MECHANISMS OF ALCOHOL-MEDIATED CD4+ T LYMPHOCYTE DEATH: RELEVANCE TO HIV AND HCV PATHOGENESIS

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1. ABSTRACT

Clinical and experimental studies have demonstrated that excessive alcohol consumption can result in impairment of the immune system, and can impact several immune functions including immune tolerance and host defense against opportunistic infections, and development of certain tumors. Although multiple factors are involved in the effects of ethanol on the immune system, several studies implicate chronic activation of immune cells and impairment of thymus-derived lymphocytes (T lymphocytes). Helper CD4+ T lymphocytes are the central regulators of the immune system and depletion of these lymphocytes is a major contributing factor in ethanol-induced immune dysfunction and exacerbation of HIV and/or HCV pathogenesis. However, the mechanisms involved in the ethanol-induced CD4+ T cell depletion have only recently begun to be elucidated. Our work demonstrates that exposure of human CD4+ T cells to physiologically relevant concentrations of ethanol leads to the (i) enhanced activation of TNFα-inducible NFκB, the transcriptional regulator of the Fas promoter and ii) increased susceptibility to Fas-and activation-induced apoptotic death via augmentation of caspase 3 activity. Work done by us, and others, on the effects of ethanol on CD4+ T cell function and survival strongly suggests that alcohol plays a significant role as a co-factor in HIV and/or HCV pathogenesis.

2. INTRODUCTION

Excessive alcohol consumption is known to have deleterious effects on the immune system. Several clinical and experimental studies suggest that dysregulation of both cell-mediated and humoral immunity is a consequence of long-term alcohol use (1-6). Clinical studies have documented that chronic alcohol use leads to increased susceptibility to opportunistic infections and development of certain tumors and is linked with alcohol induced impairment of the immune system (1, 2, 7, 8). While immune function in alcoholics can be adversely affected by associated complications including malnutrition and liver
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disease many reports indicate that ethanol per se can directly impair immune responses (9). Although multiple factors are involved in the effects of ethanol on the immune system, several observations suggest that, the major effects of ethanol involve chronic activation of immune cells and the impairment of thymus-derived lymphocytes (T lymphocytes). Specifically, experimental observations suggest that alcohol abuse can adversely affect the CD4\(^+\) T -lymphocyte population, a major regulatory arm of humoral and cell-mediated immunity, by altering their functional capacity and reducing their numbers. This review examines the mechanisms involved in alcohol mediated CD4\(^+\) T cell destruction since this has an important bearing on (i) alcohol induced immunosuppression and (ii) the role of alcohol as a potential cofactor in HIV and HCV pathogenesis.

3. ALCOHOL AND T LYMPHOCYTES

3.1. Effects on lymphocyte function and numbers

Alcohol has a profound effect on T-cell proliferation. T-cells obtained from alcohol-fed animals, show reduced proliferative responsiveness to both non-specific and specific stimuli (10-12). Studies on cell-mediated cytotoxicity suggest that ethanol can inhibit the function of specific cytotoxic T lymphocytes (CTL) (13,14). In addition to its effects on proliferative and cytotoxic function, ethanol has also been shown to affect the extent and pattern of cytokine production by T lymphocytes (15,16).

A number of experimental animal models of ethanol abuse have established that ethanol causes loss of lymphoid cells from the thymus, spleen and lymph nodes (11, 17-20). In addition to affecting the cell numbers in the lymphoid organs, ethanol has also been observed to significantly reduce numbers of T cells from the peripheral blood of human subjects (13, 21-24).

3.2. Effect on CD4\(^+\) T lymphocytes

Chronic alcohol administration in experimental animal systems results in a decrease in the absolute numbers of CD4\(^+\) T lymphocytes from the periphery and the spleen (25,26), as well as a reduction in their immune function (16). In human studies it has been found that alcoholic patients have significantly reduced numbers of CD4\(^+\) T lymphocytes (24,27-29). As mentioned above other clinical complications associated with chronic alcohol abuse can negatively influence the immune system. Several studies have shown that alcohol withdrawal results in the recovery of CD4\(^+\) T-cell count (suggesting that ethanol can directly affect CD4\(^+\) T lymphocyte survival) (27-29). In the next two sections we present several potential mechanisms, by which ethanol may cause CD4\(^+\) T lymphocyte depletion.

