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ASSOCIATION OF ALCOHOL CONSUMPTION AND EXAGGERATED IMMUNOPATHOLOGIC EFFECTS IN THE LIVER INDUCED BY INFECTIOUS ORGANISM

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1. ABSTRACT

The cause of alcoholic liver disease (ALD) is multifactorial and poorly understood. It is clear that alcohol alone is not responsible for most of the changes associated with ALD and that cofactors are involved in initiation and production of ALD. One cofactor that has received a great deal of attention recently is the concomitant infection with hepatitis C virus (HCV) and alcohol abuse. The interactive effects of HCV and alcohol abuse are still unclear, but apparently they are the result of an inability of the immune system to control the viral infection and exaggerated hepatocyte damage mediated by either the cells of the inflammatory response or factors produced by the inflammatory cells. A major effort in my laboratory has been focused on defining the effects of alcohol consumption on immunity to various infectious agents. Efforts have also been directed to elucidating the pathologic effects in the liver of inflammatory and immune responses to microorganisms that either specifically or ultimately infect the liver from an initial site of infection other than the liver. This review will focus on one aspect of the possible pathogenic effects associated with alcohol abuse and hepatic infections: the possible role of the immune system, notably the cytotoxic T lymphocyte (CTL) response. It is clear that the development of a CTL response is critical for the control of HCV and other infections, and it is also likely that this response is involved in liver damage. In this review, the evidence that shows the importance of the $CD8^+$ CTL in bacterial and viral clearance and the role for pathogenesis will be presented. Findings obtained from animal studies that support the suggestion that activated $CD8^+$ CTLs can induce liver damage will be presented, as will results of recent studies from my laboratory that provide evidence for an effect of alcohol to enhance the liver damage mediated by activated $CD8^+$ T cells.

2. INTRODUCTION

Alcoholic liver disease (ALD) is a serious consequence of long-term alcohol abuse by human beings. That quantity and duration of alcohol consumption is not a predictor for the development of ALD has led to the suggestion that there exist cofactors, in addition to alcohol abuse, necessary for the development of ALD. It is clear that a number of possible mediators of liver damage exist. One popular hypothesis is that proinflammatory cytokines induced by bacterial endotoxin [lipopolysaccharide (LPS)] released from the gastrointestinal tract is a major factor in

liver damage in ALD (1,2). Other mechanisms of liver damage have been proposed, including the possible role of metabolites of alcohol such as acetaldehyde (3-5) and reactive oxygen substances (6) directly affecting the hepatocyte. An immune response to hepatocytes altered by acetaldehyde has also been proposed as a mechanism to initiate cell destruction, ultimately leading to the various aspects of ALD (3-5)

One important agent that is involved in the development of severe ALD is infection with HCV (7-15). Alcohol abuse is reported to be associated with an increased severity of hepatitis and the development of chronic hepatitis as compared with findings for infection with the virus only (10,11,15). It has been reported that alcohol abuse is associated with an inability to control the virus (7).

As will be discussed in detail in the next section, consumption of alcohol by human beings and experimental animals results in consistent changes in the immune system (16,17). There seem to be two divergent effects of alcohol on the immune system. The first is the well-described immunosuppression associated with consumption of large amounts of alcohol (16,17). The second—of interest to this review—is that alcohol abuse is associated with a fairly consistent finding of activated lymphocytes in the peripheral blood even in the absence of liver disease (18,19). In experimental animal models, consumption of diets containing ethanol does NOT result in a decreased inflammatory response, and, in models of infections, it has been found that alcohol consumption is associated with an exaggerated inflammatory response in the liver, perhaps because of an inability to control the infection in the liver (20-22).

