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Immunology of HCV and HBV in Renal Failure and Transplantation

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Viral hepatitis has a special relationship to renal diseases. Hepatitis B and C viruses (HBV and HCV) infections are more prevalent in renal failure patients than in general population, an important cause of morbidity and mortality of renal failure patients on chronic dialysis and after renal transplantation. The association is largely due to the frequent use of blood products in patients with end-stage kidney diseases and multiple invasive medical procedures to which these patients are exposed. The effects of renal failure on the general health and immune status of patients with renal diseases also make viral hepatitis more difficult to diagnose as well as to manage. Finally, there have been few studies of the natural history and therapy of viral hepatitis in renal failure patients, making conclusions difficult. This paper will review the prevalence, incidence, clinical features, and natural histories of HBV and HCV infections and suggest recommendations for management and therapy in renal failure patients and patients undergoing renal transplantation.
Keywords: **HBV, HCV, Renal Failure, Renal Transplantation, Immunology**

Introduction

Both hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are common in patients with end-stage renal disease (ESRD) and in renal transplant recipients [1-4]. Although both viral replication and liver disease progression are accelerated after renal transplantation, the subsequent long-term impact of chronic HBV and chronic HCV is unclear. Chronic renal failure patients are at particular risk of HBV infection. Early studies have demonstrated that renal failure patients benefit from vaccination; however, not all studies have consistently shown benefit. HCV infection is common among patients with ESRD [5, 6]. However, the effect of HCV infection on survival among ESRD patients, and the impact of renal transplantation on the course of HCV infection have not been adequately defined.

The primary goal of treatment of both chronic hepatitis B and C should be eradication of the viral infection. Secondary aims are prevention of decompensated cirrhosis or hepatocellular carcinoma through sustained viral suppression. When determining the optimal treatment regimen, several questions must be addressed which antiviral agents, whom to treat, and when to start antiviral therapy. Interferon has direct antiviral and potent immunomodulatory actions [7].

Development of screening serological tests for detection of HBV and HCV infections has significantly reduced the incidence rates of these infections after renal transplantation [8]. However, chronic liver disease resulting from HBV and HCV infections is still a major concern in kidney recipients [9]. It is certain that immunosuppressive agents facilitate higher replication rates of both

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HBV and HCV in this patient group. The frequency of hepatitis B surface antigen (HBsAg) seropositivity in recipients overall is low, but the frequency of this antigen is significant in patients with aggravated HBV-related liver disease thereafter. Detection of anti-hepatitis B core (HBc) antibody in serum samples of asymptomatic renal transplants has been reported in the absence of other serological markers for HBV. It is unclear whether HCV antibody status and markers of HBV infection are connected with renal dysfunction.

Immunological pathway in viral hepatitis

The natural history of viral infections is affected by disruption of the host specific cellular immune responses. HBV belongs to a family of closely related DNA viruses called the Hepadnaviruses. Included in this family are the WHV (Woodchuck Hepatitis Virus), the DHBV (Duck Hepatitis B Virus) and several other avian and mammalian variants. All the hepadnaviruses have similar hepatotropism and life cycles in their hosts. Hepadnavirus replication is believed to be largely restricted to the liver because virus entry into hepatocytes is dependent on the presence of a receptor that is predominantly expressed on this cell type. The viral genome of HBV is a partially duplex circular DNA of 3.2 kb that encodes four overlapping open reading frames. The preS-S (presurface-surface) region of the genome encodes the three viral surface antigens by differential initiation of translation at each of three in-frame initiation codons. The most abundant protein is the 24-KD S protein (which is known as HBsAg). The preC-C (precore-core) region encodes HBcAg (Hepatitis B core antigen) and HBeAg (Hepatitis B e antigen). HBeAg is not required for viral replication and plays no role in viral assembly. The P coding region is specific for the viral polymerase, a multifunctional enzyme involved in DNA synthesis and RNA encapsidation. The X open reading frame encodes the viral X protein (HBx), which modulates host cell signal transduction and can directly and indirectly affect host and viral gene expression.

