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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

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 TNFα 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 signal 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

ethanol on Fas-mediated apoptotic death in human CD4$^+$ T cell line Jurkat (E6-1) (57). The data obtained demonstrated that FasR-triggering anti-Fas monoclonal antibody (CH11) induced significantly greater apoptotic cell death in Jurkat CD4$^+$ T cells that were treated with ethanol (25 mM) for 12-18 h as compared to the untreated cells. Fas-dependant apoptotic death of CD4$^+$ T-cells plays a major role in the depletion of CD4$^+$ T-cells and ensuing immunosuppression in several pathophysiological conditions, including HIV-1 infection. Hence the potential of ethanol to increase the susceptibility of CD4$^+$ T-cells to Fas-mediated apoptosis may allow ethanol to act as a co-factor and exacerbate the clinical conditions that lead to immunosuppression.

5.2. Alcohol and activation-induced apoptotic death in CD4$^+$ T cells

The activation of mature resting peripheral T lymphocytes typically results in their proliferation and cytokine release (58). However, T cells that have been previously activated undergo apoptosis after a second activation stimulus, a process known as activation-induced cell death (AICD) (59,60). A variety of pharmacological, mitogenic, or antigenic stimuli can be used to trigger AICD (61,62). Current knowledge indicates that AICD plays an important role in the deletion of autoreactive T-cell clones in the thymus, autoreactive T cells in the periphery, and activated T cells at the termination of the immune response (63). Furthermore, reports suggest that AICD also plays a role in various infectious disease pathologies, including the CD4$^+$ T -cell depletion seen in HIV-infected individuals (64-69).

To elucidate the mechanisms of alcohol-induced CD4$^+$ T -cell depletion, we initially examined the effect of ethanol on the activation-induced apoptotic death of human CD4$^+$ T -cell line (Jurkat E-6) and further extended these observations to human PBLs obtained from healthy volunteers (70). Pretreatment of Jurkat T cells and peripheral blood lymphocytes (PBLs) with a physiologically relevant level of ethanol (25 mM) sensitized them to apoptosis upon activation by CD3-crosslinking or stimulation with Conconavalin A (ConA) and Phytohemmaglutinin (PHA), as documented by DNA fragmentation and cell viability analysis.

5.3. Caspase-3 and alcohol induced apoptotic death in CD4$^+$ T cells

Caspase-3 is the key mediator of Fas and activation-induced apoptotic death of CD4$^+$ T -cells (71,72). Hence, we examined the effect of ethanol 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 ROIs (87-90). An important source of ROIs 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 (H_2O_2) (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 $\geq$ 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). Similarly, development of HCV-specific CD4$^+$ T cells has been shown to be important in modulating hepatic outcome following HCV recurrence with liver transplantation (110).

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

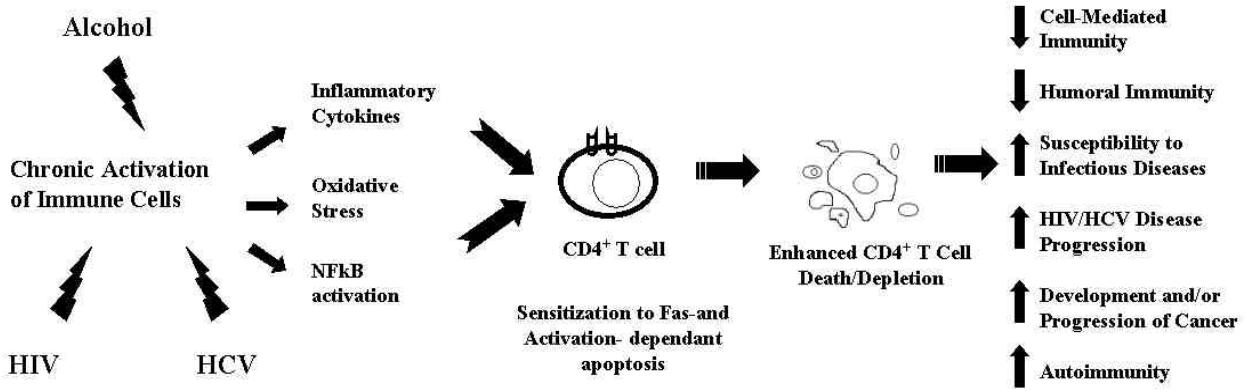

Figure 1. Alcohol and its potential interaction with HIV-1 and HCV infection: effect on CD4$^+$ T cell survival and ensuing immune dysfunction.