4. ALCOHOL, OXIDATIVE STRESS AND CD4\(^+\)T LYMPHOCYTES

Oxidative stress is an important pathological factor in chronic alcohol abuse, and the ensuing production of reactive oxygen intermediates (ROIs), such as superoxide radicals, hydroxyl radicals and hydrogen peroxide are all implicated in alcohol-induced cytotoxicity (30). The oxidative stress associated with alcohol abuse can be induced by multiple mechanisms including, among others, the metabolism of alcohol, endotoxemia, increased TNF\(\alpha\) production and release, viral infections and nutrient antioxidant depletion (31,32). Chronic ethanol abuse is also associated with a decrease in the levels of the critical intracellular antioxidant GSH (33-38). Oxidative stress and the resultant GSH depletion can adversely affect normal CD4\(^+\) T lymphocyte function, proliferation and survival (39, 40-43). In the context of CD4\(^+\) T cell survival, reactive oxygen species (ROIs) are known to play a pivotal role in the initiation of Fas-mediated apoptosis in both cultured CD4\(^+\) T cells and peripheral blood lymphocytes (44). Furthermore, repletion of intracellular GSH levels has been demonstrated to counteract Fas-mediated apoptosis in T lymphocytes (45,46).

5. ALCOHOL AND CD4\(^+\) T LYMPHOCYTE APOPTOTIC DEATH

While experimental and clinical studies have documented that alcohol intake can cause depletion of CD4\(^+\) T lymphocytes, the mechanisms underlying this alcohol effect are largely undetermined. In order to elucidate the mechanisms of alcohol-induced immunotoxicity, we examined the effect of ethanol on Fas and activation-dependant apoptotic cell death, which play a key role in the physiologic and pathophysiologic depletion of CD4\(^+\)T cells.

5.1. Alcohol and Fas-mediated apoptosis in CD4\(^+\) T cells

The Fas receptor (FasR/APO-1/CD95) has been identified as a key initiator of apoptotic death in a variety of cell types (47-49). Binding of Fas ligand (FasL) or stimulation with agonistic antibodies (e.g. CH11) leads to aggregation of the Fas receptor on the cell membrane, and the recruitment of specific intracellular signaling molecules, to form the death-inducing signaling complex or DISC (50). Recent developments in the understanding of apoptotic T cell death have shown that the FasR/Fas ligand (FasL) system plays an important role in maintaining the physiologic homeostasis of mature peripheral CD4\(^+\) and CD8\(^+\) T lymphocytes. Resting naive T lymphocytes express little surface FasR and are resistant to the cytotoxic effects of FasL or anti-Fas antibodies. FasR expression is upregulated in activated T lymphocytes and can, in conjunction with T cell receptor activation, mediate apoptotic death in antigen-primed and transformed T cells (51-53). In the early phase of activation, T cells are relatively refractory to the cytotoxic effects of Fas signaling, and this resistance correlates with the lack of recruitment of caspase-8 to the FasR signaling complex (54). The delay in sensitivity to apoptosis is important in allowing the expansion of antigen-specific T cells during an immune response. However, after sustained or repeated activation, the T cells become more sensitive to Fas-induced apoptosis (55,56).

To investigate the mechanisms involved in alcohol induced T cell toxicity, we examined the effect of
activation. Importantly, these data indicate that ethanol-mediated signaling that is associated with Caspase-3 fragmentation was completely blocked by a reversible enhancement of Caspase-3 activation and subsequent DNA stimulation of both FasR and TCR-CD3 complex. This significantly enhanced Caspase-3 activation induced by physiologically relevant concentration of alcohol to Fas receptor and TCR-CD3 complex (57,70). The data on Caspase-3 activation, occurring as a result of stimulation of the Fas receptor and TCR-CD3 complex (57,70). The data obtained showed that the exposure of CD4+ T-cells to a physiologically relevant concentration of alcohol significantly enhanced Caspase-3 activation induced by stimulation of both FasR and TCR-CD3 complex. This enhancement of Caspase-3 activation and subsequent DNA fragmentation was completely blocked by a reversible Caspase-3 specific inhibitor. These results showed that ethanol pretreatment can affect FasR and TCR-CD3 mediated signaling that is associated with Caspase-3 activation. Importantly, these data indicate that ethanol-mediated enhancement of susceptibility to Fas- as well as activation-induced apoptotic death of CD4+ T cells involves the augmentation of Caspase-3 activation, which could play a major role in the T lymphocyte defects and depletion observed in alcohol induced immunosuppression.