The host's immune attack against HBV is the cause of the liver injury (Figure 1), mediated by a cellular response to small epitopes of HBV proteins, especially HBcAg, presented on the surface of the hepatocyte. HBV infections occur in two stages: the proliferative phase and an integrative phase. During the proliferative phase there is the formation of

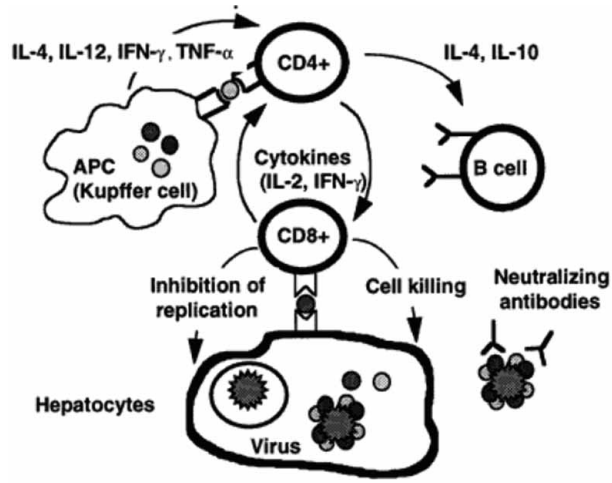

Figure 1. Immunological pathway in viral hepatitis

complete virions and formation of the antigens. The cell surface expression of the antigens leads to activation of cytotoxic $CD8^+$ T cell and hepatocyte destruction. In the integrative phase, viral DNA is taken into the host genome. HBV replicates in hepatocytes to produce HBsAg particles and virions. Both types of particle can be taken up by antigen-presenting cells, which degrade the viral proteins to peptides that are then presented on the cell surface bound to MHC-I or MHC-II (Major Histocompatibility Complex) molecules. HLA class-I-restricted $CD8^+$ cells recognize HBV peptide fragments derived from intracellular processing and presentation on the hepatocyte surface by Class I molecules. This recognition reaction can lead to either direct lysis of the infected hepatocyte or the release of IFN-γ (Interferon-γ) or TNF-α (Tumor Necrosis Factor-α), which can down regulate viral replication in surrounding hepatocytes without direct cell killing. HLA class-I pathway involves internal processing of HBcAg peptides within hepatocytes, leading to their display on the hepatocyte surface. $CD8^+$ cell recognition of peptides displayed in the HLA binding groove initiates apoptosis mediated by the FasL (Fas Ligand), cytokines, and perforin. HLA Class-II-restricted $CD4^+$ T cell recognize externally derived HBV peptide fragments derived from viral proteins presented in the antigen groove of non-hepatic antigen-presenting cells, principally macrophages. The identification of viral protein epitopes by the $CD4^+$ cell results is an increased synthesis of cytokines, which augment T cell proliferation, increase the display of HLA class-I molecules on hepatocytes, and decrease viral replication. In certain circumstances, $CD4^+$ cells may also be capable of a cytolytic attack.

Cell-mediated immunity in hepatitis C

There is accumulating evidence that failure to generate an effective immune response against HCV in the acute phase of infection is responsible for the high rate of chronicity. Most HCV proteins have been shown to be targets of helper T cell responses and cytotoxic T lymphocyte (CTL) activities (Figure 2). Strong T cell proliferative responses against HCV core, E2, NS3, NS4, and NS5 proteins have been found to be associated with self limited infection. The identified immunodominant epitopes are highly conserved among the known HCV isolates and can be presented by different human histocompatibility leukocyte (HLA) class II molecules. Among these epitopes, several highly conserved $CD4^+$ T cell immunodominant epitopes within the NS3 protein have been particularly linked to viral clearance in acute hepatitis C. In addition, the ability to generate anti-HCV multispecific T cell proliferative responses has been shown to correlate with response to interferon treatment. Thus, broadly directed and vigorous proliferative responses against structural and nonstructural proteins seem to be important in controlling HCV infection. Analysis of the cytokine profiles of HCV-specific T cells revealed that persons displaying a T helper type I profile (antigen-dependent production of interleukin-2 and interferon-γ) that promotes cellular effector mechanisms rather than humoral immune responses are more likely to experience viral clearance.

