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Hepatitis C: Current issues and the potential for feature progress

Hepatitis C'nin bugünü ve geleceği

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ÖZET: *Hepatitis C virusu ile ilgili en önemli konu, virus varlığının erken ortaya çıkarılması ve dolayısıyle tedaviye oldukça erken bir dönemde başlanabilmesidir. HCV-RNA varlığı, hastalığı otoimmun hadiselerden ayıran en önemli unsurlardan biridir. Tedavideki en son eğilimler, Interferona serum ve karaciğer dokusu HCV-RNA'sının kaybolmasına kadar devam edilmesi şeklindedir. Eğer sirozun klinik tablosu varsa ve hasta karaciğer nakline hazırlanmakta ise, serumdaki yüksek HCV-RNA seviyeleri Interferon tedavisi ile azaltılmaya çalışılabilir. Bundan amaç, transplantasyon sonrası HCV hastalığının tekrarlanma olasılığını geciktirmek veya önleyebilmektir. Bu hususdaki görüşler bu çalışmada geniş olarak irdelenmektedir.*

Anahtar Kelimeler: **Hepatitis C**

SUMMARY: *The key issue in hepatitis C virus (HCV) is its early detection, therfore leading to treatment at a reasonable early phase of the disease. Differentiation from autoimmune hepatitis will be an an important issue and detection of HCV-RNA will play an important role in this aspect. Current trends are to treat these patients with high dose of Interferon until they achive HCV-RNA negativity both in the serum and the liver tissue. If the patient has already established cirrhosis and orthotopic liver transplantation (OLTx) is in consideration, high serum titers of HCV-RNA can be decreased with pre-OLTx trial of IFN in anticipating of delaying or even preventing post-OLTx recurrence of HCV infection. These aspects are disccussed in details.*

Key words: **Hepatitis C**

HEPATİTİS C virus (HCV) infection is a major health care problem worldwide. In one study performed in the United States, of 2,523 patients tested for HCV in an urban emergency department, 18% were found to be positive. In contrast the seropositivity rates for HBV and HIV-1 were remarkable lower; 5% and 6%, respectively (1).

Detection and Methods

The quest to the agent responsible for non-A, non-B viral hepatitis came to an end in 1988 with the identification of an RNA virus (2) subsequently identified as the hepatitis C virus. The first assay for HCV utilizing the detection of antibodies to C100-3, a recombinant polypeptide derived from the NS4 region of the viral genome was introduced in 1989. In most cases of transfusion-related hepatitis C, the antibody detected with this early assay was identifiable 3 to 6 months after the clinical onset of the post-transfusion hepatitis. Occasionally it took as long as a year to become detectable. More recently, second and third generation assays for HCV, which are both more sensitive and detect anti-HCV as early as 30 to 90 days sooner than did the earlier assay) have been introduced into clinical use. These assays also detect antibodies to a variety of structural and non-structural viral proteins rather than C100-3 alone. Although considerable progress has been made relative to our ability to detect antibodies to HCV, current methods are not sufficient to identify all cases of active HCV infection and these "occult cases" currently are detectable only with HCV-RNA testing.

The detection of HCV-RNA using polymerase chain reaction (PCR) techniques is the most sensitive method for detecting HCV currently available. Using this technique, HCV-RNA can be detected within days to a few weeks of exposure to HCV. Thus, with PCR technology, HCV-RNA can be detected in the serum well before the appearance of any form of anti-HCV. More importantly, it documents active infection and persists throughout the duration of the infection. An important point about HCV-RNA is that in chronic HCV infection, the virus may be detectable only intermittently. In addition, it is now well-known that HCV can infect cells other than hepatocytes such as peripheral mononuclear cells, pancreatic

cells and salivary glands as is the case for HBV (3,4). Thus, a single negative HCV-RNA test does not rule out infection or document disease resolution.

HCV-RNA PCR technology has improved to the point that both qualitative and quantitative PCR assays are available using serum or liver tissue as reagents. At HCV-RNA levels less than $3.5x10^5$ genome equivalents/ml of serum, the current quantitative assay for HCV-RNA (branced chain DNA) does not detect virus despite the presence of an active infection. Thus at this level of infection, only the qualitative PCR assay is useful.

Despite these remarkable advances in the detection methods available for identifying cases of HCV, 5% of patients with chronic liver disease continue to have crytogenic disease. Almost half of these patients have been transfused. Thus, it is highly likely that yet another transfusion-associated virus (hepatitis non-A, non-B, non-C) exists.