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

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10. REFERENCES

1. H.G.Adams & C.Jordan: Infections in the alcoholic. *Med Clin North Am* 68, 179-200 (1984)
2. Eckardt, M.J. Harford, T.C. Kaelber, C.T. Parker, E.S. Rosenthal, L.S. Ryback, R.S. Salmoiraghi, G.C. E. Vanderveen & K.R.Warren: Health hazards associated with alcohol consumption. *JAMA* 246, 648-666 (1981)
3. E.R. Eichner: The hematologic disorders of alcoholism. *Am J Med* 54, 621-630 (1973)
4. W.D. Jr. Johnson: Impaired defence mechanisms associated with acute alcoholism. *Ann N Y Acad Sci* 252, 343-347 (1975)
5. S.S. Caiazza & S.S. Ovary Z: Effects of ethanol intake on the immune system of guinea pigs. *J Stud Alcohol* 37, 959-964 (1976)
6. N.R. Di Luzio & D.L. Williams: Enhancement of host susceptibility to Staphylococcus aureus infection by chronic ethanol ingestion--modification by glucan immunostimulation. *Alcohol Clin Exp Res* 4, 254-260 (1980)
7. F.E. Smith & D.L. Palmer: Alcoholism, infection and altered host defenses: a review of clinical and experimental observations. *J Chronic Dis* 29, 35-49 (1976)
8. MacGregor, R.R: Alcohol and immune defense. *JAMA* 256, 1474-1479 (1986)
9. R.C. Baker & T.R. Jerrells: Recent developments in alcoholism:immunological aspects. *Recent Dev Alcohol* 11, 249-271 (1993)
10. R.Domiati-Saad & T.R.Jerrells: The influence of age on blood alcohol levels and ethanol-associated immunosuppression in a murine model of ethanol consumption. *Alcohol Clin Exp Res* 17, 382-388 (1993)
11. Jerrells, T.R. W.Smith & M.J.Eckardt: Murine model of ethanol-induced immunosuppression. *Alcohol Clin Exp Res* 14, 546-550 (1990)
12. M.G.Mutchnick & H.H.Lee: Imparied lymphocyte proliferative response to mitogen in alcoholic patients. Absence of a relation to liver disease activity. *Alcohol Clin Exp Res* 12, 155-158 (1988)
13. N.H. Stacey: Inhibition of antibody-dependent cell-mediated cytotoxicity by ethanol. *Immunopharmacology* 8, 155-161 (1984)
14. A.S.Walia & E.W.Lamon: Ethanol inhibition of cell-mediated lysis of antibody-sensitized target cells at a calcium-dependent step. *Proc Soc Exp Biol Med* 192, 177-81 (1989)
15. Chadha, K.C. Stadler, I. Albini, B. S.M.Nakeeb & H.R.Thacore: Effect of alcohol on spleen cells and their functions in C57BL/6 mice. *Alcohol* 8,481-485 (1991)
16. Peterson, J.D. Herzenberg, L.A. K.Vasquez & C.Waltenbaugh: Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Natl Acad Sci U S A* 95, 3071-3076 (1998)
17. A.J.Saad & T.R.Jerrells: Flow cytometric and immunohistochemical evaluation of ethanol-induced changes in splenic and thymic lymphoid cell populations. *Alcohol Clin Exp Res* 15, 796-803 (1991)
18. S.J. Ewald: T lymphocyte populations in fetal alcohol syndrome. *Alcohol Clin Exp Res* 13, 485-489 (1989)
19. S.J.Ewald & H.Shao: Ethanol increases apoptotic cell death of thymocytes in vitro. *Alcohol Clin Exp Res* 17, 359-365 (1993)
20. Jerrells, T.R. Slukvin, I. D.Sibley & J.Fuseler: Increased susceptibility of experimental animals to infectious organisms as a consequence of ethanol consumption. *Alcohol Alcohol Suppl* 2, 425-430 (1994)
21. Brayton, R.G. Stokes, P.E. M.S.Schwartz & D.B.Louria: Effect of alcohol and various diseases on leukocyte mobilization, phagocytosis and intracellular bacterial killing. *N Engl J Med* 282, 123-8 (1970)
22. N.E.Gilhus & R.Matre: In vitro effect of ethanol on subpopulations of human blood mononuclear cells. *Int Arch Allergy Appl Immunol* 68, 382-386 (1982)
23. Smith, W.I.Jr. Van Thiel, D.H. Whiteside, T. Janoson, B. Magovern, J. T.Puet & B.S.Rabin: Altered immunity in male patients with alcoholic liver disease: evidence for defective immune regulation. *Alcohol Clin Exp Res* 4, 199-206 (1980)

