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HEPATITIS C VIRUS (HCV) AND HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) INFECTIONS IN ALCOHOLICS

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1. ABSTRACT

Approximately 400,000 individuals in the United States are co-infected with hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) and it is likely that almost one in two of these subjects consumes alcohol. The majority of these patients suffer an accelerated course of liver disease as manifested by the onset of cirrhosis within 5 to 10 years of developing HCV infection, as well as an increased risk of developing hepatocellular carcinoma (HCC). It is thought that chronic alcohol abuse mediates liver damage as a result of increased production of free radicals and proinflammatory cytokines. In the setting of chronic HCV infection, alcohol ingestion has an additional effect of diminishing immune clearance and increasing viral burden to hasten the onset of cirrhosis and HCC. Likewise, chronic HCV and HIV-1 co-infection results in a net increase in HCV burden; higher prevalence rates of HCV transmission to sexual partners and offspring, as well as an accelerated progression to end stage liver disease as compared to individuals with HCV infection alone. Thus, the synergistic effects of alcohol abuse and HIV-1 greatly impact on the morbidity and mortality for patients with HCV coinfection. Ultimately, this cumulative disease process will require far more aggressive management with abstinence and counseling for alcohol abuse; highly active antiretroviral therapy (HAART) for HIV infection and combination anti-viral therapy for HCV infection to stem the rapid progression to end stage liver disease.

2. INTRODUCTION

Hepatitis C virus (HCV) is an enveloped single-stranded positive sense RNA virus of the Flaviviridae family. Since its identification in 1989 as the major causative agent of blood-borne, non-A non-B hepatitis, HCV has emerged as the most common cause of liver disease in North America. Chronic HCV infection may lead to a wide spectrum of symptoms ranging from chronic

hepatitis to cirrhosis, hepatocellular carcinoma and end-stage liver disease (1,2). At the onset of the HIV epidemic, coinfection with HCV and HIV-1 was commonly observed in recipients of blood transfusions, hemophiliacs and intravenous drug users (IDU) because both viruses share a blood borne route of transmission (3-6). Since then, a number of cross-sectional studies have shown that coinfection with HCV and HIV-1 is associated with accelerated progression of liver disease (7-9).

Alcohol consumption is independently associated with accelerated progression of a wide spectrum of liver diseases including cirrhosis and hepatocellular carcinoma (HCC) (10,11). Alcoholics have been found to develop severe hepatitis and rapid progression to end stage liver disease in the presence of HCV infection (12-15). There are also reports that in chronic HCV-infected individuals coinfected with HIV, alcohol intake may further modify the course of liver injury (16,17). Accordingly, in this article we review the role of HIV-1 and alcohol as cofactors in the natural history of HCV infection.

3. IMPACT OF ALCOHOL ON HCV PATHOGENESIS

3.1. HCV and liver disease

HCV infection is recognized as a leading cause of cirrhosis, liver failure and HCC. An estimated 170 million people worldwide and 3.9 million in the United States have been infected with the virus (18). Early estimates suggested that between 50 % to 85% of individuals exposed to HCV developed chronic infection, approximately 20% to 30% progressed to cirrhosis and 5% developed hepatocellular carcinoma after 20 to 30 years (19). In the United States, an estimated 8,000 to 10,000 people die annually from HCV-associated chronic liver disease (20).

Prior to 1990, HCV was most commonly transmitted by transfusion and parenteral contact with blood products (21) and the risk of acquiring infection from a blood transfusion from an anti-HCV antibody-positive donor was more than 80% (22). This is evident from the high post-transfusion seroconversion rate of 0.45% in the United States (per unit blood transfused), prior to the initiation of routine screening of blood for HCV antibodies and surrogate markers (23). However, since the implementation of donor screening for surrogate markers as well as serologic testing for HCV, this rate has fallen to as low as 1 in 100,000 (24).

Although the incidence of transfusion-associated hepatitis has declined, it is thought that the significant increase in HCV infection is now related to intravenous drug abuse from shared use of contaminated needles and syringes (21). According to a report from 1993, 86% of injecting drug users in the United States tested positive for anti-HCV antibodies (25). It is not surprising that HIV-1 infection, exposure to hepatitis B virus and a positive rapid plasma reagin test have also been identified as risk factors for HCV transmission in injection drug users. It has been shown that HCV is transmitted more readily by injection drug users than is HIV-1 (26, 27) and needle-stick exposure constitutes a 2% to 10% risk of seroconversion in health care workers (28). The rate of seroconversion may depend on several factors including virus titer and the quantity of virus transferred to the recipient.