The Virus

HCV is a linear, single-stranded RNA virus with positive polarity consisting of 9,400 nucleotides and a single open reading frame that codes for a viral protein of approximately 3,000 amino acids (2,5). Although six distinct major genotypes have been identified, recently numerous sub-species (quasi-species) of HCV have been defined by nucleotide mapping. All currently recognized genotypes of HCV are antigenically identical for the purpose of detection of disease. The 5' end of the genome consist of an untranslated highly conserved region adjacent to the genes for various structural proteins such as the nucleocapsid core and the viral surface protein. On the other hand, the envelope proteins are encoded for by a hypervariable region, which varies markedly from viral isolate to viral isolate and sometimes even within viral agents isolated from the same patient at different time periods during the course of infection (quasi-species). This marked variability in protein expression may allow the virus to evade the host's immunologic defense mechanisms directed at the viral envelope proteins.

The 3' end of the genome is responsible for the genes that regulate the expression of various nonstructural (NS) proteins. The NS5 region encodes for an RNA-dependent RNA polymerase which enables the HCV to replicate. The NS4 region encodes the c-100-3 protein.

Being an RNA virus, HCV is quite different from HBV. Specifically, it does not replicate via a DNA intermediate. A minus strand of RNA is produced from the RNA with the help of a RNA dependent RNA polymerase. This reaction RNA to RNA differs from the usual flow of genetic information within the cell (DNA to RNA being the normal flow of information). Moreover, because it is an RNA virus, without a DNA intermediate, it cannot integrate into the host genome.

Unlike Retroviruses, HCV does not contain a reverse transcriptase (5). Thus, HCV is included in the family of Flaviviridae as a new genus. Additionally, in contrast to HBV, HCV circulates at lower viral loads (10^5 to 10^7 virions per milliliter). Because HCV-RNA cannot integrate into the host's genome, it remains a mystery how HCV leads to the development of hepatocellular carcinoma (6). Nonetheless, there is good epidemiologic data to support the concept that HCV infection is a potent predisposing factor in the development of hepatocellular carcinoma (HCC).

Disease Mechanisms

In contrast to HBV, HCV is thought to be directly cytopathic for hepatocytes. This conclusion is based upon the observation that with interferon treatment responders typically experience a rapid decline in their serum ALT level. This normalisation of the serum ALT level is thought to represent the direct antiviral action of interferon inhibiting a cytopathic virus. There are numerious problems with this conclusion however. First, unlike a classic cytopathic virus, the HCV persists for decades in an infected host. In contrast cytopathic viruses produce sufficient cell injury that they tend to "burn out". Secondly, the injury in most cases of HCV disease is low grade and chronic, rather than severe or fulminant. In fact fulminant hepatitis due to HCV if it ever occurs is rare. Thirdly, despite great efforts to identify HCV in infected tissue, no confirmed reports of findings the HCV using electron microscopy in tissues known to be infected with the virus or in cells undergoing changes consistent with focal cell death as a result of viral induced cytotoxicity have been reported. Finally, immune suppression, as occurs universally following solid organ transplantation, accelerates the natural history of the disease.

The role of the immune system in HCV disease is rather unclear. Recent observations in immunosuppressed patients with chronic hepatitis C (7) and cell culture studies (8,9) suggest that HCV by itself may not be cytopathic. Thus, HCV associated liver disease may not be due solely to a direct cytopathic effect but rather, also be a consequence

of an immune-mediated injury, as is the case for HBV. It is widely believed that the host's immune response to HCV envelope epitopes is the driving force for the numerious quasi species that exist and enable the virus to persist despite an immune response directed against it. Another interesting question about HCV is whether or not immunity occuring after an earlier HCV infection persists. Based upon studies performed in animals, it appears as if antibodies to HCV are not protective and simply represent a marker of an earlier infection. Specifically recurrent infection with an identical HCV genotype as well as other genotypes has been reported in experimental animals. Relevant to this latter issue, it has been established that HCV exists in plasma in two distinct forms: free virus and virus complexed to antibodies (10,11). The possibility exists that the latter form may be neutralized virus. Thus it is possible that neutralizing antibodies, should they exist, may increase during early infection in some individuals (the few who clear virus) and limit the spread of infection to uninfected cells. It also seems likely that the formation of neutralizing anti-HCV antibodies might prevent the spread of infection to neonates from infected mothers accounting for the low rate of vertical transmission of hepatitis C as compared to HBV.

Subtypes of Hepatitis C Virus

HCV is the most variable virus among the hepatitis viruses. The hypervariable region of the virus genome codes for its envelope proteins. As a result, these proteins vary markedly from isolate to isolate and even within the same patient studied across time. Isolates of HCV also vary considerably in certain other parts of their nucleotide sequences such that the identification of individual genotypes is possible (12-15). Genotyping may be important clinically as different genotypes may have different clinical features and lead to quite different clinical outcomes in terms of their disease activity, potential for chronicity, replication efficiency and response to medical treatment (16). Interestingly, the currently recognized genotypes appear to be distributed geographically and may determine disease severity although this latter issue is not fully resolved.