24. Young, G.P. Van der Weyden, M.B. I.S.Rose & F.J.Dudley: Lymphopenia and lymphocyte transformation in alcoholics. *Experientia* 35, 268-269 (1979)
25. Helm, R.M. Wheeler, G. Burks, A.W. R.Hakkak & T.M.Badger: Flow cytometric analysis of lymphocytes from rats following chronic ethanol treatment. *Alcohol* 13, 467-471 (1996)
26. J.E.Shellito & R.Olariu: Alcohol decreases T-lymphocyte migration into lung tissue in response to Pneumocystis carinii and depletes T-lymphocyte numbers in the spleens of mice. *Alcohol Clin Exp Res* 22, 658-663 (1998)
27. Roselle, G.A. Mendenhall, C.L. C.J.Grossman & R.E.Weesner: Lymphocyte subset alterations in patients with alcoholic hepatitis. *J Clin Lab Immunol* 26, 169-173 (1988)
28. Pol, S. Artru, P. Thepot, V. P.Berthelot & B.Nalpas: Improvement of the CD4$^+$ cell count after alcohol withdrawal in HIV-positive alcoholic patients. *AIDS* 10, 1293-1294 (1996)
29. Laso, F.J. Madruga, J.I. Lopez, A. Ciudad, J. Alvarez-Mon, M. J.San Miguel & A.Orfao: Distribution of peripheral blood lymphoid subsets in alcoholic liver cirrhosis: influence of ethanol intake. *Alcohol Clin Exp Res* 20, 1564-1568 (1996)
30. A.I. Cederbaum: Role of lipid peroxidation and oxidative stress in alcohol toxicity. *Free Radic Biol Med* 7, 537-539 (1989)
31. McClain, C.J. Barve, S. Deaciuc, I. M.Kugelmas & D.Hill: Cytokines in alcoholic liver disease. *Semin Liver Dis* 19, 205-219 (1999)
32. Hill, D.B. L.S.Marsano & C.J. McClain: Increased plasma interleukin-8 concentrations in alcoholic hepatitis. *Hepatology* 18, 576-580 (1993)
33. Honchel, R. Ray, M.B. Marsano, L. Cohen, D. Lee, E. S.Shedlofsky & C.J.McClain: Tumor necrosis factor in alcohol enhanced endotoxin liver injury. *Alcohol Clin Exp Res* 16, 665-669 (1992)
34. Koch, O.R. Galeotti, T. G.M.Bartoli & A.Boveris: Alcohol-induced oxidative stress in rat liver. *Xenobiotica* 21, 1077-1084 (1991)
35. Fernandez-Checa, J.C. Kaplowitz, N. Garcia-Ruiz, C. Colell, A. Miranda, M. Mari, M, E.Ardite & A.Morales: GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect. *Am J Physiol* 273(1 Pt 1), G7-17 (1997)
36. J.F.Wang & J.J.Spitzer: Alcohol-induced thymocyte apoptosis is accompanied by impaired mitochondrial function. *Alcohol* 14, 99-105 (1997)
37. Shan, X.Q. T.Y.Aw & D.P.Jones: Glutathione-dependent protection against oxidative injury. *Pharmacol Ther* 47, 61-71 (1990)
38. S.H. Thomas: Paracetamol (acetaminophen) poisoning. *Pharmacol Ther* 60, 91-120 (1993)
39. Peterson, J.D. Herzenberg, L.A. K.Vasquez & C.Waltenbaugh: Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Natl Acad Sci U S A* 95, 3071-3076 (1998)
40. D.L.Hamilos & H.J.Wedner: The role of glutathione in lymphocyte activation. I. Comparison of inhibitory effects of buthionine sulfoximine and 2-cyclohexene-1-one by nuclear size transformation. *J Immunol* 135, 2740-2747 (1985)
41. R.K.Fidelus & M.F.Tsan: Enhancement of intracellular glutathione promotes lymphocyte activation by mitogen. *Cell Immunol* 97, 155-163 (1986)