HCV can also be transmitted by sexual and perinatal exposure. Sexual transmission of HCV infection in heterosexual couples is less frequent (<3%) compared with HIV-1 transmission (~ 100%) (29,30). The risk for sexual transmission of HCV increases, however, with other factors such as promiscuous sexual behavior, concomitant IDU and the presence of sexually transmitted infections including HIV-1 infection (29,31-33). Approximately 15% to 20% of individuals with acute HCV in the United States report sexual exposure in the absence of other risk factors.

The rate of chronic infection following perinatal exposure has been estimated to range from 0% to 7 % and depends upon several factors including the maternal HCV viral loads, mode of delivery, and coinfection with HIV-1 (34-40). HCV transmission from mother to infant primarily occurs at the time of delivery and depends upon the mode of delivery. For example, in one study, the HCV transmission risk was found to be significantly lower for delivery by elective Caesarean-section than for vaginal delivery or emergency Caesarean-section (34). The risk of perinatal transmission is significantly increased in mothers who are coinfected with HIV (see Section 4.1).

HCV accounts for approximately 20% of cases of acute hepatitis in the United States. The estimated incubation period to onset of symptoms is 7 weeks with a range of 4 to 20 weeks. Viral RNA can be detected in the serum prior to peak alanine aminotransferase levels, which rapidly decline to non-detectable levels in cases of acute self-limiting disease where the serum ALT levels return to normal within 2 to 6 months (2,38).

At least 85% of patients acutely infected with HCV develop chronic HCV infection. Although symptoms of acute hepatitis resolve, ALT levels remain above normal and viral RNA can be detected in the serum (41-43). The histologic appearance of the liver in these patients ranges from normal to changes consistent with chronic hepatitis.
The major complication of chronic HCV infection is the development of cirrhosis, which reportedly occurs in 20% to 30% of patients (44-46). In certain circumstance, cirrhosis can also develop more rapidly, within 1 to 2 years of exposure (2), whereas in low risk cohorts, the development of cirrhosis appears to be markedly attenuated. For example, only 2% of patients were found to have cirrhosis in a cohort of women with HCV infection 17 years after receiving HCV-contaminated anti-D immune globulin (47), which underscores the important role of contributory factors that hasten the onset of progressive disease.

Several predictive factors have been suggested that contribute to more severe or more rapidly progressive liver disease. These factors include age at the time of infection (infants and age greater than 40 years), duration of HCV infection (more than 20 years), host immune

status, male gender, infection with genotype 1, coinfection with another hepatitis virus or HIV-1, heavy alcohol intake (> 50 g/day) and iron overload (17,48-50). Among these factors, the relationship of alcohol and disease progression has been consistently demonstrated in multiple studies (51-53). In one study, the relative risk of developing cirrhosis in HCV positive people who abused alcohol (175 g/day) was found to be 147 fold, using HCV negative and teetotalers as the reference population (51). The relative risk of developing cirrhosis in HCV negative alcohol abusers (175 g/day) and HCV positive teetotalers was only 15 and 9 fold, respectively.

Cirrhosis per se is the single major risk factor for HCC that accounts for nearly 6% of all human cancers (54,55). The annual risk of primary HCC in HCV-related cirrhosis ranges from 1% to 4% (56). Although several lines of evidence indicate an association between HCV and HCC, the strongest evidence comes from Japan, where HCC is the leading cause of cancer deaths in men (56,57). The prevalence of HCV antibody-positive patients with HCC in Japan has been reported to be 70% to 90% (56). In one study, anti-HCV was found in 94% of patients with HCC (27). In contrast, the prevalence of HCV antibody-positive patients with HCC in the United States was found to be only 29% (56,57). Most cases were unrelated to either HCV or hepatitis B virus, implicating other etiologies. The prevalence of HCV-associated HCC in Europe and in South Africa has been reported to be 62-65%, and 29%, respectively (56). Thus, it is conceivable that there are, as yet unknown, genetic factors and environmental cofactors that play an important role in the development of HCC.

As mentioned earlier, a number of host or environmental factors are important in the progression of liver disease. These factors include older age, male gender, coinfection with another hepatitis virus or HIV-1, and heavy alcohol intake. Among the viral factors, the influence of a specific HCV genotype in the evolution of HCC has not been observed in some studies (6,58-60), whereas other reports have suggested that HCV 1b is the most important risk factor for HCC (61,62). HCV genotype 1b is the most prevalent genotype worldwide.

The persistent generation of hepatocytes may be a key feature of HCC associated with cirrhosis (63). Evidence indicates that the risk of HCC is particularly high in patients with histological detection of dysplasia, increased hepatocute proliferation or abnormal liver cell growth (64-67). In an Italian study with 176 cirrhotic patients, 60% of the patients (n = 42) with a high hepatocyte proliferative state index developed HCC compared with only 17% of the patients (n = 134) with a low index (66).