In one recent American study (17), samples obtained from 97 individuals with chronic hepatitis C were genotyped. Type I was found in 74 patients (76%); type II was found in 16 (16%); type III was found in 6(6%); and type IV in only 1(1%). The authors were able to distinguish demographic, clinical, biochemical and histological features of chronic hepatitis C among their American subjects based upon HCV genotype. A second group of investigators, however, were unable to report any clear-cut differences between cases based upon the HCV genotype except that individuals infected with type 1b(II) appeared to have more severe liver disease and have more markedly elevated ALT levels than did those infected with other genotypes. All fifteen of their cases with type 1b(II) HCV had either moderate or severe chronic active hepatitis. Some had cirrhosis. In contrast, only 57 of 74 (79%) patients with type 1a genotype had advanced liver disease. Obviously, more data comparing the genotype of HCV with the natural history are needed before these issues can be resolved fully.

HCV Genotyping

A major controversial point relative to HCV genotyping is the method and nomenclature to be used. Several different methods for genotyping HCV have been described. These include type-specific oligonucleotide sequencing, restriction fragment length polymorphism (RFLP) analysis (18), immunologic and serologic methods (19,20). The gold standard for HCV genotyping has yet to be identified but probably will be based upon the virus' oligonucleotide sequence. The disadvantage of this method however is that it is not applicable to a large number of samples. Despite the many methods currently being used for genotyping and the lack of a specific gold standard, a recently developed consensus system has been reported (21).

Although the clinical importance of genotyping is not well established, it is likely that major antigenic differences exist between various genotypes and may importantly determine disease outcomes. Accordingly, antibodies elicited as a result of infection with one form of HCV being specific for genotype specific envelope protein fail to neutralize other virus genotypes. This phenomenon has important implications not only for the development of vaccines but also for understanding disease progression, and the response of different genotypes and quasi species to treatment as well as the risk of a second infection.

In the recently developed consensus system, the various HCV genotypes have been numbered in the order of their discovery using Arabic numerals starting with la and extending through 6a. Subtypes within a genotype are identified by lower-case letters that follow the major type classification, again in the order of their discovery, such as la-c, 2a-b, 4a, 5a and finally 6a. Using this classification, the genotype first cloned by Choo et al. has been identified as subtype la.

Liver Disease Caused by HCV

Viral Liver Disease, Most viral infections are self-limited. In contrast, hepatitis C virus infection becomes chronic in more than 80% of the patients infected with the virus (22). Moreover, chronic HCV infection is a frequent cause of end stage liver disease in developed countries and is present in 25 to 40% of the patients undergoing liver transplantation at most American centers. Although the natural history of HCV disease in individual cases are not well-defined, it is well-known that chronic hepatitis C is associated with the development of both liver cirrhosis and hepatocellular carcinoma (HCC) (6,22).

The slowly progressive nature of HCV liver disease in obvious from retrospective studies of patients, who have acquired HCV as a result of a blood transfusion (6,22). The mean time of development of chronic hepatitis C, cirrhosis and hepatocellular carcinoma are 10,21 and 28 yearsrespectively in the best such study performed to date (22). Although current knowledge relative to the pathogenicity of HCV infection is quite rudimentary, the following mechanisms appear to be likely: i) HCV is directly cytotoxic; ii) cytotoxic T cell reactivity to HCV determinants occurs and may also determine disease activity or outcome (8,9); iii) the high mutation rate in the hypervariable region of the viral genome (the Es/NS1 region) allows the virus to escape the host's humeral and possibly cellular immune defense systems; and, iv) monocytes and macrophages infected with HCV may indirectly contribute to the hepatocyte injury caused by the virus by secreting cytokines and other small molecules that further enhance liver cell injury (23,24).

Clinical Presentation of HCV Related Liver Disease

Clinically, hepatitis C resembeles hepatitis B except that it has a greater tendency to progress to be a chronic ilness and, finally cirrhosis. This progression is generally occult, causing low grade symptoms, such as chronic fatique and listlessness.

Jaundice is detected in only 5% of patients with acute HCV infection. The risk of fulminant hepatic failure with acute icteric hepatitis C is minimal, being less than 1: 10.000 (25).

Laboratory evidence of HCV Disease: a low grade HCV infection is defined by aminotransferase levels of 100 IU/L or less. In severe cases, the ALT or AST levels may be a high a 300 to 400 IU/L. Chronic infection is characterized by cycles of fluctuating serum aminotransferase levels. Even in patients with histologic evidence of chronic active hepatitis, aminotransferase levels are higly variable (26). Moreover, serum aminotransferase levels may be normal or near normal in patients with cirrhosis caused by HCV.