The premise that will be put forward in this article is that one aspect of ALD involves an infection that affects the liver [including, but not limited to, the hepatitis viruses (B, C, and others, as well as many bacteria)]. The immunosuppressive effects of alcohol limit the ability of the host to clear the infection, which results in an increased nonspecific inflammatory response. This nonspecific inflammatory response includes macrophages and lymphocytes that produce proinflammatory cytokines [e.g., tumor necrosis factor (TNF)] that induce nonspecific liver damage. Further, many agents that infect the liver (notably hepatotropic viruses) induce an immune response characterized by a predominant $CD8^+$ CTL response (23,24). Although the immune response is suppressed by alcohol, this is not a complete suppression, and it is proposed that antigen-specific CTLs are produced that infiltrate the liver. Recognition of infected cells by the antigen-specific CTL results in the induction of hepatocyte apoptosis through interaction of Fas ligand (Fas-L) expressed on the activated CTL and Fas on the hepatocyte (25), [this may be enhanced by alcohol abuse (26)], as well as by TNF (and perhaps other cytokines) produced by the activated CTL. Findings reported in the recently published literature show alcoholic hepatitis is associated with a marked increase in expression of Fas on hepatocytes (27). It has also been shown that alcohol-associated liver injury is accompanied by an increase in the expression of mRNA for Fas-L (28,29). The increased expression of Fas and Fas-L by hepatocytes would increase their sensitivity to activated lymphocytes. Hepatocyte damage by CTL is also mediated by perforin and granzyme-mediated mechanisms (30), as well as by Fas–Fas-L interactions. The perforin–granzyme-mediated hepatocyte damage would most likely be an antigen-specific mechanism. There are study results that show an increased expression of class I major histocompatibility molecules on cells from human beings who abuse alcohol (31,32), and if this finding is extended to the liver, this would, at least in theory, increase class I major histocompatibility–restricted epitopes.

If a viral infection of the liver is not controlled, the interactions of lymphocytes and hepatocytes that are described above will continue and result in a chronic hepatitis. It has been shown, in some individuals, that the immune response wanes before the viral infection is cleared, and this is associated with the development of a chronic viral infection (24). Immunosuppression by alcohol may result in such a situation.

3. IMMUNITY TO HEPATITIS C VIRUS

The immune response to HCV has been relatively well defined in the past few years. It is clear that the most important immune response involves the production of $CD8^+$ CTLs (23-25). In an interesting series of experiments it has been shown that interferon-gamma (IFN-gamma) which is produced by both $CD4^+$ and $CD8^+$ T cells, can not only restrict replication of viruses but also noncytolytically clear virus from infected cells (33). It is thought that the IFN- gamma produced by $CD8^+$ T cells is important in the immunity to hepatotropic viruses. It has been shown that $CD4^+$ T cells are also important in immunity to HCV (23,34), but it is unclear exactly what role they play.

It is also clear that the antigen-specific CTLs produced in response to HCV infection clear infected hepatocytes by cytolytic mechanisms (i.e., perforin and granzyme and Fas–Fas-L), which is a major pathologic mechanism (26, 35, 36). If the virus growth is not controlled and the inflammatory response continues, it is logical for one to suggest that continued destruction of hepatocytes will continue as well.

There are reports of T cells with γ/δT-cell receptors that infiltrate the liver or increase in numbers in the livers of individuals with HCV infections (37), but it is not clear whether these cells are involved in antiviral effects, pathogenic effects, or immunoregulatory functions or in all these possible functions. Similarly, recent reports have shown in at least some experimental infections of the liver that a population of resident cells with characteristics of both T cells and natural killer cells (NK T cells), may be important in some infections by performing functions similar to those functions performed by traditional NK cells (38). It remains to be shown whether these cells are important in the antiviral immune responses or the pathogenic effects associated with hepatic infections of human beings.