6. ALCOHOL, CD4+ T LYMPHOCYTE DEATH AND HIV PATHOGENESIS

In the U.S. it has been found that HIV+ individuals are more likely to consume alcohol and be categorized as alcohol abusers than the general population (73). While it has been proposed that alcohol consumption may increase HIV-1 induced immunosuppression and disease progression, the mechanisms that underlie the potential interactions between these two common immunosuppressive conditions are just beginning to be elucidated (57,70,74). The immunological hallmark of HIV-1 infection is the progressive depletion of CD4+ T lymphocytes (75). This decrease in CD4+ T lymphocytes is preceded by early T-cell functional defects characterized by a loss of cell-mediated delayed type hypersensitivity reactions (in vivo), and by a failure of T cells to proliferate in response to T-cell receptor stimulation by recall antigens or mitogens (in vitro) (76-78). Several mechanisms have been proposed to account for the loss of CD4+ T lymphocytes during an HIV-1 infection. Both clinical, and experimental studies implicate enhanced apoptosis of peripheral uninfected CD4+ T lymphocytes as a key mechanism in CD4+ T-cell depletion during the course of HIV-1 disease (79-81). Accumulating data indicate that the depletion of CD4+ T-cells in HIV-1 infected individuals is due to an aberrant upregulation of the physiological mechanisms controlling peripheral CD4+ T-cell apoptosis, including, enhanced expression of, and susceptibility to, FasR signaling (82-86).

HIV-1 infected patients have chronic ongoing inflammation, which is associated with high plasma levels of proinflammatory cytokines, and the production of ROS (87-90). An important source of ROS is provided at the very early stages of HIV-1 infection by activated polymorphonuclear neutrophils. Additionally, Tat, the HIV-1 viral protein, is responsible for an endogenous cellular increase of ROI. Recent ex vivo experiments have clearly demonstrated that the oxidative stress produced as a result of HIV-1 infection participates in CD4+ T lymphocyte depletion through increased apoptosis, particularly Fas-induced apoptosis (88).

Oxidative stress influences the regulation of T cell receptor (TCR) mediated signaling and activation of oxidative stress sensitive transcription factor NfκB (91). In T lymphocytes, NfκB is a vital control element in T cell activation, and it is induced by inflammatory cytokines (e.g. TNFα), several T cell mitogens, and by antibodies against cell surface markers that mimic physiologic T cell activation (92-94). Recently, we have shown that alcohol can enhance TNFα inducible NfκB activation and HIV-1-LTR transcriptional activity in CD4+Jurkat T cells and that oxidative stress may be a major factor in this effect of ethanol (74). Similarly, CD4 cross-linking by the HIV
envelope protein gp 120 or heat inactivated HIV-1 activates NFκB as well as amplifies TNFα- induced NFκB activation via formation of reactive oxygen intermediates such as hydrogen peroxide (H₂O₂) (95-97). Importantly, recent reports show that NFκB is a critical transcriptional regulator of fas promoter and hence FasR expression (98). Since intracellular GSH levels can modulate TCR signaling and activation of NFκB, it is likely that ethanol induced GSH depletion can enhance stimulation dependent NFκB activation with a subsequent up-regulation of FasR expression on CD4⁺ T lymphocytes.

Our observations on the effect of alcohol on the critical pathogenic mechanisms during HIV infection, namely, Fas-mediated and activation induced apoptotic death of CD4⁺ T Lymphocyte, as well as TNFα-inducible NFκB activation and viral replication (57,70,74), strongly suggest that alcohol may play a significant role as a cofactor in HIV pathogenesis.

7. ALCOHOL, CD4⁺ T LYMPHOCYTE DEATH AND HEPATITIS C (HCV) PATHOGENESIS

Patients with chronic hepatitis C appear to have accelerated disease progression with alcohol consumption. Clinical studies suggest that alcoholics with chronic hepatitis C present with severe disease at a younger age, and chronic hepatitis C patients who consume in excess of 10 g of alcohol per day, have higher liver enzyme levels than patients who do not consume alcohol (99). Poynard et al in a study of over 2000 hepatitis C patients showed that alcohol consumption of ≥ 50 g daily was associated with more rapid fibrosis progression (100). Additionally, The amount of alcohol consumed has been reported to positively correlate with HCV RNA levels in the serum. This interaction between alcohol and HCV can impair immune mediated viral killing mechanisms and can elevate viral gene expression (101,102). Moreover, it has been found that the inflammatory response in the liver is greater in hepatitis C patients who drink alcohol. Lastly, not only does alcohol consumption appears to accelerate hepatitis C progression, it may also depress the response to interferon therapy (103).