Histologic evidence of HCV Disease: Chronic hepatitis C has characteristic histologic features (27). Typical findings include the presence of prominent lymphoid follicles and focal, injury to bile ducts, with bile duct proliferation. These histologic findings occurring, in combination with a positive serologic response to one or more autoantibodies provides a useful diagnostic tool for differentiating chronic hepatitis C from low grade autoimmune chronic hepatitis.

Disease course: HCV infection is a strikingly pernicious disease as compared to HBV disease. Once HCV infection becomes chronic, only 2% of patients experience a spontaneous remission. With HBV infection, spontaneous remission occurs in more than 60% of patients with progressive time. Certain factors tend to enhance the virulance of HCV and the likelihood that a HCV infection will become chronic. These include concomitant alcohol abuse, the presence of HBV, or HIV infection, and relativelly older age at the onset of the infection. The lack of HCV remission emphasizes the importance of early and aggressive treatment of HCV infection before advanced liver disease develops. In fact, there are reports of patients who have experienced an apperent clinical remission of chronic hepatitis C but with subsequent follow up appears to undergo a spontaneous reactivation of the disease.

Determination of the extent of infection:

A PCR testing has become more widely available, it may become possible to distinguish between the concepts of HCV contamination, infection and disease. This is especially helpful, since anti-HCV antibodies are not always detectable in the serum.

PCR testing can distinguish HCV contamination from HCV infection because with infection both positive and negative strands of HCV virus RNA are detectable. In the setting of contamination, viral replication does not occur; therefore, only the positive strand of the HCV-RNA is detected.

In contrast to infection, HCV liver disease in identified by liver biopsy and the detection of elevated levels of hepatic enzimes.

PCR testing distinguishes persons who carry rep-

licating virus and are infectious from individuals with prior infection who have had an immune response to HCV and may have cleared the virus. In 70% to 90% of patients with an acute HCV infection, liver enzyme levels remain elevated 12 months after the onset of the acute ilness; by definition, these patients have chronic HCV related disease. Many of these cases are free of symptoms, even though their aminotransferase levels are high and a biopsy if obtained documents results show evidence of chronic hepatitis. In about 80% of patients with chronic disease, the serum is positive for anti-HCV. Antibodies to c33c and c22 appear approximately 12 weeks after the liver enzyme levels become elevated. Antibodies to c100-3 appear by week 15(28).

Immunocompromised individuals such as those who have undergone liver transplantation, received chemotherapy or corticosteroids or other immunosupressive agents, often do not develop anti-HCV antibodies despite the presence of active disease. For these individuals, PCR testing remains the only means of detecting the virus and an ongoing infection.

Differentiation of HCV infection from other types of hepatitis

Since HCV infection does not have a clinical distinct presentation, it can be quite difficult to differentiate it from other forms of hepatitis on clinical grounds alone. For example, the incidence of positive anti-HCV reaction in patients with autoimmune hepatitis may be as high as 15%. Most of these cases are thought to be false-positive reaction a result of the hypergammaglobimemima that is characteristic of their of autoimmune hepatitis.

To lessen the possibility of misdiagnosis, all patients with putative HCV disease should be screened for the presence of other autoimmune diseases with the following serologic tests; antinuclear antibody, anti-smooth muscle antibody, anti-thyroglobulin antibody, and antimicrosomal antibody. In addition, human leukocyte antigen (HLA) typing to detect HLA antigen B8 and DR3, which are common in individuals with autoimmune disease might be recommended. Moreover, thyroid function assessment consisting of measurement of serum levels of thyroid hormone (T4) and TSH, are recommended to identify cases with autoimmune throid disorders (29).

If the results of any of these serologic tests are positive, the potential for autoimmune hepatitis as the cause of the liver disease or as a confounding liver disease is real and must be considered in the treatment and follow-up of a give patient. Other autoimmune diseases such as diabetes mellitus, thyroiditis, autoimmune hemolytic anemia, alveolitis, pulmonary fibrosis, pulmonary hypertention, psoriasis, rheumatoid arthritis and monoclonal gammopathy can occur. In these setting, interferon alfa therapy must be used carefully as it can aggravate the associated autoimmune disease. Bear in mind, however, that some markers believed to suggest autoimmune disease such as anti-Liver Kidney-microsome antibody (anti-LKM antibody), anti-GOR (Garnier, Osguthorpe-Robson) antibody have been associated with HCV infection; their presence does not necessarly imply that the patient has a confounding autoimmune disease process (30).