HLA class I-restricted CTLs can directly kill virus-infected cells and produce potent antiviral cytokines and therefore are crucial in clearing viral infections. However, CTL-mediated lysis of virus-infected host cells, if inefficient, can result in persistent infection and chronic tissue injury. In HCV-infected patients, $CD8^+$ T cell responses are directed against structural and non-structural proteins in the context of different HLA molecules. Chronic hepatitis C occurs despite a polyclonal and multispecific HCV-specific CTL activity that can be found in the peripheral blood and in the liver. CTL escape mutants, including CTL antagonists, may contribute to the manifestation of chronic infection [10]. On the other hand, studies showed an inverse correlation between levels of HCV-specific CTL activity and viral loads, suggesting that HCV can be controlled to some extent by CTLs. This observation is confirmed by studies in chimpanzees showing that during acute infection, $CD8^+$ CTL activities correlated better with protection than the antibodies. Additional support for this evidence comes from studies in agammaglobulinemic children in whom resolution of HCV infection can occur independently of antibodies. Thus, the vigor and character of CTL responses in the early phase of infection are probably crucial in clearing the virus, whereas in the later phase insufficient viral-specific CTL responses may contribute to hepatocellular injury.

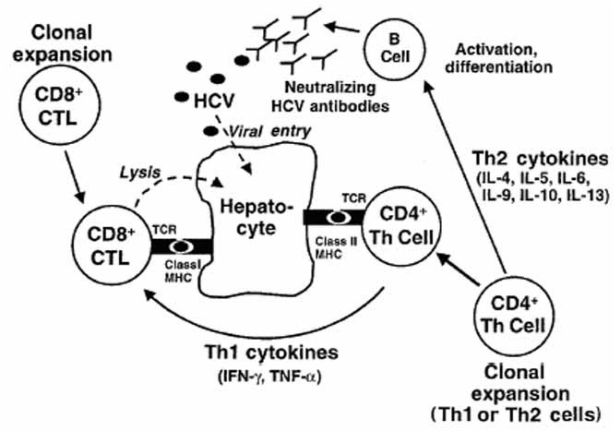

Figure 2. Components of the antiviral immune response: although the hepatocyte is depicted as the target cell of HCV-specific immune response here, other cells, including dendritic cells and macrophages, are also important in antigen presentation to the immune system (CTL: cytotoxic T cell; IL: interleukin; MHC: major histocompatibility complex; TCR: T cell receptor; Th: T helper; Th1: T helper cells with a type 1 cytokine profile; Th2: T helper cells with a type 2 cytokine profile; TNF: tumor necrosis factor)

Effects of HCV before and after renal transplantation

The natural history of HCV in renal transplant recipients remains poorly understood for lack of good longitudinal histologic data. Most early studies have used serum alanine aminotransferase (ALT) elevation rather than protocol biopsies to determine onset and severity of hepatitis after renal transplantation despite the lack of correlation between ALT and histologic activity. Although elevated ALT levels were observed in 50-100% of renal transplant recipients with HCV infection, no associated impact on graft or patient survival could be demonstrated [6, 11]. However, longer follow-up studies have demonstrated reduced patient survival in the second decade after transplantation [12, 13]. Reduced survival in HCV positive renal transplant recipients is attributable to increased liver-related and sepsis-related mortality [14, 15]. The observed increase in gram-negative infection is usually attributed to translocation from the gut because of

impaired immune defenses in advanced liver disease [16, 17]. Both liver-related and sepsis-related deaths in HCV-renal transplant recipients are likely to be direct consequences of cirrhosis. In a recent case-control study, cirrhosis was the most important independent predictor of death after renal transplantation [13]. Despite the increased liver-related and sepsis-related deaths in HCV-renal transplant recipients, renal transplantation does improve overall survival in HCV-patients on hemodialysis [18, 19]. In view of the lengthening waiting lists for renal transplantation, it is important to optimize outcomes by identifying those pre-transplant predictors of poor outcome post-transplant.