Although, the precise mechanism by which HCV infection results in HCC is not known, there is evidence that the viral proteins may have a direct carcinogenic effect. The HCV genome encodes a large precursor polypeptide of about 3,000 amino acids, which undergoes post-translational cleavage by both cellular and viral proteases into structural proteins (Core, E1, E2) and nonstructural proteins (NS2-NS5) (68). The core protein transforms rat embryo fibroblasts to the malignant phenotype, immortalizes primary human hepatocytes, and inhibits apoptotic cell death in culture (69-71). There is also evidence suggesting that HCV core protein may function as a transcription regulator to promote cell growth by down-regulating p53 expression (72).

The *in vivo* oncogenic role of HCV core protein has been evaluated in mice transgenic for the HCV core gene. Although some investigators did not find core protein cytopathic for the liver cells, others have reported induction of HCC (73-75). Other regions of the HCV genome may contribute to the development of HCC. The truncated NS3 serine protease gene can transform rodent cells in culture, causing tumor formation in nude mice (76,77).

The host immune system plays a very important role in determining the outcome of HCV infection in infected individuals. Immunocompromised patients including those with organ transplantation, HIV-1 infection and hypogammaglobulinemia show more rapid progression of the liver disease than immunocompetent subjects. (8,78,79). Although, HCV elicits antibody responses in most infected individuals, it is well known that such antibodies fail to induce viral clearance in almost 85% of the cases with chronic infection (2). The mechanism by which HCV establishes a chronic infection is not well understood. However, studies in animals and humans have suggested that a primary determinant of chronicity is the development of viral quasispecies, which allows other strains to emerge that escape previous immune response (80-82). Cellular immune responses have been strongly implicated as important factors in the protection against HCV infection. Studies have shown that MHC class 1 restricted, polyspecific CTL are the major effector cells in providing such protection. In one animal study, chimpanzees that successfully cleared acute HCV infection had strong CTL but poor antibody response (83). On the other hand, all the chimpanzees that progressed to chronic infection generated weaker CTL responses. A number of studies on humans with self-limited HCV infection are also consistent with the chimpanzee data. CTL responses were more common in patients who had cleared viremia than in those with persistent infection (84-86).

$CD4^+$ helper T lymphocytes play a critical role in the activation of $CD8^+$ CTL by antigen presenting cells (87). There is evidence that an effective $CD4^+$ T-lymphocyte response is critical for a positive $CD8^+$ CTL response (85). In other studies, a strong $CD4^+$ proliferation response has been correlated with improved outcome in patients with acute infection (88,89). Patients who were unable to maintain this immune response developed chronic hepatitis (89). In additional studies, it has been shown that patients who cleared the virus infection had a predominant T-helper Th1 cytokine response with less of a contribution from the Th2 related cytokines (90). In contrast, patients with chronic infection had a predominant Th2 response. The Th1 response is characterized by production of IL-2 and IFN-gamma, which are required for

cell-type-specific immune response for CTL generation and NK cell activation whereas, the Th2 response is mainly characterized by production of IL-4, IL-5 and IL-10, which help to augment humoral responses (91). Napoli et al. (92) have examined the role of cytokines in liver injury and found that the progressive liver injury in chronic HCV was associated with increased production of intrahepatic IL-2 and IFN-alpha and decreased production of IL-10, suggesting that Th1 cytokines may be involved in mediating hepatocellular damage. Plasma levels of TNF-alpha are also increased in patients with chronic HCV infection and correlate with biochemical markers of hepatocellular damage.

3.2. Alcohol and liver disease

According to the National Epidemiology Survey, almost 1 in 2 Americans (44.3%) actively consume alcoholic beverages, and more than 2 million people are affected by alcoholic liver disease (93,94). It is well known that heavy alcohol consumption can lead to a variety of abnormalities in the liver that include steatosis, alcoholic hepatitis and hepatic fibrosis that precede the development of alcoholic cirrhosis. These alcoholic liver disorders are primarily the result of alcohol metabolism itself in the liver. Although alcohol affects all cell types in the liver, the major target of ethanol-induced injury are the hepatocytes, which account for almost 65% of total cell population and 85% of total volume of the liver (11).

Two major enzyme systems are involved in the metabolism of alcohol in the liver: alcohol dehydrogenases (ADH) and the microsomal ethanol-oxidizing system (MEOS).

ADH has a high affinity for alcohol and accounts for essentially all alcohol metabolism when blood and tissue concentrations are low. However, when the concentrations are high, MEOS with a lower affinity for ethanol also contributes to ethanol metabolism.