After establishing a firm diagnosis of chronic hepatitis C, three major issues related to treatment should be considered carefully: These are i) dosage, ii) duration of treatment, and iii) how to monitor response to treatment. Generally it is believed that 3-5 million unites of IFN alfa 3-7 times a week for 6-12 months is the appropriate dose and duration of treatment. The high rate of disease reactivation with lowe doses and shorter periods of therapy have stimulated hepatologist to use higher doses for longer periods of time up to 18-24 months. The third issue, how to monitor the effectiveness of therapy is especially important. Serum ALT levels have been used widely to monitor the response. It is often thought that persistent normalization of the serum ALT level is associated with viral clearance and such has been reported a loss of detectable anti-E2/NS1 and anti-c 100/NS4 antibodies. However, serum and liver tissue HCV-RNA negatively are required to document disease resolution. In a small retrospective study, of 10 patients treated with IFN alfa for 3 to 6 year, a sustained loss of serum HCV-RNA was associated with long-term remission. Relapses when they occur usually are preceded by reappearance of HCV-RNA in the serum. In a larger, multicenter trial, disappearance of HCV-RNA in the serum by the fourth week of treatment was found to be a prerequisete for a sustained clinical response (32). Based on all of the available evidence, it is recommended that the following variables be measured in all patients being treated with interferon for HCV infection: HCV-RNA, aminotransferases, hepatic iron, and glutamyl transpeptidases. These tests assess viral carriage, and virus-induced liver and bile duct damage and provide data relative to the likehood of IFN treatment to be effective.

Autoimmune Disorders

A puzzling phenomenon relative to HCV infection is the role of the immune response and autoimmunity to HCV associated liver disease. A major question is whether or not an association between autoimmune hepatitis (AH) and chronic hepatitis C due to HCV infection exists. If so, current therapeutic approaches to HCV associated AH and possibly chronic hepatitis C (CHC) may need to be revised. Moreover whether certain genotypes or subtypes of HCV are more likely to be associated with autoimmune phenomenon than others, remains to be determined. Nonetheless, many studies have shown that patients with chronic hepatitis C have detectable autoantibodies and other features suggesting the existance of confounding autoimmune process (33-43). The highest frequency of such phenomenon has been reported in German and Italian patients who also have a unique liver kidney microsomal (LKM) autoantibody (34-36). Additionally, antinuclear (ANA) and smooth muscle antibodies have been reported to occur at higher frequencies in individuals with CHC than in others with liver disease. The prevalance of these autoantibodies in cases of HCV disease ranges from 44% to 80% (33-37). Additional observations relative to the role of HCV and autoimmune liver injury are that there is a strong relationship between seropositivity for antibody to GOR and LKM antibodies and HCV infection (35); GOR is a naturally occurring pentadecapeptide that is readily recognizable by the host's cellular immune responses and anti-GOR is detectable in the majority of patients with HCV infection (36,37) but very rarely in patients with other forms of liver disease.

How can this association between HCV infection and the presence of these markers of autoimmunity be explained? a structural similarity between the targets of these antibodies and proteins produced by the HCV appears most likely. As a result, a HCV infection can initiate an immune response to structurally similar host antigens converting them into an auto-antigens which are capable, at least in some cases, of triggering an autoimmune reaction. A host of putative immunologic disorders such as Sjogren's syndrome, lichen planus, Hashimoto's thyroiditis, membranous glomerulonephritis, polyarteritis nodosa, cryoglobulinemia and even autoimmune hepatitis have been described in individuals with co-existing active HCV infections and represent examples of immune mediated disease occuring in a HCV infected individual (38-41).

Exacerbations of autoimmune hepatitis have been observed in persons given interferon as a result of erroneously diagnosed HCV disease. This effect has been atributed to the fact that IFN increases expression of HLA class I and class II antigens on liver cells. The result is an exaggerated presentation of these antigen to both helper and cytotoxic lymphocytes, which leads to exacerbation of the underlying autoimmune disease. When there is evidence of autoimmune disease in addition to hepatitis C, patients should be treated with interferon only after they have been informed that the autoimmune disease might be exacerbated and that they are willing to accept this additional risk. Should treatment be undertaken, close monitoring is nesessary; if autoimmune disease becomes clinically active or exarcerbated, consideration of stopping IFN therapy is essential. It is usually wise to pretreat such patients for their autoimmune response and then to initiate interferon therapy only after the autoimmune disease has been controlled. The autoimmune therapy must be maintained throughout the time period of interferon administration (44).

HCV Disease

Obviously, it is essential for clinicians to reach a definitive diagnosis of the type of hepatitis before initiating a specific course of treatment. A major issue relative to this statement is whether or not IFN treatment has or will produce a deleterious effect in patients with a coexistent autoimmune disease and/or reactivity. Moreover, steroids increase viral replication while IFN therapy may exacerbate a pre-existing autoimmune disease proces. Thus, the wrong treatment for hepatitis might adversely affect the individual's condition by increasing the underlying disease activity. Consequently, it is essential to determine a precise diagnosis and to investigate the relationships between HCV and autoimmunity before starting any treatment. Each of the following questions bear specific investigation relative to this issue: i) are immunologic abnormalities more frequent in patients with chronic hepatitis due to HCV as compared to normal subjects with other forms of viral and non-viral liver disease; ii) is the cellular and/or humoral immune response to HCV damaging to cells; and, iii) is the immune response to HCV capable of inducing or unmasking a latent autoimmune disease process. All three of these points remain to be answered fully, but appear to be answered by a yes in at least some cases if not a majority. The clinical differentiation of autoimmune hepatitis from HCV infection is a major clinical issue and may not be entirely resolvable

in a given case. Some investigators have suggested that serum anti-LKM1 positivity identifies a distinct autoimmune disorder that is due to HCV (autoimmune hepatitis type II).