In patients without ESRD, interferon-α monotherapy achieves long-term viral eradication (sustained response) in 15% of patients after 6 months of therapy and 25% after 12 months. This efficacy increases in ESRD: 20-42% sustained response after months and 30-70% after 12 months of therapy [7], despite the high occurrence of normal ALT in hemodialysis patients with HCV infection usually associated with lack of response. Sustained responses achieved before renal transplant are maintained post-transplant. Of note, pre-transplant interferon-α therapy has no detrimental effect on subsequent renal allograft survival [20]. Side effects of interferon-α, including a flu-like syndrome, weight loss, and dose-related myelosuppression, are increased in patients on hemodialysis and necessitate dose reduction in 20-60% and withdrawal in 10-45%. Depression occurs in 20% of patients on interferon-α, which may cause depletion of CNS synaptic serotonin. Preemptive therapy with selective serotonin reuptake inhibitors (SSRIs) usually permits completion of interferon. The increased efficacy and side-effects of interferon-α in patient with end-stage renal disease probably reflect higher plasma levels from reduced renal clearance.

Two recent advances in the treatment of chronic HCV are ribavirin and pegylated interferon. Ribavirin has an immunomodulatory rather than an antiviral effect, promoting a switch from predominantly TH2 (viral persistence) to TH1 (viral clearance) cytokine profile. When combined with interferon-α, ribavirin prevents post-treatment relapse, thereby increasing the sustained response rate almost 3-fold. This combination is now the accepted treatment of choice for chronic HCV infection [21]. However, the major adverse effect of ribavirin is dose-related hemolysis. Unfortunately, ribavirin is contraindicated in renal failure because reduced renal clearance leads to rapid accumulation of this drug, resulting in life-threatening hemolysis. Combinations of interferon-α with other less toxic immunomodulators, including levovirin, mycophenylate, and histamine are currently under study in non-renal failure patients.

Pegylation of interferon-α is produced by the addition of multiple polyethylene glycol moieties to a parent drug molecule. The much larger pegylated interferon molecule has reduced volume of distribution and prolonged half-life, enabling once weekly administration. The fluctuations in serum interferon levels are thus avoided, thereby reducing side effects and increasing antiviral efficacy. Viral clearance occurs more rapidly than with standard interferon-α in most responders PCR negative by 4 weeks. In patients without ESRD, the sustained response rate with pegylated interferon is 39%, more than double that of standard interferon [22]. Pegylated interferon does not undergo extensive renal clearance and should be safe in dialysis patients. Unlike standard interferon-α, pegylated interferon is effective in patients with established cirrhosis (sustained response of 45% vs. 4%). However, both interferons may precipitate encephalopathy or variceal hemorrhage in patients who have already developed decompensated cirrhosis. These patients should therefore be considered for combined liver-kidney transplantation rather than antiviral therapy (Figure 3).

Antiviral therapy of HCV positive renal transplant recipients is limited by poor efficacy and safety. Although interferon-α therapy achieves clearance of serum HCV-RNA in 25-50%, all rapidly relapse after treatment withdrawal. This lack of sustained response reflects high pretreatment viral load. Interferon-α therapy in renal transplant recipients is also associated with increased rate of allograft rejection and graft loss [23]. Interferon-α with or without ribavirin is therefore not currently