42. Droge, W. Pottmeyer-Gerber, C. H.Schmidt & S.Nick: Glutathione augments the activation of cytotoxic T lymphocytes in vivo. *Immunobiology* 172, 151-156 (1986)
43. H.Gmunder & W.Droge: Differential effects of glutathione depletion on T cell subsets. *Cell Immunol* 138, 229-237 (1991)
44. Banki, K. Hutter, E. Colombo, E. N.J.Gonchoroff & A.Perl: Glutathione levels and sensitivity to apoptosis are regulated by changes in transaldolase expression. *J Biol Chem* 271, 32994-3001 (1996)
45. Delneste, Y. Jeannin, P. Sebille, E. J.P.Aubry & J.Y.Bonnefoy: Thiols prevent Fas (CD95)-mediated T cell apoptosis by down-regulating membrane Fas expression. Eur J *Immunol* 26, 2981-2988 (1996)
46. Watson, R.W. Rotstein, O.D. Jimenez, M. J.Parodo & J.C. Marshall: Augmented intracellular glutathione inhibits Fas-triggered apoptosis of activated human neutrophils. *Blood* 89, 4175-4181 (1997)
47. Galle, P.R. W.J.Hofman & H.Walczak: Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. *J Exp Med* 182, 1223-1230 (1995)
48. Krammer, P.H. Dhein, J. Walczak, H. Behrmann, I. Mariani, S. Matiba, B. Fath, M. Daniel, P.T. E.Knipping & M.O.Westendorp: The role of APO-1-mediated apoptosis in the immune system. *Immunol Rev* 142, 175-91 (1994)
49. Ju, S.T. Panka, D.J. Cui, H. Ettinger, R. el-Khatib, M. Sherr, D.H. B.Z.Stanger BZ & A.Marshak-Rothstein: Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 373(6513), 444-448 (1995)
50. Kischkel, F.C. Hellbardt, S. Behrmann, I. Germer, M. Pawlita, M. P.H.Krammer & M.E.Peter: Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J* 14, 5579-5588 (1995)
51. Alderson, M.R. Tough, T.W. Braddy, S. Davis-Smith, T. Roux, E. Schooley, K. R.E.Miller & D.H.Lynch. Regulation of apoptosis and T cell activation by Fas-specific mAb. *Int Immunol* 6, 1799-1806 (1994)
52. Brunner, T. Mogil, R.J. LaFace, D. Yoo, N.J. Mahboubi, A. Echeverri, F. Martin, S.J. Force, W.R. D.H.Lynch & C.F.Ware: Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 373, 441-444 (1995)
53. Ramsdell, F. Seaman, M.S. Miller, R.E. Picha, K.S. M.K.Kennedy & D.H.Lynch: Differential ability of Th1 and Th2 T cells to express Fas ligand and to undergo activation-induced cell death. *Int Immunol* 6, 1545-1553 (1994)
54. Peter, M.E. Kischkel, F.C. Scheuerpflug,C.G. Medema, J.P. K.M.Debatin & P.H.Krammer: Resistance of cultured peripheral T cells towards activation-induced cell death involves a lack of recruitment of FLICE (MACH/caspase 8) to the CD95 death-inducing signaling complex. *Eur J Immunol* 27, 1207-1212 (1997)
55. Combadiere, B. D'Sousa, C.R. R.N.Germain & M.J. Lenardo: Selective induction of apoptosis in mature T lymphocytes by variant T cell receptor ligands. *J Exp Med* 187, 349-355 (1998)
56. Combadiere, B. Freedman, M. Chen, L. Shores, E.W. P.Love & M.J.Lenardo: Qualitative and quantitative contributions of the T cell receptor zeta chain to mature T cell apoptosis. *J Exp Med* 183, 2109-2117 (1996)