4. MODELS OF CD8$^+$ T CELL–MEDIATED PATHOGENICITY IN THE LIVER

It has been shown that CD8$^+$ T cells that have been activated by antigen in peripheral lymphoid organs (i.e., spleen and lymph nodes) home specifically to the liver where they are killed, presumably by Fas–Fas-L interactions (39,40). In this model, the activated CD8$^+$ T cell expresses Fas-L (39,40), and the interaction of the Fas-L on the T cell with Fas on the hepatocytes induces apoptosis of the T cell. It is important to note that this phenomenon is restricted to CD8$^+$ T cells (39-40). This would support the suggestion that CD4$^+$ T cells activated in the periphery may not be involved in liver damage. In fact, results from Budd's laboratory, obtained with the use of animals transgenic for the T-cell receptor for epitopes of ovalbumin that are restricted either to class II major histocompatability (DO11.10) or to class I major histocompatibility (OT-1) complex molecules, show that activation of the CD8$^+$ T cells in the OT-1 mice with ovalbumin resulted in demonstrable liver damage (41). It was clear from this paper that activation of CD4$^+$ T cells by administration of ovalbumin to DO11.10 mice did not affect the liver, at least as reflected by serum levels of alanine aminotransferase (ALT) (41). These data would raise questions regarding the validity of the concanavalin A model used by others as a model system for immune-mediated alcoholic hepatitis (42) as liver damage is associated in this model with activation of CD4$^+$ T cells.

In a more relevant study, it was shown that mice that are transgenic for core antigen of the hepatitis B virus expressed specifically in the liver show a remarkable amount of liver damage when antigen-specific CD8$^+$ T cells were injected into these animals (43). One remarkable finding from these studies was that the initial response was focal areas of apoptosis in the liver that resulted in an inflammatory cascade ranging from a neutrophil infiltration to ultimately a mononuclear cell infiltration that ultimately ended in fulminant liver damage (43). From these findings it can be concluded that the expression of antigen in hepatocytes, presumably by class I major histocompatibility complex molecules, results in recognition by antigen-specific CTLs.

A question that naturally arises is, exactly how do the antigen-responsive T cells encounter parenchymal cells in the liver that express the specific viral epitope? Recent study findings have shown that, in fact, lymphocytes randomly migrate through the liver parenchyma as they do in other tissue (44). It is also important to mention recent studies, results of which show that there is a population of dendritic cells that is resident in the liver and capable of picking up antigen and migration to draining lymph nodes where the initial immune response is produced (45-47). The immune response in the lymph nodes would, of course, include both CD4$^+$ and CD8$^+$ T cells, but it seems that the CD8$^+$ cells traffic specifically to the liver, probably because of the overwhelming expression of class I major histocompatability complex molecules on infected cells.

5 EXPERIMENTAL EVIDENCE FOR ALCOHOL ENHANCEMENT OF CD8$^+$ T CELL–MEDIATED LIVER DAMAGE

One model in my laboratory that has been used to study the effects of ethanol on host defense mechanisms is infection with the facultative intracellular bacterium *Listeria monocytogenes*. Results of the first studies showed that mice fed an ethanol diet were significantly more susceptible to the infection, as evidenced by an inability to control the growth of the bacteria in the liver (20). The infection resulted in a great deal of liver damage that characteristically consisted of a mixture of neutrophils and mononuclear cells (20). Because of the high number of bacteria in the liver it was difficult to determine whether the liver damage was mediated by the cytotoxicity of the bacterial growth in hepatocytes, effects of inflammatory cells, or both. Interestingly, immune mice were susceptible to reinfection if fed ethanol (20), which might support the suggestion of a role for the recall immune response in liver damage, but the immune system still could not control the growth of the bacteria. Thus, interpretation of these findings is difficult. To make the experiments easier to interpret we challenged mice immune to *L. monocytogenes* with a dose of bacteria less than a lethal dose (i.e., 0.5 LD_{50}), as well as an avirulent strain of *L. monocytogenes* (DPL 1942; Act A$^{-/-}$) that would be cleared by the existing immune response. Results of these studies showed that liver damage was evident in these mice if ethanol fed, and the damage peaked at a time when the inflammatory response peaked (22). We interpreted these findings to indicate that the inflammatory response has at least some role in the liver damage associated with this infection. In an attempt to define the role of inflammatory cells in liver damage, we took advantage of an observation that was made in the laboratory: Injection of mice immune to *L. monocytogenes* with heat-killed bacteria was associated with a significant increase in serum ALT levels only in the immune, ethanol-fed group. Further evaluation of these mice showed that the response to heat-killed antigen resulted in a severe steatosis that was evident as early as 6 h after antigen injection (22). Very high levels of ALT and inflammatory cell infiltration of the liver were seen as early as 18 h after antigen injection (22).

