herpes, influenza, pneumonia, HPV, HIV, malaria, that could be prevented with an effective vaccine program. Unfortunately, poor immune responses to vaccines have been demonstrated in hazardous alcohol users from the general population and HIV infected individuals are also poor vaccine responders. It needs to be highlighted that alcohol use is widespread among HIV infected subjects. Poor or non-vaccine responders (i.e. alcohol users) need vaccine protocol modifications such as high or multiple doses. Thus, patients with comorbidity (HIV+ alcohol) are at significant risk for deficient response to vaccines. No studies to date have evaluated vaccine responses to hepatitis, herpes, influenza, pneumonia, HPV, HIV, malaria in patients with dual comorbidity (HIV+ alcohol+). Consequently, vaccine protocols that are currently being evaluated have not controlled for, or adjusted their scheme to dual comorbidity.

The suppressive action of alcohol on some aspects of the immune system, particularly on the humoral and cellular immune responses has been shown in numerous studies. Of interest, animal studies have documented a limited immune response in animals consuming ethanol before and during vaccination times. Incomplete immune response has been observed with vaccines that are dependent on the cellular as well as the humoral system. In addition, a recent pneumoccocal vaccination trial involving an Australian cohort had three Indigenous Australian adults died of invasive pneumococcal disease, despite vaccination with serotypes that were included in the vaccine. Two of the adults were alcoholics and the other had recently consumed large amounts of alcohol.

Similar results have been observed in clinical trials with hepatitis B vaccine. While the seroconversion rate following hepatitis B vaccine in nonalcoholic subjects is at least

90%,[11] response of alcoholic patients, using standard vaccination schedules, range from 43 to 70%.[12-16] The seroconversion is even lower in alcoholic patients with advanced liver disease.[12,13,17] Thus, it can be hypothesized that alcohol consumption may down regulate antigen presentation and antibody production to any vaccine. Alcohol-induced suppression of cellular responses may also reduce the ability of the host to mount a strong immune response and therefore to resist HIV-1. Furthermore it can also be suggested that different doses or an additional boost to the vaccine schedule may need to be provided in alcoholic patients. Nonetheless, this strategy has several potential implications involving 1) more side effects, 2) poorer final responses, and 3) increased costs. The public health implications, and possible optimal vaccination schedules to minimize vaccine failures, are of critical importance for effective health strategies.

Alcohol and HIV

As the prevalence of both HIV and alcohol is high in both developed nations and particularly in developing countries it is not surprising that these two co-morbidities would appear in the same individuals. The overlap of HIV infection and alcohol abuse in any one person is especially significant because of the deleterious effects of them on oxidative stress and immune response.

Alcohol is one of the most frequently abused substances by HIV infected individuals. In North America alone, it has been estimated that 650,000-920,000 people are living with HIV/AIDS, and a significant number of these individuals are suffering from alcohol abuse.[18] According to Meyerhoff, approximately 29 to 60 percent of HIV-infected patients develop an alcohol use disorder at some point during their lives, a rate that is approximately three times as high as that of the general U.S. population.[19] Additionally, current alcohol use disorders among the HIV infected population is twice that of the general population (12-15%). Nevertheless, almost no research has directly addressed the effects of heavy alcohol use on the severity of HIV disease and its progression to AIDS.

Alcohol Use and Immune Response in HIV

Experimental animal and human studies have demonstrated that chronic, and even acute, alcohol consumption results in significant changes in the immune system. A variety of short and long-term alcohol-induced effects on both cell mediated and humoral immune response, have been observed. Impaired host defense after alcohol exposure appears to be linked to a combination of cellular defects, altered cytokine production, and humoral disarrangements.

At the cellular level, in non-HIV infected individuals, decreased antigen presentation appears to be a key element in the ethanol-induced decrease in cell-mediated immunity.[20] Several studies have demonstrated that chronic alcohol use results in low lymphocyte numbers. Although the exact mechanism is yet to be defined, apoptosis has been suggested as the main mechanism. Lowered proliferative response of lymphocytes to mitogens and T lymphocyte expression of receptors can also be directly affected by ethanol.[20] Alcohol effects on T cell proliferation are dose-dependent, and seem to be

associated with inhibition of early signaling events of calcium mobilization and/or decreased interleukin production.[21] Current research on cytokine imbalance produced by alcohol is leading to new insights on the regulation of the immune system in alcoholics. The alcohol- induced impact on cytokine production seems to be mediated by activation of Nf-kappa B leading to increases in pro-inflammatory cytokine mRNA expression. A preferential TH2 cytokine response has been associated with alcohol use.[22-24] This is of particular concern in HIV/AIDS, since high levels of TH2 interleukins have been associated with oxidative-stress induced damage and increased viral replication.[20,23,25]