Although, alcohol intake is associated with the development and rapid progression of liver disease in hepatitis C, the mechanism(s) for this alcohol-mediated effect, are poorly defined (reviewed in 104). Increasing evidence suggests that cell-mediated immune responses play a critical role in the pathogenesis of HCV infection, and that quantitative differences in these responses can influence the outcome of infection (103). While antiviral CD8⁺ cytotoxic T cells are present in HCV patients, their presence during chronic HCV infection concomitant with weak or absent antiviral CD4⁺ T cell responses indicates that CD4⁺ T cells are essential for effective HCV elimination/control by CD8⁺ T cells. Studies that have evaluated patients with self-limited vs. chronic HCV infection show that there are differential proliferative responses and cytokine production levels by antigen specific CD4⁺ T cells (105,106). Patients with self-limited acute hepatitis, and those responding to interferon α treatment, display more vigorous proliferative CD4⁺ T cell responses which are directed mainly against the nonstructural proteins of the HCV virus and result in viral clearance and resolution of the disease (105). These responses are sustained predominantly by CD4⁺ T cells of the Th1 phenotype by secreting proinflammatory cytokines such as (interferon gamma ([IFN-γ]) and interleukin-2 ([IL-2]). In contrast to the patients with self-limiting acute hepatitis, patients with chronic evolution of HCV infection show a weaker and less effective CD4⁺ T cell response with a Th2 type cytokine profile (interleukin-4 ([IL-4]) and interleukin-10 ([IL-10]) (105). The viral specific CD4⁺ T cell response persists for several years following clinical, and virological, remission (106,107). Recent data obtained from the largest cohort of patients published to date has shown that antigen specific CD4⁺ T cell responses are not only critical in the elimination of the virus during acute infection, but also play a definitive role in the long-term viral control during chronic HCV infection (108,109).

The relevance of CD4⁺ T cells in HCV pathogenesis is further underscored by studies on HCV-HIV co-infected patients, since the immunosuppression associated with HIV infection significantly alters the natural history and clinical course of HCV infection. Studies on HCV-HIV co-infected patients show that the enhanced risk of hepatic decompensation and liver failure associated with the presence of HIV infection correlates with the declining CD4⁺ cell counts (111). Recent studies examining liver fibrosis progression in a cohort of HCV-HIV co-infected patients suggest that HIV-infection induced CD4⁺ cell depletion is independently associated with the severity of liver fibrosis in chronic HCV infection (111,112). Since (i) alcohol has the potential to enhance Fas-mediated, and activation induced apoptosis in CD4⁺ T Lymphocytes and (ii) polyclonal multispecific and viral specific CD4⁺ T cell responses are critical for viral elimination during acute hepatitis C and required for modulating liver disease in chronic hepatitis C infection, it is likely that the alcohol induced loss of CD4⁺ T lymphocytes plays a critical role in the development of chronicity, and rapid progression of liver disease in alcoholic HCV patients.

8. PERSPECTIVE

A critical component of the alcohol-induced immunosuppression is the reduction in number and function, of the thymus derived T lymphocytes in general and helper CD4⁺ T lymphocytes in particular. As shown in the model Figure 1, an important aspect of the immunosuppressive function of alcohol abuse is the chronic activation of the immune system leading to an increase in inflammatory cytokines, ROIs and NFκB activation and ultimately susceptibility of CD4⁺ T lymphocytes to Fas- and activation-induced apoptotic death. Consequently, alcohol induced loss of CD4⁺ T lymphocytes may not only mediate the immunosuppression associated with chronic alcohol abuse but also play a major
Alcohol and CD4+ T cell apoptotic death

Figure 1. Alcohol and its potential interaction with HIV-1 and HCV infection: effect on CD4+ T cell survival and ensuing immune dysfunction.

role as a co-factor in other immunosuppressive conditions such as the HIV-1 and/or HCV infection.

9. ACKNOWLEDGEMENT

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10. REFERENCES


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