Classical autoimmune hepatitis (autoimmune hepatitis type I) is characterized by female predilection, young age, high titer of anti-smooth muscle and ANA, clinical response to steroids and absence of HCV-RNA in the serum. Conversely, chronic hepatitis C is characterized by a relatively older age prevalence, a male predominance, serum HCV-RNA positivity, a response to interferon treatment and occasionally anti-LKM1 autoantibody positivity.

Almost 35% of the patients with essential cryoglobulinemia are anti-HCV and HCV-RNA positive (45-47). In several studies, both HCV antibodies and HCV-RNA have been shown to exist as part of the cryoglobulins (54). Additionally, clinical cryoglobulinemia is reported to occur in as many as 30% of patients with HCV infection. In contrast, the frequency of cryoglobulinemia in patients with hepatitis B is only 15%. It is also well known that HCV is associated with cryoglobulin and immunecomplex deposition glomerulonephritis, polyneuritis multiplex and periarteritis nodosa (45-48). Considering the high prevalence of anti-GOR positivity in both chronic hepatitis C and autoimmune hepatitis, some sort of cross-recognition between the GOR protein and an HCV nucleocapsid protein epitope is likely (36).

Tissue- Antigens and Hepatitis C Virus Infection

It is possible that HCV infected plasma cells and persistent HCV infection facilitate the expression of B cell associated immunologic processes leading to immunoglobulin production which have cryoglobulin and rheumatoid factor activity. HCV infection also enhances the expression of HLA antigens on infected cells, the number of activated T lymphocytes within infected tissues and the presentation of autoantigens to both T and B cells. The role of HLA antigen specific mechanisms in the pathogenesis of HCV disease, however, remains a continuing enigma.

The emergence of viral antigens as neoantigens is mediated by their presentation on the surface of infected liver cells in conjunction with HLA antigens. Manns et al. examined the relationship between autoimmune liver disease and various class II (HLA-DR) antigens (49).

They observed that the major histocompatibility complex (MHC) class III allele C-4 A-QO is increased in prevalence in individuals with autoimmune hepatitis type I and II as well as in primary biliary cirrhosis (49). Further studies should pursue the question of whether or not patients with hepatitis C and this particular allelic marker have a higher incidence of specific other immunologic abnormalities. The variability of the HCV genome, potential homologies between the HCV and potential targets of an abnormal immune response and the role of a genetic predisposition to both HCV disease and autoimmunity are questions which are currently being investigated aggressively.

The potential inductive effect of HCV on autoimmune processes is a extremely important in the field of liver transplantation. Moreover, questions such as: 1) What is the incidence of recurrent hepatitis in transplant recipients; 2) What should an individual's viral status be before liver transplantation; 3) What can be done to reduce the recurrence rate of HCV infection post-transplantation; 4) What is the long-term outcome for transplant recipients with chronic hepatitis C, who also require continuous immunosuppressive therapy; and, 5) What immunosuppressive and/or antiviral treatment protocols would be ideal for use in transplant recipients with intercurrent hepatitis C are each critically important in the feature application of liver transplantation for HCV related disease.

Medical Treatment Modalities

There are three reasons to treat A HCV infection: I) To prevent progression to serious viral liver disease. II) To reduce the risk of hepatocellular carcinoma, and III) To eliminate an infection. Interferons (IFN) are endogenous, naturally occurring glycoproteins that possess antiviral, antiproliferative, and immunomodulatory properties. Three types of IFN alfa are available commercially. Two are recombinant forms; they are designated interferon alfa 2a and interferon alfa 2b. A naturally occurring form of IFN alfa called interferon alfa-NL, is also available. The specific goals of treatment with interferon can be summarized as follows: 1) To eliminate HCV-RNA from serum and liver tissue 2) To diminish the patient's infectivity level; and, when possible, achieve a cure; 3) To normalize the aminotransferase levels; 4) To reduce the level of hepatic inflamation and liver cell death; 5) To relive symptoms; finally 6) To improve the patient's overall health and enhance survival with an improved life quality.

IFN Mechanism of Action

HCV is thought to be directly cytotoxic to liver cells; in contrast, hepatitis B virus damages the liver via an immune-mediated response. Therefore, IFN is believed to halt progression of HCV disease by inhibiting viral replication rather than by enhancing the immune response. The interaction between IFN and its receptors results in endocytosis of the interferon-receptor complex into the liver cell and activation of various intracellular proteins. That promote the biologic actions of interferon which include antiproliferative, cytostatic/cytostatic, antiviral, and immunomodulatory activities.