57. Donohue, T.M.Jr. Clemens, D.L. Galli, A. Crabb, D. Nieto, N. J.Kato & S.S.Barve: Use of Cultured Cells in Assessing Ethanol Toxicity and Ethanol-Related Metabolism. *Alcohol Clin Exp Res* 25(5 Suppl), 87S-93S (2001)
58. Meuer, S.C. Hodgdon, J.C. Hussey, R.E. Protentis, J.P. S.F.Schlossman & E.L.Reiherz: Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J Exp Med* 158, 988 –993 (1983)
59. Alderson, M.R. Tough, T.W. Davis-Smith, T. Braddy, S. Falk, B. Schooley, K.A.
Goodwin, R.G. Smith, C.A. F.Ramsdell & D.H.Lynch: Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* 181, 71–77 (1995)
60. Brunner, T. Mogil, R.J. LaFace, D. Yoo, N.J. Mahboubi, A. Echeverri, F. Martin, S.J. Force, W.R. D.H.Lynch & C.F.Ware: Cell autonomous Fas(CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 373, 441–444 (1995)
61. Kabelitz, D. T.Pohl & K.Pechhold: Activation induced cell death of mature peripheral T lymphocytes. *Immunol Today* 14, 338 –339 (1993)
62. O.M. Pitts: ConA cytotoxicity: a model for the study of key signaling steps leading to lymphocyte apoptosis in AIDS? *Med Hypotheses* 45, 311–315 (1995)
63. D.R.Green & D.W.Scott: Activation-induced apoptosis in lympho-cytes. *Curr Opin Immunol* 6, 476 –487 (1994)
64. Groux, H. Torpier, G. Monte, D. Mouton, Y. A.Capron & J.C.Ameisen: Activation-induced death by apoptosis in CD4$^+$ T-cells from human immunodeficiency virus infected asymptomatic individuals. *J Exp Med* 175, 331–340 (1992)
65. Kaneko, H. Saito, K. Hashimoto, H. Yagita, H. K.Okumura & M.Azyma: Preferential elimination of CD28 T-cells in systemic lupus erythematosus (SLE) and the relation with activation induced apoptosis. *Clin Exp Immunol* 106, 218 –229 (1996)
66. Li, B. Bassiri, H. Rossman, M.D. Kramer, P.H. Eyubloglu, A.F. Torres, M. Sada, E. T.Imir & S.R.Carding: Involvement of the Fas/Fas ligand path-way in activation induced cell death of mycobacteria reactive human γ? T cells: a mechanism for the loss of γ? T cells in patients with pulmonary tuberculosis. *J Immunol* 161, 1558 –1567 (1998)
67. Meyaard, L. Otto, S.A. Joner, R.R. Mijnster, M.J. R.P.M.Keet & F.Miedema: Programmed cell death of T-cells in HIV-1 infection. *Science* 257, 217–219 (1992)
68. Oyaizu, N. McCloskey, T.W. Coronesi, M. Chirmule, N. V.S.Kalyanaraman & S.Pahwa: Accelerated apoptosis in peripheral blood mononuclear cells (PBMCs) from human immunodeficiency type-1 infected patients and in CD4 crosslinked PBMCs from normal individuals. *Blood* 82, 3392–3400 (1993)
69. Sarin, A. Clerici, M. Blaat, S.P. Hendrix, C.W. G.M.Shearer & P.A.Henkart:
Inhibition of activation induced programmed cell death and restoration of defective immune responses of HIV$^+$ donors by cysteine protease inhibitors. *J Immunol* 153, 862–872 (1994)
70. Kelkar, S. Dong, Q. Xiao, Y. Joshi-Barve, S. C.J.McClain & S.S.Barve: Ethanol enhances activation-induced caspase-3 dependent cell death in T lymphocytes. *Alcohol Clin Exp Res* 26, 363-370 (2002)