The steatosis induced by activation of memory T cells in this system was essentially eliminated by injection of an antibody specific for TNF, but the inflammatory cell infiltration still occurred. Interestingly, neutralization of TNF did not completely eliminate liver damage, as measured by serum ALT levels (22). It has not been determined what cells produce the TNF in this system, but inflammatory T cells, macrophages, or, perhaps, Kupffer cells are possibilities.

It has been well established that the long-term immunity to *L. monocytogenes* is mediated by CD8$^+$ T cells (48) that respond rapidly to a second challenge with bacteria. Although my coinvestigators and I have not shown definitively that the liver damage in the results described above is mediated by activated peripheral CD8$^+$ T cells, this seems to be the most likely mechanism. Studies

are in progress to deplete specifically the $CD8^+$ T cells with monoclonal antibody to show definitively that this is the mechanism. Regardless of the cell that mediates liver damage, it is clear from the findings published in this paper (22), that ethanol consumption sensitizes the hepatocytes to the effects of a recall immune response by some unknown mechanism. This seems to be the crux of the matter. The immune system is essentially under constant activation by various infectious stimuli, and, under normal conditions, few or no pathologic effects in the liver are evident. It is postulated that alcohol abuse, on the other hand, sensitizes the liver such that when activated $CD8^+$ T cells migrate to the liver they induce damage of hepatocytes rather than simply dying by apoptosis, as is the normal effect.

A recently published article (21) shows that other pathogenic bacteria that gain access to circulation can infect the liver and induce pathologic effects. The study results presented in the article by Sibley and Jerrells show that alcohol-fed mice infected orally with *Salmonella typhimurium*, ultimately develop a much more severe hepatitis than control mice infected with the same dose of bacteria. The inflammatory lesions consisted of predominantly mononuclear cells (macrophages and lymphocytes), which was somewhat surprising. It is not known exactly what mononuclear cell types (i.e., T cells, macrophages, and others) infiltrated the liver in response to infection by *Salmonella*, but both $CD4^+$ and $CD8^+$ T cells respond to antigens of the bacterium. The situation is further complicated by the host response to endotoxin (LPS) on this Gram negative organism. Nonetheless, these data show, again, that alcohol consumption adversely affects the ability of host defense mechanism to control hepatic infections.

Also of interest is the hypothesized sensitivity of hepatocytes to damage by the immune system associated with alcohol abuse in the context of a viral infection of the liver. To model this situation, my colleagues and I have established a system that uses murine cytomegalovirus (MCMV) to induce hepatitis in mice. Under normal conditions, this virus produces a self-limiting hepatitis that is mediated by proinflammatory cytokines—especially TNF (38,39)—and to an extent by lymphocytes, including NK cells (50) and $CD8^+$ T cells (51-53). The ultimate control of the virus in the liver depends on the production of antigen-specific $CD8^+$ T cells (52). To investigate the effects of ethanol consumption on the pathogenic effects in the liver of MCMV, C57Bl/6 mice were provided a liquid diet providing 36% ethanol-derived calories in a pair-feeding protocol essentially as described before (54). Mice were infected with a sublethal dose of MCMV (0.5 LD_{50}) and monitored over time to determine the extent of liver damage by measuring serum ALT levels and evaluating histologic changes in the liver. Serum levels of proinflammatory cytokine (TNF) and immune cytokines [IFN-gamma and interleukin (IL)-12] were also determined. The findings (Jerrells et al., Influence of Ethanol Consumption on Experimental Viral Hepatitis, submitted for publication, *Alcoholism: Clinical and Experimental Research*, 2002) show that the infection in the ethanol-fed group of animals resulted in a hepatitis that was not resolved at the end of the experiment, whereas the control animals showed the typical self-limiting hepatitis associated with this virus. The prolonged hepatitis in the ethanol-fed mice was evidenced by an elevated serum ALT level, as well as by a mononuclear cell (i.e., macrophages and lymphocytes) inflammation in the liver determined with the use of histologic techniques. It was found that virus was still recoverable from the livers of ethanol-fed animals throughout the study, which may be responsible for the continued mononuclear inflammatory response. Ethanol feeding was associated with an early suppression of IFN-gamma and IL-12 and a continued suppression of IFN-gamma throughout the infection. A very interesting finding that resulted from this study was that an attenuated stock of MCMV induced immunopathologic effects in the liver in mice only in the alcohol-fed group.