When IFN was initially used to that patients with hepatitis C infection, a responce was defined as normalization of the ALT level in serum. Responses occurred in 45% of patients given 3 million units of IFN TIW (three times per week), but in only 28% of those given 1 MU TIW, and 8% of untreated patients. Serial liver biopsy specimen revealed a marked reduction in the lobular inflamation and a trend toward less periportal inflamation among the patients who received the higher dose regimen. In contrast, no histologic changes were observed in those receiving the low-dose and untreated groups. More recent studies and follow-up of the patients in these early studies have shown that a normalization of the ALT without clearance of the virus (HCV-RNA) does not document resolution of the infection. Relapses occur weeks to months after IFN withdrawal if HCV-RNA is negative in the serum and liver of IFN treated patients.

Liver Transplantation

Liver transplantation is the only therapeutic option for the patients with end-stage liver disease for whom alternative medical and surgical treatments have been exhausted. In this life-saving procedure, the issue of recurrence of hepatitis C after liver transplantation is an important one. Recurrent infection jeopardizes the donor liver not only as a result of a viral infection perse but may also be associated with an increased rate of rejection (50). To prevent both acute and chronic allograft rejection, life-long immunosuppression treatment is mandatory following liver transplantation regardless presence or absence of HCV the problem is that immune suppression increases HCV-RNA levels (49). Although the precise relationship of HCV-RNA levels to hepatic injury is not well-understood, it is widely thought to be adverse. In one recent transplant study, no strong relationship between the level of the viremia and the degree of hepatic injury could be demonstrated (49) and a benign "carrier state" was thought to be possible in at least some transplant recipients. The majority of transplant cases however progress insidously to cirrhotic liver disease over a time period of 3-5 years.

It is well-established that many liver transplant recipients with and without overt clinical (biochemical) hepatitis are viremic (51, 52). Moreover, the majority (>95%) of those who are viremic pre-transplant will be viremic after transplantation (51-54). In cases transplanted prior to the wide-spread assessment of HCV-RNA testing pretransplantation, it has been difficult to differentiate between a recurrent infection and an intercurrent infection (51). With current techniques, absolute proof of disease recurrence requires verification by high-sequence homology of the hypervariable domain of the E2/NS1 region in paired pre-transplantation and post-transplantation viral samples (54). This is what is seen in the vast majority of cases that have been studied. Interestingly, after transplantation, in patients with recurrent hepatitis receiving immunosuppressive treatment, HCV-RNA levels have been found to increase 16 fold or more relative to pre-transplant levels (49). Fortunately, recurrent HCV infection progresses at a much slower rate than does recurrent post-OLTx HBV infection. Despite high levels HCV-RNA, only half of the patients with recurrent infection have histologic hepatitis at 1-2 years post-OLTx (51,52). In 65% of OLTx recipients with recurrent HCV, ALT levels are within normal limits. Whether this lack of ALT abnormality indicates a lack of disease progression, however, is highly suspected however based upon the fact that non-transplant patients who are HCV-RNA positive with normal enzymes can be shown to have histologic CAH alone or with cirrhosis (51-54). The same appears to be true in transplant cases. Moreover the 3-5 year survival rate of individuals transplanted for HCV related disease is reduced beyond to that other groups including cases of HBV disease and hepatic cancer. Moreover, the increase in death rate and organ loss is due to recurrent liver disease, not graft rejection.

Although most cases of HCV seen in transplant recipients are due to a prior HCV infection, acquisition of de novo HCV from a donor organ or transfused blood is possible. In one study, the precalence of HCV-RNA in cadaveric organ donors was found to be 2.4% (55). As would be expected recipients of anti-HCV positive donor organs with detectable HCV-RNA are more likely to

develop clinical hepatitis post-transplantation than are recipients of anti-HCV positive, but HCV-RNA negative donor organs (55).