ADH converts alcohol to acetaldehyde by removing hydrogen. Then a second enzyme aldehyde dehydrogenase in hepatic mitochondria oxidizes acetaldehyde to acetate by removing additional hydrogen and adding oxygen. Acetaldehyde is a potent toxic metabolite of alcohol and contributes to liver injury in several different ways (10). Acetaldehyde promotes cell death by depleting reduced glutathione levels and inducing oxidative damage (95). Acetaldehyde binds to specific amino acid residues on structural and functional proteins of the cells to form acetaldehyde-protein adduct leading to cellular injury (96). These adducts have been detected by immunohistochemical staining of the liver biopsy specimens of individuals with early-stage liver disease (97,98) as well as in alcohol-fed rat livers (99,100). For example, by binding to the highly reactive lysine residue on the cytoskeletal protein tubulin, acetaldehyde can impair microtubule assembly and function (101,96). Acetaldehyde-protein adducts also activate hepatic stellate cells to produce excess extracellular matrix protein leading to alcoholic fibrosis (102).

In ADH-mediated alcohol metabolism, oxidized nicotinamide-adenine dinucleotide (NAD^+) is converted to reduced NAD (NADH) during the production of acetaldehyde, leading to a shift in the redox state of the hepatocytes which, in turn, results in the inhibition of fatty acid oxidation that favors steatosis and hyperlipidemia (10,103).

The other alcohol oxidation pathway MEOS also converts alcohol to acetaldehyde. The critical component that mediates this conversion is cytochrome P-450 (CYP) 2E1, which is induced predominantly in the hepatocytes by ethanol (104). CYP2E1 utilizes reduced niotinamide-adenine dinucleotide phosphate NADPH, which is converted to $NADP^+$ by NADPH oxidation with the release of oxygen-derived free radicals as by-product. Thus induction of CYP2E1 can result in greater rates of NADPH oxidation leading to increased production of reactive oxygen species (ROS). A number of *in vitro* studies have shown that induction of CYP2E1 by ethanol is associated with generation of ROS, lipid peroxidation and mitochondrial damage (104-106). This is also supported by a number of animal studies showing a correlation between CYP2E1 induction and alcohol induced liver disease (107-109). However, the precise role of CYP2E1 from these studies remains unclear in view of the evidence that alcoholic liver injury in experimental animals can be prevented despite the induction of CYP2E1 (110). Koop et al. (110) have reported that liver pathology was prevented by gadolinium chloride ($GdCl_3$), an inactivator of Kupffer cells, in alcohol and alcohol plus $GdCl_3$ fed rats although the induction of CYP2E1 was similar in both groups. Results of another study indicate that destruction of Kupffer cells by $GdCl_3$ decreases both free radical formation and hepatic damage in alcohol-fed rats (111).

Evidence from a number of studies indicates that Kupffer cells play a pivotal role in ethanol-induced hepatotoxicity. Activation of Kupffer cells by endotoxins can result in the activation of NADPH oxidase, a major oxidant-generating enzyme leading to the release of ROS which, in turn, activates inflammatory cytokines and chemokines that have deleterious effects on hepatocytes (112). NADPH-oxidase deficient mice treated with alcohol do not develop liver pathology in contrast to the alcohol-fed normal mice (113). The NADPH-oxidase-deficient mice did not show ethanol-induced increases in free radical production, TNF-alpha transcripts and activation of NF-kappa B, suggesting that NADPH-oxidase plays an important role in alcoholic liver injury.

Aberrant expression of a variety of proinflammatory cytokines and chemokines including IL-1, IL-6, TNF-alpha and IL-8 is associated with alcoholic liver disease, which plays a critical role in liver injury (114). Increased serum levels of TNF-alpha, IL-6 and IL-8 have been shown in the sera of patients with alcoholic liver cirrhosis (114,115). Although acute ethanol exposure, per se, does not appear to be directly involved in the induction of these cytokines in the liver, alcohol contributes to enhanced translocation of gut-derived endotoxins into the circulation. Endotoxins are taken up by the Kupffer cells

for increased ROS generation and release of cytokines and chemokines (112,116). In addition, ROS produced via alcohol metabolism may itself stimulate the production and release of cytokins and chemokines.

A critical role for TNF-alpha, produced primarily by Kupffer cells, in alcoholic liver injury has been demonstrated in a number of studies (114). Plasma TNF-alpha levels in patients with alcoholic hepatitis correlate with severity of disease and risk of mortality (117,118). TNF-alpha can induce cytotoxicity by activating the TNF receptor p55 (TNFR p55) or TNF receptor 1 (TNF-R1), and by initiating signaling through the death domain (119,120). Consistent with these mechanisms, TNF-R1 mice have been shown to be resistant to alcoholic liver injury, and in another study plasma levels of TNFR p55 have been shown to negatively correlate with total blood glutathione in patients with acute alcoholic hepatitis (121). The Kupffer cell derived TNF-alpha can also induce hepatocytes to produce IL-8 and macrophage inflammatory protein-2 (MIP-2), the potent chemoattractants for neutrophils which facilitates the interaction between inflammatory leukocytes and target cells, and exacerbate cytotoxicity (122,123).