On the basis of these findings, it is suggested that alcohol consumption has a twofold effect. First, the immunosuppressive effects of ethanol limit the ability of the infected host to control the virus, essentially establishing a chronic viral infection in the liver. Recently published findings from my laboratory (55) show that inflammatory macrophages isolated from ethanol-fed mice do not respond as well as macrophages obtained from control animals to IFN- gamma in terms of antimicrobial activity. This would limit the ability of inflammatory macrophages that have infiltrated the liver to kill bacteria and viruses. If hepatocytes are similarly affected by ethanol, the inability to respond to IFN- gamma would limit the ability of this cytokine to noncytolytically clear virus from the infected cell, which seems to be an emerging function for this cytokine (33). Second, the effects of ethanol include sensitization of hepatocytes to the immune-mediated response to the virus. It is still unclear whether the pathogenic effect of the response to the virus is mediated by proinflammatory cytokines such as TNF or by direct interactions of lymphoid cells (NK cells or CTLs) with infected hepatocytes.

6 SUMMARY AND CONCLUSIONS

In summary, data are being produced to support the concept that activated lymphocytes, especially $CD8^+$ T cells, damage hepatocytes by both nonspecific and antigen-specific mechanisms. This can occur through a variety of mechanisms to include production of proinflammatory, cytolytic cytokines such as TNF, which has been suggested to be an important nonspecific mechanism for the liver damage seen in ALD [see McClain et al. (1)]. Other potential mechanisms include the antigen-specific induction of apoptosis of hepatocytes through Fas–Fas-L interactions and by a perforin–granzyme-mediated pathway. It is also possible that other lymphoid cells participate in the liver damage associated with hepatic infections to include NK and NK T cells, and γ/δ T cells, as well as macrophages that would damage hepatocytes through similar mechanisms, as described earlier in this article. It is also possible (perhaps likely) that ethanol somehow sensitizes the hepatocyte, perhaps by metabolites of ethanol such as acetaldehyde or oxidative stress, to exaggerate the pathologic effects of immune responses that

occur in the liver. This effect may be associated with depletion of glutathione or other protective molecules from the hepatocytes.

As with most effects associated with alcohol abuse, the situation is complex and multifactorial. Through careful experiments it will be possible to unravel the mystery and reach mechanistic conclusions, which are necessary to define new therapeutic approaches for the management of ALD.

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8. REFERENCES

1. McClain C. J, D. B. Hill, Z. Song, I. Deaciuc & S. Barve: Monocyte activation in alcoholic liver disease. *Alcohol 27*, 53-61 (2002)

2. Yin M, M. D. Wheeler, H. Kono, Bu. U. Bradford, R. M. Gallucci, M. I. Luster & R. G. Thurman: Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology 117*, 942–952 (1999)

3. Hoerner M, U. J. Behrens, T. M. Worner, I. Blacksberg, L. F. Braly, G. Schaffner & C. S. Lieber: The role of alcoholism and liver disease in the appearance of serum antibodies against acetaldehyde adducts. *Hepatology 8*, 569–574 (1988)

4. Israel Y, E. Hurwitz, O. Niemela & R. Arnon: Monoclonal and polyclonal antibodies against acetaldehyde-containing epitopes in acetaldehyde–protein adducts. *Proc Natl Acad Sci U S A 83*, 7923–7927 (1986)

5. Roman J, A. Colell, C. Blasco, J. Caballeria, A. Pares, J. Rodes & J. C. Fernandez-Checa: Differential role of ethanol and acetaldehyde in the induction of oxidative stress in HEP G2 cells: effect on transcription factors AP-1 and NF-κB. *Hepatology 30*, 1473–1480 (1999)