71. Cohen, J. J. Duke, R. C. V.A.Fadok & K.S.Sellins: Apoptosis and programmed cell death in immunity. *Annu Rev Immunol* 10, 267 (1992)
72. J. H. Russell: Activation-induced death of mature T cells in the regulation of immune responses. *Curr Opin Immunol* 7, 382 (1995)
73. Lefevre, F. O'Leary, B. Moran, M. Mossar, M. Yarnold, P.R. G.J.Martin & J.Glassroth: Alcohol consumption among HIV-infected patients. *J Gen Intern Med* 108, 458-460 (1995)
74. Dong, Q. Kelkar, S. Xiao, Y. Joshi-Barve, S. C.J.McClain & S.S.Barve: Ethanol enhances TNF-alpha-inducible NFkappaB activation and HIV-1-LTR transcription in CD4+ Jurkat T lymphocytes. *J Lab Clin Med* 136, 333-343 (2000)
75. A. S. Fauci: Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science* 262, 1011–1018 (1995)
76. Lane, H. C. J. M. Drepper, W. C. Greene, G. Whalen, T. A. Waldmann & A. S. Fauci: Qualitative analysis of immune function in patients withand the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. *N Eng. J Med* 313, 79–84 (1985)
77. Miedema, F. Petit, A.J. Terpstra, F.G. Schattenkerk, J.K. de Wolf, F. Al, B.J. Roos, M, Lange, J.M. S.A.Danner & J.Goudsmit: Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. *J Clin Invest* 82, 1908-1914, (1988)
78. Shearer, G.M. Bernstein, D.C. Tung, K.S. Via, C.S. Redfield, R. S.Z.Salahuddin & R.C.Gallo: A model for the selective loss of major histocompatibility complex self-restricted T cell immune responses during the development ofacquired immune deficiency syndrome (AIDS). *J Immunol* 137, 2514-2521 (1986)
79. Meyaard, L. Otto, S.A. Jonker, R.R. Mijnster, M.J. R.P.Keet & F.Miedema: Programmed cell death of T cells in HIV-1 infection. *Science* 257, 217 (1992)
80. Estaquier, J. Idziorek, T. de Bels, F. Barre´ Sinoussi, F. Hurtrel, B. Auburtin, A.M. Venet, A. Mehtali, M. E.Muchmore & P.Michel: Pro-grammed cell death and AIDS: Significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. *Proc Natl Acad Sci USA* 91, 9431 (1994)
81. Finkel, T.H. Tudor Williams, G. Banda, N.K. Cotton, M.F. Curiel, T. Monks, C. Baba, T.W. R.M.Ruprecht & A.Kupfer: Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nat Med* 1, 1129 (1995)
82. Aries, S.P. Schaaf, B. Müller, C. R.H.Dennin & K.Dal-hoff: Fas (CD95) expression on CD4 1 T cells from HIV-infected patients increases with disease progression. *J. Mol. Med.* 73, 591–593 (1995)
83. Silvestris, F. Cafforio, P. Frassanito, M.A. Tucci, M. Romito, A. S.Nagata & F.Dammacco: Overexpression of Fas antigen on T cells in advanced HIV-1 infection: differential ligation constantly induces apoptosis. *AIDS (Phila.).* 10, 131–141 (1996)
84. Gehri, R. Hahn, S. Rothen, M. Steuerwald, M. R.Nuesch & P. Erb: The Fas receptor in HIV infection: expres-sion on peripheral blood lymphocytes and role in the depletion of T cells. *AIDS (Phila.).* 10, 9–16 (1996)
85. Katsikis, P.D. Wunderlich, E.S. Smith, C.A. L.A.Herzen-berg & L.A. Herzenberg: Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immu-nodeficiency virus-infected individuals. *J. Exp. Med.* 181, 2029–2036 (1995)