Plasma levels of IL-6 (hepatocyte stimulating factor) are elevated in patients with alcoholic hepatitis and correlate with severity of liver disease (124,125). A recent study suggested that IL-6 in alcoholic liver disease was involved in mediating anti-apoptotic signals to overcome alcohol-induced hepatic apoptosis (126). The study demonstrated that livers from chronically ethanol-fed IL-6 (-/-) mice but not IL-6 (+/+) mice showed significant apoptosis. The expression of anti-apoptotic proteins Bcl-2 and Bcl-x (L) was also markedly elevated in the livers of ethanol-fed IL-6 (+/+) mice, suggesting that IL-6 prevents alcohol-induced apoptosis by induction of Bcl-2 and Bcl-x (L). In a number of studies TGF-beta expression was elevated in patients with alcoholic liver disease (127- 129). TGF-beta increases collagen gene expression and collagen synthesis in hepatic cells (130,131). Patients with active liver disease showed a 97% increase in type 1 collagen synthesis in liver biopsy specimens (127), suggesting that TGF-beta may be the key factor in the development of hepatic fibrosis in alcoholic liver disease.

3.3. Effect of alcohol on HCV-induced liver disease

Alcohol intake has been implicated as the major independent risk factor in the progression of HCV-associated liver disease. Approximately 18% to 25% of alcoholics in the United States are infected with HCV, and the seropositivity rate among alcoholics with liver disease is as high as 40% or even higher (15,132,133). Other studies have reported a strong association of alcohol consumption in promoting progression of liver disease among persons with chronic HIV-1 infection (13, 49, 52,53, 134-137). Harris et al. (137) have reported a 4-fold increased risk for cirrhosis in patients with HCV infection and a history of heavy alcohol abuse (80 g/day). In a population-based study in Italy, the incidence of liver cirrhosis was 10-fold higher in chronic alcoholics infected with HCV compared with non-infected alcoholics (138). Furthermore, HCV infection causes more severe liver disease in alcoholic patients than in nonalcoholic patients. In a large multicenter study, anti-HCV positivity in the sera of alcoholic patients correlated significantly with clinical severity of the liver disease characterized by the presence of periportal inflammation, cirrhosis, piecemeal necrosis and cellular unrest (15). Antibodies to hepatitis B did not correlate with the severity of the disease. Alcoholic patients with HCV antibodies are also likely to have HVC RNA in serum, indicating active viral replication (139). In another study based on HCV antibodies and RNA levels to correlate HCV infection with severity of liver disease, the investigators found that patients with detectable viral RNA and antibodies in sera had more severe liver disease, namely chronic hepatitis and cirrhosis, than those with undetectable levels (140). Patients with cirrhosis and HCV RNA also showed higher alanine aminotransferase activity than patients without HCV RNA. Consistent with this study, in another study alcoholic patients with chronic hepatitis and hepatocellular carcinoma had much higher prevalence rates of HCV RNA (84% and 100%, respectively) compared with those with fatty liver (4.3%), hepatic fibrosis (10.1%) and alcoholic hepatitis (22.2%) (141). Alcoholics with chronic HCV infection also show more rapid progression to end-stage liver disease than non-alcoholics. In one study 58% of the patients in the alcohol group (>40g alcohol/day in women and >60 g of alcohol/day in men) developed cirrhosis by the second decade of exposure to HCV as opposed to only 10% in the non-alcohol group (53).

Total lifetime alcohol consumption is also a risk factor for the disease progression. Patients who progressed to cirrhosis had an average 1.5 –fold higher total lifetime alcohol consumption than those with chronic hepatitis (52). However, other risk factors may have contributed to these differences. The cirrhotic patients were older (> 50 years) at the time of infection and had a longer duration of infection opposed to the patients with chronic hepatitis. The total average alcohol consumption of the cirrhotic patients during the period of infection was also much higher (~ 1.6 times) than for those with chronic hepatitis. Other investigators have also reported association of similar risk factors including male gender with the increased rate of fibrosis progression in chronic hepatitis C patients (13). However, neither of the two studies found significant association between viral genotype with disease progression.