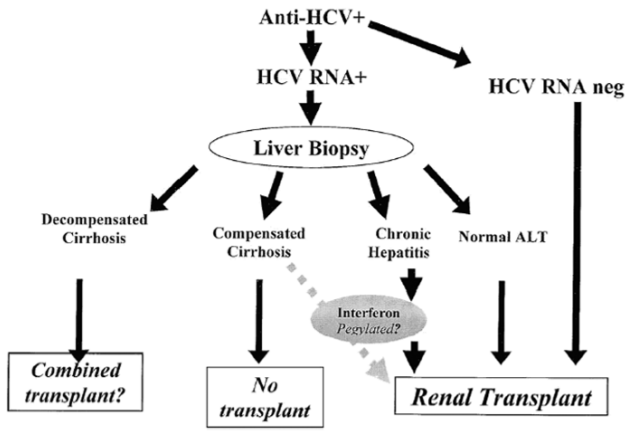

Figure 3. Algorithm for HCV positive renal transplant candidate

recommended in renal transplant recipients with HCV infection. However, recent reports of successful rescue of transplant recipients with severe cholestatic HCV would support the cautious use of combination interferon/ribavirin in this desperate situation. Immunosuppression has an important role in the accelerated natural history of HCV infection observed in transplant recipients. Viremia levels rise 10 to 100 fold after both liver and renal transplantation, reflecting similar primary and adjuvant immunosuppression regimens. Calcineurin inhibitors do not directly enhance HCV replication in non-transplant patients [24]. In contrast, corticosteroid therapy is associated with enhanced viral replication and more rapid progression to cirrhosis before and after liver transplantation. Anti-lymphocyte antibodies are also associated with more rapid progression to cirrhosis after liver transplantation. Although no such studies have been performed in renal transplant recipients, these observations would support steroid-sparing protocols and the avoidance of induction anti-lymphocyte antibodies in all transplant recipients with chronic HCV infection. Mycophenolate mofetil (MMF), an inosine monophosphate dehydrogenase inhibitor like ribavirin, has potent intrinsic antiviral activities and may reduce HCV replication after liver transplantation. Further data is required in renal transplant recipients with HCV infection [25]. Recent studies provide conflicting data on the effect of anti-IL2R antibodies on HCV.

Effects of HBV before and after renal transplantation

Lethal outbreaks of HBV infection in hemodialysis units were common before the introduction of CDC guidelines for infection control in 1977 [26]. The incidence of nosocomial infections fell after the recommendation for routine vaccination of all susceptible patients and hemodialysis staff, in 1982 [27]. The prevalence of HBV infection has fallen by 90% in dialysis units in Europe and North America [28], where outbreaks are now limited to units that have not adopted this simple and cost-effective measure. However, HBV remains a major problem in the Asia-Pacific region where the prevalence of HBsAg in CAPD and hemodialysis patients directly reflects that in the local population (8-20%) [29]. Vertical or early horizontal transmission is the usual route of infection within these hyperendemic countries. Nosocomial infection during dialysis is, therefore, uncommon because most HBsAg negative patients have natural immunity from previous exposure.

Since testing for HBsAg became available in 1969, post-transplant HBV infection has been recognized as an important cause of morbidity and mortality after renal transplantation. Early studies reported an association between HBsAg positivity and reduced survival after renal transplantation, because of a 5- to 10-fold increase in liver-related mortality [30]. In one case-control study, HBsAg positive patients who underwent renal transplantation had a significantly worse outcome than matched HBsAg positive patients maintained on hemodialysis [31]. These observations led to the proposal that HBV infection should be considered an absolute contraindication to renal transplantation. However, this proposal was made without a clear understanding of the natural history of post-transplant HBV infection and before the availability of safe and effective antiviral therapies against HBV.