In order to determine the recurrence rate of HCV infection among transplant recipients. PCR for HCV-RNA prior to liver transplantation is essential. In one study using these techniques, the HCV recurrence rate was 96% while the rate of newly acquired HCV rate was 35% (51). In another large series, the incidence of anti-HCV positivity following liver transplantation was found to be 13.6% (56). The concordance rate between an HCV positive serologic and histologic hepatitis is quite low (49,51,56). On the other hand, in recent studies (51,52,54), a high incidence of recurrent liver disease in anti-HCV positive liver transplant recipients (40% after 1 year) has been reported. Feray et al. analyzed the long-term consequences of acquired and recurrent HCV infection after liver transplantation in a large population using serology, nested polymerase chain reaction (PCR) and branched-DNA technology (54). The actuarial recurrence rate of hepatitis C virus-related hepatitis was 72% at 1 year. The incidence of newly acquired HCV infection was 20% at 4 years. A major unsolved issue is the origin of the recurrent viral infection after the recipient's own liver has been removed. Replicative forms of HBV have been demonstrated in tissues other than the liver and are thought to be the source of at least some post-transplant HBV reinfections. The same may be true for HCV (54,56). The only way to eliminate the problem of recurrent HCV disease appears to be to eradicate the infection prior to OLTx. To achieve this goal, the patient's infection needs to be treated until the serum, peripheral mononuclear cells and the liver are all HCV-RNA negative prior to transplantation. This may be very difficult to achieve in a time frame of an individual's need for a transplant. A further drawback to pre-transplant IFN treatment is that the effectiveness of IFN is reported to be reduced in cirrhotics. However, unless the infection is eliminated pre-transplant, the required post-transplant immunosuppression may prohibit the elimination of the infection after transplantation (49,53).

Pathology of Post-Transplant HCV Infection

Despite high levels of HCV-RNA seen post-transplantation, ALT levels often remain within normal limits in more than half of the cases (49,51,56). Thus, liver histology is essential to identify whether or not post-transplant hepatitis is present. In one study of 43 transplanted HCV-RNA positive patients at one year post-OLTx, mild chronic hepatitis was found in 18 and progressive liver injury with chronic active hepatitis and bridging fibrosis was found in only 4 (57).

In evaluating recurrent hepatitis C, rejection needs to be considered as some of the histologic features of the two conditions overlap. These include the presence of a mononuclear infiltrate in the portal areas and bile ducts. Fourtunately, there are some histologic features which are typical of recurrent HCV infection. These include fatty infiltration, a parenchymal mononuclear infiltrate and diffuse focal hepatocyte necrosis. Lymphoid aggregates are a common finding of hepatitis C, non-transplant cases but only rarely seen in transplant cases, moreover, the portal inflamation in transplant cases, characteristic of HCV in non transplant cases, can be minimal to non existant post transplantation.

Following transplantation, the immunosuppression required to prevent rejection enhances HCV replication leading to very high HCV-RNA levels. This coupled with the concept that HCV is directly cytopathogenic should lead to a more severe hepatic injury than actually occurs. The explanation for this paradox is not readily available. However, the viral genotype may be an important factor in determining the severity of viral liver disease post-transplantation. Type 1b(II) HCV appears to be more aggressive than other genotypes in liver transplant recipients. This same genotype in non-transplant cases is associated with more severe liver disease.

Medical Treatment of HCV Infection Following Liver Transplantation

The treatment of chronic hepatitis C with interferon with or without the use of other agents is well-established (58,59). The response rates achieved differ considerably from one center to another because different doses and protocols of Interferon therapy are utilized. In a pilot study, Interferon 3 times a week lowered HCV-RNA levels but after cessation of Interferon, HCV-RNA levels returned to pretreatment values (60). Moreover, there was no significant change in liver histology. Because post-OLTx hepatitis can progress in the presence of normal serum enzyme values, the only way to determine the rate of progression of chronic hepatitis in a liver allograft is repetitive liver biopsies.

Intercurrent hepatitis C following liver transplantation is acquired as a result of the use of infected blood products. Improvements in surgical tech-

nique that reduce the need for blood and the use of current HCV antibody assay systems have reduced the rate of de novo HCV disease in all transfusion recipients including those who receive transplants. The current risk of a post-transfusion hepatitis following a single unit of blood is 0.07%. Recent data suggest that as many as approximately one-third of the individuals referred for liver transplantation have evidence of hepatitis C infection (61). In contrast to the many studies addressing HCV reinfection, there are only a few studies that address the long-term clinical course of hepatitis C acquired following liver transplantation.

As noted earlier, because IFN therapy has the potential to produce allograft rejection and is only minimally effective, HCV infection should be treated prior to OLTx rather than after transplantation (62,63). Attempts to accomplish this seal are still only being reported preliminarily. It is well-known, however, that individuals with cirrhosis (who are candidates for transplantation) are less likely than others to respond to Interferon therapy. The treatment of HCV post liver transplantation has been uniformly unsuccesful at eliminating the HCV and achiving a HCV-RNA negative state. The best that has been reported is a stabilization and less after, a redaction in the serum ALT levels. Despite either a reduction or stabilization of the serum ALT level, clinical course of post transplant HCV disease appearars to be progressive over 3-5 years leading to recurrent cirrhosis, an enhanced frequency of rejection episodes, more episodes of severe rejection and on overall poor long term prognosis. As a result, both recurrent and newly acquired hepatitis due to HCV infection post-transplantation remain as major challenge for physicians and surgeons involved in liver transplantation.

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