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
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It has also been shown that alcohol-associated liver injury literature show alcoholic hepatitis is associated with a as by TNF (and perhaps other cytokines) produced by the (25), [this may be enhanced by alcohol abuse (26)], 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 γδT-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.

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.

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).

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).

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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 histocompatibility (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 histocompatibility 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 LD50), 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
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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 LD50) 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 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 γδ 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
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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.

7. ACKNOWLEDGMENTS

My research reviewed in this article was supported by grants AA12450 and AA07731 from the National Institute on Alcohol Abuse and Alcoholism. The technical assistance of Debbie Hoerman, Jackie Pavlik, and Jennifer Jerrells is appreciated. The initial work with MCMV was done by Kristen Mitchell, Pharmacology and Toxicology Graduate Program, Washington State University, Pullman, WA.

8. REFERENCES


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**Key Words:** Alcoholic liver disease; Cytotoxic T cells; CD8⁺ T cells; CD4⁺ T cells; Tumor necrosis factor; Interferon gamma; Fas; Fas ligand; Perforin; Granzyme, Review

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