In addition to accelerating the development of cirrhosis, alcohol consumption also appears to hasten the appearance of HCC after cirrhosis is established. In a study of patients with established cirrhosis, 10-year cumulative occurrences rate of HCC was 80.7% in anti-HCV-positive patients with alcoholic cirrhosis compared with 56.5 % in nonalcoholic patients with HCV-associated cirrhosis and 18.5% in anti-HCV-negative patients with alcoholic cirrhosis (142). Another study has reported an almost 6-fold higher risk of HCC in HCV-positive patients with alcohol-induced cirrhosis than HCV-negative patients with alcohol-induced cirrhosis (143). Heavy alcohol intake also shortens the period of time to the development of HCC.

Among patients who consume alcohol at a rate of 80g/day or more, the median disease-free survival time was 12.6 months compared with 25.4 months for patients with alcohol intake of less than 80 g/d (144). In another study, patients with an alcohol intake of 46 g/day or more developed HCC in an average of 26 years compared with 31 years for those who consumed less than 45 g/day (49).

3.4. Alcohol-HCV interactions

Several studies have reported a correlation between HCV titers in the blood and the amount of alcohol intake (134, 145-147), suggesting that heavy alcohol intake enhances the ability of the virus to either replicate or persist in the body. Pessione et al conducted a cross-sectional study in 233 HCV carriers to determine the relationship between HCV levels in the blood and self-reported alcohol consumption (SRAC) (134). The amount of alcohol consumption significantly correlated with the viral RNA titers in the blood. An average consumption of 35 (range 1-69) grams of alcohol/week to 105 (range70-139) grams/week increased viral titers almost 3-fold in the blood. The degree of hepatic fibrosis correlated with patients age and alcohol consumption. In another study, moderation of alcohol consumption has been shown to decrease HCV titers in the blood and the severity of liver disease (146), further suggesting that there is a clear relationship between alcohol consumption and virus replication or clearance.

Although the interaction between alcohol and HCV is not fully understood alcohol can affect virus infection in many ways (148). The immunosuppressive environment created by alcohol can result in faster replication and longer survival of the virus than in an intact immune system. This can, in turn, lead to evolution of a more heterogenous population of viral quasispecies with greater pathogenic potential. In one study designed to examine the quasispecies complexity in alcoholics and nonalcoholics, alcoholic patients demonstrated significantly more diverse quasispecies in the serum than nonalcoholic patients (149).

Alcohol-mediated decreases in the antigen presentation capacity of monocytes can inhibit antigen-specific T lymphocyte proliferation (150), leading to impaired virus-specific CTL response. In addition, alcohol-induced monocyte production of IL-10 and TGF-beta , both of which have immunomodulatory function can contribute to viral persistence associated with Th2 response (151,152,90). Chronic alcohol use can also affect cellular immune responses by altering the cytokine expression pattern in proliferating T cells from Th1 to a predominant Th0 subtype. This switch from Th1 to Th0 has been associated with decreased cellular immune responses to viral structural proteins (153). Further evidence that alcohol can alter immune regulation has arrived from studies showing that increased adenosine deaminase activity in alcoholic cirrhotic patients results in blunted anti-inflammatory properties of adenosine (154). Since adenosine is a crucial factor for the proliferation of CTL (155), decreases in endogenous adenosine could contribute to impaired CTL functions in these patients. Another mechanism by which alcohol consumption may increase HCV replication and/or persistence in infected individuals is through oxidative stress associated with alcohol metabolism in the liver (95). As such, there is evidence that HCV infection itself induces oxidative stress, and very likely, contributes to virus persistence in infected persons (156). Taken together, chronic alcohol consumption can contribute to the natural course of HCV infection through several different mechanisms.

4. IMPACT OF ALCOHOL AND HIV-1 INFECTION ON HCV PATHOGENESIS

4.1. HCV as an opportunistic pathogen in persons with HIV-1 infection

In the United States, an estimated 900,000 people are currently living with HIV-1 infection or AIDS and nearly 400, 000 of them are coinfected with HCV (157). The coinfection with these two viruses is common because both viruses share blood-borne and sexual routes of transmission. The prevalence in IDU is greater than 80% and rises to almost 100% in the hemophiliac population (4,158-160). Although not common, HCV is also transmitted through sexual contact and vertical transmission. In the United States, sexual transmission accounts for approximately 10% of the reported cases of acute HCV infection (29). However, earlier studies have suggested that additional factors such as the presence of HIV-1 infection or other sexually transmitted infections increase the rate of sexual transmission of HCV infection (31-33). In one study, HCV seroprevalence in stable heterosexual partners with HIV-1 was almost 30% higher than in the stable heterosexual partners of individuals with HCV infection (33). The risk of vertical transmission of HCV is also increased in mothers coinfected with HIV-1 (37,39,162). The rate of HCV infection in HIV-1-infected infants is approximately 5-fold higher than among HIV-1-uninfected infants (162). Another study based on the evaluation of worldwide published and unpublished HCV vertical transmission studies showed that the overall transmission rate of HCV from HIV-1-coinfected mothers was 5-fold higher than from the HIV-1-noninfected mothers (163).