86. Estaquier, J. Idziorek, Zou, W. Emilie, D. Farber, C.M. J.M.Bourez & J.C.Ameisen: T helper type1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)- mediated apoptosis of CD4 1 T cells from human immu-nodeficiency virus-infected persons. *J. Exp. Med.* 182, 1759–1767 (1995)
87. Elbim, C. Pillet, S. Prevost, M.H. Preira, A. Girard, P.M. Rogine, N. Matusani, H. Hakim, J. N.Israel & M.A.Gougerot-Pocidalo. Redox and activation status of monocytes from human immunodeficiency virus-infected patients: relationship with viral load. *J Virol* 73:4561-6, 1999.
88. N.Israel &M.A.Gougerot-Pocidalo: Oxidative stress in human immunodeficiency virus infection. *Cell Mol Life Sci* 53, 864-870 (1997)
89. Macchia, D. Almerigogna, F. Parronchi, P. Ravina, A. E.Maggi & S.Romagnani: Membrane tumour necrosis factor-alpha is involved in the polyclonal B-cell activation induced by HIV-infected human T cells. *Nature* 363, 464-466 (1993)
90. Bilello, J.A. Stellrecht, K. G.L.Drusano & D.S.Stein: Soluble tumor necrosis factor-alpha receptor type II (sTNF alpha RII) correlates with human immunodeficiency virus (HIV) RNA copy number in HIV-infected patients. *J Infect Dis* 173, 464-467 (1996)
91. Staal, F.J. Anderson, M.T. Staal, G.E. Herzenberg, L.A. C.Gitler & L.A.Herzenberg: Redox regulation of signal transduction: tyrosine phosphorylation and
calcium influx. *Proc Natl Acad Sci U S A* 91, 3619-3622 (1994)
92. M.J. Lenardo & D. Baltimore: NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 58, 227-229 (1989)
93. R. Sen & D. Baltimore: Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell* 47, 921-928 (1986)
94. Tong-Starkesen, S.E. P.A.Luciw & B.M.Peterlin: Signaling through T lymphocyte surface proteins, TCR/CD3 and CD28, activates the HIV-1 long terminal repeat. J Immunol 142, 702-707 (1989)
95. Briant, L. Reynes, J. Coudronniere, N. J.P.Benezech & C.Devaux: HIV-1 reactivation in resting peripheral blood mononuclear cells of infected adults upon in vitro CD4 cross-linking by ligands of the CDR2-loop in extracellular domain 1. *J Acquir Immune Defic Syndr* 21, 9-19 (1999)
96. Briant, L. Coudronniere, N. Robert-Hebmann, V. M.Benkirane & C.Devaux: Binding of HIV-1 virions or gp120-anti-gp120 immune complexes to HIV-1-infected quiescent peripheral blood mononuclear cells reveals latent infection. *J Immunol* 156, 3994-4004 (1996)
97. Shatrov, V.A. Ratter, F. Gruber, A. W.Droge & V.Lehmann: HIV type 1 glycoprotein 120 amplifies tumor necrosis factor-induced NF-kappa B activation in Jurkat cells. *AIDS Res Hum Retroviruses* 12, 1209-1216 (1996)
98. Chan, H. D.P.Bartos & L.B.Owen-Schaub: Activation-dependent transcriptional regulation of the human Fas promoter requires NF-kappaB p50-p65 recruitment. *Mol Cell Biol* 19, 2098-2108 (1999)