6. Nieto N, S. L. Friedman & A. I. Cederbaum: Cytochrome P450 2E1-derived reactive oxygen species mediate paracrine stimulation of collagen I protein synthesis by hepatic stellate cells. *J Biol Chem 277*, 9853-9864 (2002)

7. Anand B. S. & M. Velez: Influence of chronic alcohol abuse on hepatitis C virus replication. *Dig Dis 18*, 168–171 (2000)

8. Anderson S, C. L. Nevins, L. K. Green, H. El-Zimait & B. S. Anand: Assessment of liver histology in chronic alcoholics with and without hepatitis C virus infection. *Dig Dis Sci 46*, 1393–1398 (2001)

9. Brillanti S, C. Masci, S. Siringo, G. Di Febo, M. Miglioli & L. Barbara: HCV infection and chronic active hepatitis in alcoholics. *Arch Virol Suppl 4*, 212–214 (1992)

10. Corrao G, & S. Arico: Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. *Hepatology 27*, 914–919 (1998)

11. Regev A & L. J. Jeffers: Hepatitis C and alcohol. *Alcohol Clin Exp Res 23*, 1543–1551 (1999)

12. Rosman A. S, A. Waraich, K. Galvin, J. Casiano, F. Paronetto & C. S. Lieber: Alcoholism is associated with hepatitis C but not hepatitis B in an urban population. *Am J Gastroenterol 91*, 498–505 (1996)

13. Schiff, E. R: The alcoholic patient with hepatitis C virus infection. *Am J Med 107*, 95S–99S (1999)

14. Takase S, N. Takada, M. Sawada, M. Tsutsumi & A. Takada: Relationship between alcoholic liver disease and HCV infection. *Alcohol Alcohol Suppl 1A*, 77–84 (1993)

15. Tanaka T, T. Yabusako, T. Yamashita, K. Kondo, S. Nishiguchi, T. Kuroki & T. Monna: Contribution of hepatitis C virus to the progression of alcoholic liver disease. *Alcohol Clin Exp Res 24*, 112S–116S (2000)

16. Cook R. T: Alcohol abuse, alcoholism and damage to the immune system—a review. *Alcohol Clin Exp Res 22*, 1927–1942 (1998)

17. Jerrells T. R & S. B. Pruett: Immunotoxic effects of ethanol. In J. H. Dean, M. I. Luster, A. E. Munson, & I. Kimber (Eds.), *Immunotoxicology and Immunopharmacology* pp. 323–347. New York: Raven Press (1994)

18. Cook R. T: Cytoplasmic cytokines in the T cells of chronic alcoholics. (Letter). *Alcohol Clin Exp Res 24*, 241–243 (2000)

19. Song K, R. A. Coleman, C. Alber, Z. K. Ballas, T. J. Waldschmidt, F. Mortari, D. R. LaBrecque & R. T. Cook: T_H1 cytokine response of $CD57^+$ T-cell subsets in healthy controls and patients with alcoholic liver disease. *Alcohol 24*, 155–167 (2001)

20. Saad A. J, R. Domiati-Saad & T. R. Jerrells: Ethanol ingestion increases susceptibility of mice to *Listeria monocytogenes*. *Alcohol Clin Exp Res 17*, 75–85 (1993)

21. Sibley D & T. R. Jerrells: Alcohol consumption by C57BL/6 mice is associated with depletion of lymphoid cells from the gut-associated lymphoid tissues and altered resistance to oral infections with *Salmonella typhimurium*. *J Infect Dis 182*, 482–489 (2000)

22. Slukvin I. I, P. J. Boor & T. R. Jerrells: Initiation of alcoholic fatty liver and hepatic inflammation with a specific recall immune response in alcohol-consuming C57Bl/6 mice. *Clin Exp Immunol 125*, 123–133 (2001)

23. Chang K. M, R. Thimme, J. J. Melpolder, D. Oldach, J. Pemberton, J. Moorhead-Loudis, J. G. McHutchison, H. J. Alter & F. V. Chisari: Differential $CD4^+$ and $CD8^+$ T-cell responsiveness in hepatitis c virus infection. *Hepatology 33*, 267–276 (2001)