Evidence is emerging on the immunological abnormalities induced by alcohol use in non-HIV infected individuals and in AIDS murine models. Information related to alcohol use and HIV infection in humans is limited and controversial. Watson and colleagues[26,27] suggested that alcohol may accelerate the development of AIDS and case reports have confirmed these data.[28] Nevertheless, a prospective study by Kaslow[29] failed to demonstrate a relationship between percentage of CD4 cells, progression to AIDS and alcohol use. Another investigation conducted by Chandiwana and colleagues[30] similarly revealed no significant differences in mean CD4 counts between alcohol users and "non-users"; in fact, most of the patients with CD4 <200 cells did not use alcohol ($p = 0.023$). Crum and colleagues[31] confirmed that there were no significant differences in CD4 cell counts or cell decline among different alcohol categories during 5 years of follow-up. They demonstrated, however, that between 2 to 5 years post-seroconversion, there was a statistically significant increase in CD8 cell count among the heaviest drinkers (21 drinks/week). In contrast, Bagsara and collaborators[32] showed that alcohol use was associated with an increased inhibition of CD8 function and increased HIV replication. Recently, CD8 levels of activation (CD38+) have been recognized as strong independent predictors of disease progression and viral suppression in HIV infected subjects receiving HAART.[33] Clearly, further research is needed to understand the immunological implications of alcohol use during the course of HIV/AIDS.

HIV Vaccines and Alcohol

Considering the fact that there is no cure for HIV currently available, extensive research is being conducted to produce a viable vaccine against HIV disease. Vaccine-induced immune responses can vary substantially depending on the nature of the immunogen. The immune response generated by live-attenuated vaccines is generally very similar to that elicited by natural infection and, thus, includes both antibodies able to prevent infection of target cells (neutralizing antibodies) and cell-mediated immunity. Killed virus vaccines and purified synthetic proteins preferentially elicit neutralizing antibodies and $CD4^+$ T-cell responses but not $CD8^+$ cytotoxic T lymphocytes (CTLs). Replication-defective virus-based vectors alone, and to a greater extent in combination with DNA, predominantly stimulate CTLs and $CD4^+$ T-cell responses, although they are less efficient at eliciting antibodies. The importance of both humoral and cellular responses has been recognized during vaccine trials. Indeed, there is wide consensus that antibodies are crucial for preventing chronic infection, whereas cell-mediated responses can potentially control the infection in instances where chronicity is not abrogated. Antibodies may also have beneficial effects against disease progression.

Over the last ten years, it has also become clear that HIV-1 evades neutralizing antibodies through a variety of mechanisms,[34-37] and there is active research aimed at discovering ways to overcome the apparent difficulty in stimulating a broad neutralizing antibody response to HIV-1. Interestingly enough, however, despite the fact that studies have reported alcoholics to be poor HUMORAL (antibody) responders to vaccinations, **the correlation between HIV vaccine ineffectiveness and alcohol abuse has not been addressed**. The only exception is a study that examined the effect of ethanol ingestion on the antibody and cellular specific response to gp120. Several clinical trials have been performed in developing countries where the prevalence of alcohol consumption is the highest without considering the impact of alcohol use on efficacy.

Alcohol and Hepatitis Vaccine in HIV Infected Subjects

In general, it is well accepted that HIV infected children and adults have suboptimal responses to both live and synthetic antigens as the immunodeficiency progresses. In one study, ID immunization with three doses of recombinant HB vaccine induced a positive antibody response to HB in 7 (39%) of 18 HIV-positive subjects with CD4+ counts ≥200 cells/mm^3. Responses were boosted in 3 (50%) of 6 subjects with a history of prior HB immunization and new responses were induced in 4 (33%) of 12 subjects with no history of prior HB immunization. The latter results are comparable to published reports of primary HB immunization via the IM route in HIV-positive subjects. Six clinical trials, conducted among men having sex with men (119 HIV+, 365 HIV-controls), have investigated the effects of IM immunization with 3 doses of HB vaccine.[38-42] The overall response rate among the HIV-infected group was 35% compared to 89% among controls. Only one study has evaluated immune response to a vaccine among participants with drug abuse as an additional risk factor. In this report, an even poorer immune response was demonstrated. To the best of our knowledge no study has examined the effect of alcohol on the response to hepatitis vaccine in HIV infected adults.

VI

APPENDICES

APPENDIX A. REFERENCES

I. OVERVIEW AND INTRODUCTION

I. A: Dimensions of the Epidemic

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I.B: Context for Research: Major Themes of the NIH Plan

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II. NIAAA ALCOHOL AND HIV/AIDS BIOMEDICAL RESEARCH

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APPENDIX C: RECENT PUBLICATIONS

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