99. Cromie, S.L. Jenkins, P.J. S.Bowden & F.J.Dudley: Chronic hepatitis C: effect of alcohol on hepatitic activity and viral titre. *J Hepatology* 19, 821-926 (1996)
100. Poynard, T. P.Bedossa & P.Opolon: Natural history of liver fibrosis progression in patients with chronic hepatitis C. The fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINVIR, and DOSVIRC groups. *Lancet* 349, 825-832 (1997)
101. Management of Hepatitis C. NIH Consensus Statement, 1997. March 24-26; 15(3):1-41.
102. Geissler, M. A.Gesien & J.R.Wands: Inhibitory effects of chronic ethanol consumption on cellular immune responses to hepatitis C virus core protein are reversed by genetic immunizations augmented with cytokine-expressing plasmids. *J Immunol* 159, 5107-5113 (1997)
103. Izumi, N. Enomoto, N. Uchihara, M. Murakami, T. Ono, K. Noguchi, O. Miyake, S. Nouchi, T. Fujisawa, K. F.Marumo & C.Sato: Hepatic iron contents and response to interferon-α in patients with chronic hepatitis C. Relationship to genotypes of hepatitis C virus. *Dig Dis Sci* 41, 989-994 (1996)
104. E.R. Schiff: The alcoholic patient with hepatitis C virus infection. *Am J Med* 107, 95S-99S (1999)
105. Tsai, S.L. Liaw, Y.F. Chen, M.H. C.Y.Huang & G.C.Kuo: Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology* 25, 449-458 (1997)
106. Diepolder, H.M. Zachoval, R. Hoffmann, R.M. Wierenga, E.A. Santantonio, T. Jung, M.C. D.Eichenlaub & G.R.Pape: Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. *Lancet* 346, 1006-1007, (1995)
107. Ferrari, C. Valli, A. Galati, L. Penna, A. Scaccaglia, P. Giuberti, T. Schianchi, C. Missale, G M.G.Marin & F.Fiaccadori: T-cell response to structural and nonstructural hepatitis C virus antigens in persistent and self-limited hepatitis C virus infections. *Hepatology* 19, 286-295 (1994)
108. Gerlach, J.T. Diepolder, H.M. Jung, M.C. N.Gruener & W.W.Schraut: Recurrence of hepatitis C virus after loss of virus-speicifc CD4+ T-cell response in acute hepatitis C.
109. Hoffmann, R.M. Diepolder, H.M. Zachoval, R. Zwiebel, F.M. Jung, M.C. Scholz, S. Nitschko, H. G.Riethmuller & G.R.Pape: Mapping of immunodominant CD4+ T lymphocyte epitopes of hepatitis C virus antigens and their relevance during the course of chronic infection. *Hepatology* 21, 632-638 (1995)
110. Gerlach, J.T. Diepolder, H.M. Jung, M.C. Gruener, N.H. Schraut, W.W. Zachoval, R. Hoffmann, R. Schirren, C.A. T.Santantonio & G.R. Pape: Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. *Gastroenterology* 117, 933-941 (1999)
111. Makris, M. Preston, F.E. Rosendaal, F.R. Underwood, J.C. K.M.Rice & D.R.Triger: The natural history of chronic hepatitis C in haemophiliacs. *Br J Haematol* 94, 746-752 (1996)
112. Telfer, P. Sabin, C. Devereux, H. Scott, F. G.Dusheiko & C.Lee: The progression of HCV-associated liver disease in a cohort of haemophilic patients. *Br J Haematol* 87, 555-561 (1994)

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