4.2. Effect of HIV-1 infection on HCV disease

HIV-1 infection significantly affects the clinical course of HCV infection. HIV-1 infection is associated with higher HCV burden and a more rapid progression of HCV-related liver disease compared with HIV-1-negative individuals (8,7,39,157,164). A number of studies have shown that HCV replication is significantly increased in individuals who are co-infected with HIV-1 (163-165). For example, HCV RNA levels showed nearly an 8-fold increase in hemophiliacs co-infected with HIV-1 and HCV than in these who remain HIV-1 negative, and in a 10-year follow-up study, liver failure was seen exclusively in individuals infected with both viruses (165,166). Another study also indicates that the risk of progressive liver disease is 7-fold higher for hemophiliacs coinfected with HIV-1 than for those with HCV infection (167).

Other studies indicate that HIV-1 coinfection accelerates the incidence and the severity of the liver

disease in HCV-infected individuals. HIV-1-infected individuals with chronic hepatitis C show increased liver fibrogenesis (7, 168-170). Although Serfaty et al (168) did not find a significant correlation between CD4 cell count and the rate of progression of fibrosis, in other studies HIV-infection-induced CD4 depletion is associated with higher fibrosis progression rates (7,169,170), suggesting that immune status plays an important role in HCV-induced liver disease.

The prevalence of liver cirrhosis is also significantly increased in HCV and HIV-1 coinfected individuals. In an autopsy study the incidence of liver cirrhosis was 1.5 times greater in HCV and HIV-1 coinfected hemophiliacs than in individuals infected with HCV alone (171). The mean time for disease progression according to another study was 6.9 years in HIV-1 positive individuals versus 23.2 years in HIV-1 negative individuals (8).

In some patients HCC is a terminal complication of chronic hepatitis C. Although a number of malignancies are associated with chronic HIV-1 infection, the incidence of HCC is rare in HIV-1-infected individuals (172). Information on the role of HIV-1 as a cofactor in the morbidity of HCV-infected individuals is also limited (174). Despite the increased risk of liver fibrosis and cirrhosis with HIV-1 coinfection, the lack of association with HCC is poorly understood.

The mechanism(s) by which HIV-1 infection may increase HCV burden and the progression of liver disease remains unclear. However, it is conceivable that impaired humoral and cellular immune responses in HIV-1 infections also contribute to the more aggressive course of HCV disease. Earlier studies have suggested that the control of HCV infection requires $CD4^+$ Th1 cell-dominated immune response which activates cytotoxic T-lymphocyte response, and Th2 immune response which is linked to the production of antibodies against HCV (175). Since progressive loss of $CD4^+$ lymphocytes is a hallmark of HIV-1 infection, this might directly impair the immune responsiveness of the host to HCV infection. In HIV-1-coinfected individuals, HCV viral load increased with decreasing CD4 lymphocyte counts (163). In addition, a number of proinflammatory cytokines are activated during both *in vitro* and *in vivo* HIV-1 infection, including TNF-alpha, IL-6 and TGF-beta (176). These cytokines are known to promote hepatic fibrosis (30,177). The levels of these cytokines might be higher in HCV-infected individuals who are coinfected with HIV-1, resulting in rapid progression to the liver disease. TGF-beta mRNA expression in patients with chronic hepatitis strongly correlates with lobular necrosis (178). Serum TGF-beta levels are also higher in patients with HCC compared with that of chronic hepatitis C, suggesting that TGF-beta may also play a role in the induction or progression of HCV-associated HCC (179). An *in vitro* study suggests that TGF-beta may also contribute to HCV pathogenesis by inhibiting the HCV-specific cytotoxic T lymphocytes (CTL) activity (180). A number of studies indicate that IL-10 expression is increased in HIV-1 infected cells (181,182). Since IL-10 is a down-regulator of immune responses, it could impair the cytotoxic function of CTL and contribute to virus persistence. The influence of HCV genotype on the clinical course of the disease in HIV co-infected individuals has not been observed to be important in many studies (39,171,173). However, one study has reported that patients co-infected with HIV-1 and HCV showed more rapid progression of HCV infection and AIDS than those infected with HIV and other genotypes (183).