24. Gruener N. H, F. Lechner, M. C. Jung, H. Diepolder, T. Gerlach, G. Lauer, B. Walker, J. Sullivan, R. Phillips, G. R. Pape & P. Klenerman: Sustained dysfunction of antiviral $CD8^+$ T lymphocytes after infection with hepatitis C virus. *J Virol 75*, 5550–5558 (2001)

25. Chisari F. V: Cytotoxic T cells and viral hepatitis. *J Clin Invest 99*, 1472–1477 (1997)

26. Pianko S, S. Patella, G. Ostapowicz, P. Desmond & W. Sievert: Fas-mediated hepatocyte apoptosis is increased by hepatitis C virus infection and alcohol consumption, and may be associated with hepatic fibrosis: mechanisms of liver cell injury in chronic hepatitis C virus infection. *J Viral Hepat 8*, 406–413 (2001)

27. Natori S, C. Rust, L. M. Stadheim, A. Srinivasan, L. J. Burgart & G. J. Gores: Hepatocyte apoptosis is a pathologic feature of human alcoholic hepatitis. *J Hepatol 34*, 248–253 (2001)

28. Deaciuc I. V, F. Fortunato, N. B. D'Souza, D. B. Hill, J. Schmidt, E. Y. Lee & C. J. McClain: Modulation of caspase-3 activity and Fas ligand mRNA expression in rat liver cells in vivo by alcohol and lipopolysaccharide. *Alcohol Clin Exp Res 23*, 349–356 (1999)

29. Galle P. R, W. J. Hofmann, H. Walczak, H. Schaller, G. Otto, W. Stremmel, P. H. Krammer & L. Runkel: Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. *J Exp Med 182*, 1223–1230 (1995)

30. Balkow S, A. Kersten, T. T. Tran, T. Stehle, P. Grosse, C. Museteanu, O. Utermohlen, H. Pircher, F. von Weizsacker, R. Wallich, A. Mullbacher & M.M. Simon: Concerted action of the FasL/Fas and perforin/granzyme A and B pathways is mandatory for the development of early viral hepatitis but not for recovery from viral infection. *J Virol 75*, 8781–8791 (2001)

31. Parent L. J, R. Ehrlich, L. Matis & D. S. singer: Ethanol: an enhancer of major histocompatibility complex antigen expression. *FASEB J 1*, 469–473 (1987)

32. Singer D. S, L. J. Parent & M. A. Kolber: Ethanol: an enhancer of transplantation antigen expression. *Alcohol Clin Exp Res 13*, 480–484 (1989)

33. Guidotti L. G & F. V. Chisari: Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol 19*, 65–91 (2001)

34. Gerlach J. T, H. M. Diepolder, M. C. Jung, N. H. Gruener, W. W. Schraut, R. Zachoval, R. Hoffmann, C. A. Schirren, 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)

35. Cerny A. & F. V. Chisari: Pathogenesis of chronic hepatitis C: immunological features of hepatic injury and viral persistence. *Hepatology 30*, 595–601 (1999)

36. Chang K. M., B. Rehermann & F. V. Chisari: Immunopathology of hepatitis C. *Springer Semin Immunopathol 19*, 57–68 (1997)

37. Tseng C. T, E. Miskovsky, M. Houghton & G. R. Klimpel: Characterization of liver T-cell receptor $\gamma\delta$ T cells obtained from individuals chronically infected with hepatitis C virus (HCV): evidence for these T cells playing a role in the liver pathology associated with HCV infections. *Hepatology 33*, 1312–1320 (2001)

38. Salazar-Mather T. P, J. S. Orange & C. A. Biron: Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1α (MIP-1α)–dependent pathways. *J Exp Med 187*, 1–14 (1998)

39. Crispe I. N, & W. Z. Mehal: Strange brew: T cells in the liver. *Immunol Today 17*, 522–525 (1996)

40. Huang L, G. Soldevila, M. Leeker, R. Flavell & I. N. Crispe: The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. *Immunity 1*, 741–749 (1994)

41. Russell J. Q, G. J. Morrissette, M. Weidner, C. Vyas, D. Aleman-Hoey & R. C. Budd: Liver damage preferentially results from $CD8^+$ T cells triggered by high affinity peptide antigens. *J Exp Med 188*, 1147–1157 (1998)

42. Cao Q, R. Batey, G. Pang & R. Clancy: Ethanol-altered liver associated T cells mediate liver injury in rats administered Concanavalin A (Con A) or lipopolysaccharide (LPS). *Alcohol Clin Exp Res* 23, 1660-1667 (1999)

43. Ando K., :Mechanisms of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. *J Exp Med 178*, 1541–1554 (1993)

44. Luettig B, L. Pape, U. Bode, E. B. Bell, S. M. Sparshott, S. Wagner & J. Westermann: Naive and memory T lymphocytes migrate in comparable numbers through normal rat liver: activated T cells accumulate in the periportal field. *J Immunol 163*, 4300–4307 (1999)

45. Galle M. B, R. M. DeFranco, D. Kerjaschki, R. G. Romanelli, P. Montalto, P. Gentilini, M. Pinzani & P. Romagnoli: Ordered array of dendritic cells and $CD8^+$ lymphocytes in portal infiltrates in chronic hepatitis C. *Histopathology 39*, 373–381 (2001)

46. Lu L, C. A. Bonham, X. Liang, Z. Chen, W. Li, L. Wang, S. C. Watkins, M. A. Nalesnik, M. S. Schlissel, A. J. Demestris, J. J. Fung & S. Qian: Liver-derived $DEC205^+B220^+CD19^-$ dendritic cells regulate T cell responses. *J Immunol 166*, 7042–7052 (2001)

47. Trobonjaca Z, F. Leithauser, P. Moller, R. Schirmbeck & J. Reimann: Activating immunity in the liver. I. Liver dendritic cells (but not hepatocytes) are potent activators of IFN-γ release by liver NKT cells. *J Immunol 167*, 1413–1422 (2001)

48. Harty J. T & M. J. Bevan: $CD8^+$ T cells specific for a single nonamer epitope of *Listeria monocytogenes* are protective in vivo. *J Exp Med 175*, 1531–1538 (1992)

49. Orange J. S, T. P. Salazar-Mather, S. M. Opal & C. A. Biron: Mechanisms for virus-induced liver disease: tumor necrosis factor–mediated pathology independent of natural killer and T cells during murine cytomegalovirus infection. *J Virol 71*, 9248–9258 (1997)

50. Biron C. A: Activation and function of natural killer cell responses during viral infections. *Curr Opin Immunol 9*, 24–34 (1997)

51. Del Val M, H. Volkmer, J. B. Rothbard, S. Jonjic, M. Messerle, J. Schickedanz, M. J. Reddehase & U. H. Koszinowski: Molecular basis for cytolytic T-lymphocyte recognition of the murine cytomegalovirus immediate-early protein pp89. *J Virol 62*, 3965–3972 (1988)

52. Reddehase M. J, W. Mutter, K. Munch, H. J. Buhring & U. H. Koszinowski: CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. *J Virol 61*, 3102–3108 (1987)

53. Ye M, C. S. Morello & D. H. Spector: Strong CD8 T-cell responses following coimmunization with plasmids expressing the dominant pp89 and subdominant m84 antigens of murine cytomegalovirus correlate with long-term protection against subsequent viral challenge. *J Virol 76*, 2100–2112 (2002)

54. Jerrells T. R, W. Smith & M. J. Eckardt: Murine model of ethanol-induced immunosuppression. *Alcohol Clin Exp Res 14*, 546–550 (1990)

55. Sibley D. A, N. Osna, C. Kusynski, L. Wilkie & T. R. Jerrells: Alcohol consumption is associated with alterations in macrophage responses to interferon-γ and infection by *Salmonella typhimurium. FEMS Immunol Med Microbiol 32*, 73–83 (2001)

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