4.3. Effect of alcohol and HIV infection on HCV disease

Alcohol consumption has been implicated as an independent risk factor in the progression of HCV-related liver disease. Although some studies have found no significant association between alcohol consumption and the severity of liver disease in HIV-HCV-infected individuals (17,168), others have shown that alcohol consumption is associated with accelerated disease progression (7,169). Heavy alcohol consumption (>50 g/day), low CD4 cell count (<200 cells/microliter) and age at HCV infection (>25 years old) were found to be the independent risk factors in coinfected individuals contributing to the higher fibrosis progression rates (7). The study predicted that individuals with these risk factors may develop cirrhosis in 16 years compared with 36 years in a person who drinks 50 g or less alcohol per day and has a CD4+ cell count of more than 200 cells/microliter. Alcohol may also worsen the progression of HCV disease by its influence on HIV-1 infection. The combined impact of alcohol and HIV-1 on HCV-induced hepatitis is likely to occur because alcohol and HIV-1 are involved in immune suppression and are known to increase HCV replication (see Sections 3.4 and 4.2). Prolonged alcohol consumption has also been shown to enhance the production of cytokines that promote inflammation and fibrosis (see Section 3.2). Thus, the combination of these two important factors is expected to play an important role in the reduced ability of the host to eliminate HCV and to build protective immunity. Furthermore, altered polarization of Th1 and Th2 responses during HIV-1 and alcohol consumption is likely to contribute to these phenomena, as well.

4.4. Immunocompetent cells in the liver.

The persistence of hepatitis during HCV infection with or without HIV-1 is likely to be controlled by numerous pro-inflammatory factors secreted by immunocompetent and parenchymal cells in the liver. The secretion of these factors, particularly chemokines during the course of HCV infection is not well described or understood. However, the role of chemokines in HIV-1 infection has been gaining considerable attention, because of the ability of HIV-1 to induce the production of chemokines and other cytokines (184). Paradoxically, chemokines may be protective during the early stages of HIV-1 infection, but may be detrimental during the later stages of infection. The anti-HIV-1 property of chemokines is attributed to their inherent ability to block the binding of the viral envelope gp 120 to the chemokine coreceptors on target cells (185, 186). During HCV infection superimposed by HIV-1, these factors may also be further magnified to further compromise the liver to a profound inflammatory reaction. Chronic alcohol use is also

associated with significant increases in the secretion of chemokines (187). Increased chemokine release and CTL abundance in the livers of SIV-infected rhesus monkeys have been observed (188). The liver is a very rich source of chemokines, because its cellular components are capable of secreting these mediators under a wide variety of pathophysiological conditions. Hepatic injury in the absence of overt infection can also elicit the secretion of these factors (189).

Kupffer cells are the cells primarily responsible for the clearance of infectious agents including HCV and HIV-1. After prolonged alcohol use, they are compromised. As a result, their phagocytic, chemotactic and microbicidal actions are attenuated. These conditions may allow the HCV to proliferate within the liver. During chronic alcohol use a similar mechanism is likely to occur. Hepatic sinusoidal endothelial cells are also capable of producing chemokines. Chemokines are expected to attract leukocyte infiltration in the liver that could contribute to hepatic injury. During HCV-induced hepatitis, leukocyte infiltration and proliferation may be sustained by mediators and growth factors released locally by immigrant leukocytes and resident cells, i.e., Kupffer cells, endothelial cells, stellate cells and hepatocytes.

5. SUMMARY AND PERSPECTIVE

The combination of alcohol abuse coupled with HIV and HCV coinfection is sadly a common and often devastating condition. In these patients, several distinct but related processes converge to produce cirrhosis and liver failure within a short period of time. These pathologic pathways include the cytopathic effects of HCV replication in the liver and the production of reactive oxidative species from alcohol abuse as well as the activation of proinflammatory cytokines and cellular immune response towards HCV, which all contribute to propagating the liver damage. However, the central theme from the numerous studies reviewed revolves around the ability of the immune system to prevent the progressive damage inflicted by uncontrolled HCV infection on the liver. It is clear that young women infected following childbirth seldom if ever develop cirrhosis 30 years after contracting HCV infection. Those individuals with limited immune responses, which includes infants, organ transplant recipients maintained on immuno-suppressive regimens and those compromised by alcohol abuse and HIV-1 infection often develop progressive liver disease within a short period of time.

Accordingly, an organized effort should be made to prevent or treat alcohol abuse as well as HIV-1 and HCV coinfection as early as possible to prevent progressive disease. In the first instance education and community programs can be instituted to prevent alcohol-related disease and to stem the tide of the blood borne virus infection by intravenous drug abuse. In addition, more aggressive antiviral regimens with both HAART to limit the immunosuppressant effects of HIV infection as well as combination treatment for HCV infection with long acting interferon alpha and with Ribavirin should be instituted prior to the development of cirrhosis, as patients are more likely to clear HCV when treated early in the course of the disease. If patients can become abstinent from alcohol and receive treatment prior to the onset of CD4 depletion and cirrhosis, the balance will be redressed considerably in their favor to stem the tide of progressive liver disease and the sequelae of liver failure.

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