Serial biopsies in HBsAg positive renal transplant recipients with elevated ALT levels demonstrated histologic progression in 85%, of whom 20% progressed to cirrhosis within 5 years [32]. Protocol liver biopsies in all HBsAg positive patients before and at regular intervals after transplant are needed to accurately determine the natural history of hepatitis B infection after renal transplantation. Until this data is available, many lessons can be learned from liver transplantation for HBV-cirrhosis. To determine which HBsAg positive patients may be suitable for renal transplantation, attempts have been made to identify whether pre-transplant viral (DNA level, genotype) and host (serum ALT level, histologic stage) factors will accurately predict poor post-transplant outcome. There is little correlation between pre-transplant and post-transplant serum HBV-DNA status: although 100% of renal transplant candidates with detectable HBV-DNA in serum before transplant remained HBV-DNA positive after transplant, more than 90% of HBV-DNA negative candidates also became DNA positive post-transplant [33]. This is because corticosteroids markedly enhance HBV replication in all patients via the glucocorticoid-responsive element on the HBV genome. High pre-transplant viral load (either HBeAg positive or DNA>10^5 copies mL) has been associated with reduced 10-year survival after renal transplantation [34], reminiscent of the effect of pre-transplant viral load on recurrent hepatitis B after liver transplantation. Pre-transplant serum ALT level before transplant does not correlate with severity of liver disease, either before or after transplantation. Reports of death from decompensated cirrhosis and hepatocellular carcinoma within 3 years of transplant implied that

many patients had advanced HBV-related liver disease at the time of transplant. In fact, recent studies have confirmed that pre-transplant cirrhosis is the most important predictor of post-transplant mortality in HBsAg positive renal transplant recipients. In a large, long-term follow-up study, although liver-related deaths accounted for almost 40% of post-transplant deaths in HBsAg positive renal transplant recipients; these deaths were limited to patients with cirrhosis. The overall mortality in non-cirrhotic HBsAg positive renal transplant recipients was no different from that in HBsAg negative renal transplant recipients (Figure 4).

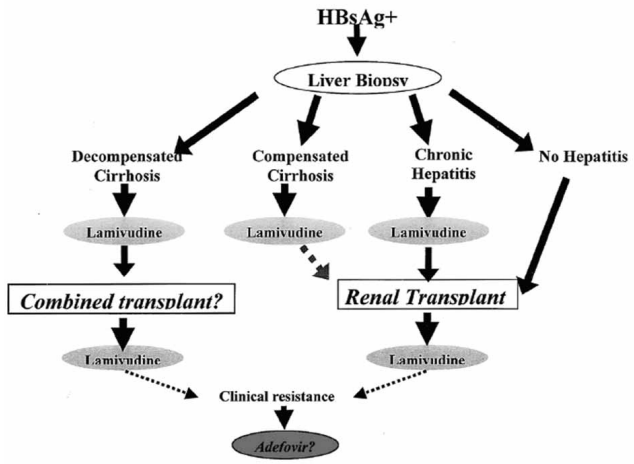

Figure 4. Algorithm for HBsAg positive renal transplant candidate

HBV vaccination in renal transplant candidates

Although universal neonatal vaccination will eventually eradicate HBV infection, targeted vaccination of high-risk groups is currently recommended. Routine vaccination of all patients with end-stage renal disease should rapidly reduce the incidence of HBV infection in dialysis units. This will also reduce post-transplant HBV infection, including de novo HBV infection from an anti-HBc positive donor kidney. Unfortunately, vaccination rates remain low in hemodialysis patients [27]. Additionally, response rates are reduced by renal failure specific defects in cellular and humoral immune responses. Protective anti-HBs responses are achieved in only 60% of CAPD patients, 50% of hemodialysis patients, and less than 20% of renal transplant recipients after conventional vaccination schedules [35, 36]. Double-dose regimens and drug combinations with IL-12 and GM-CSF provide minor additional benefit. Intradermal administration of recombinant HBV vaccine enhances HBV-specific B cell and T cell responses to recombinant vaccine, which is attributed to high concentrations of dendritic cells and memory T cells within the dermis [37]. Intradermal administration appears to be more effective than conventional intramuscular vaccination in healthy volunteers. In patients with ESRD, low-dose intradermal vaccination (3 to 6 doses of 5 mg twice per week) achieved protective levels of anti-HBs in more than 90% [38]. In addition, intradermal vaccination may also be effective after renal transplantation [39]. Although the durability of anti-HBs response in these studies was superior to intramuscular vaccination, monitoring patients every 6 months is recommended with boosters at 1 year or when anti-HBs falls below 10 IU/L. Intradermal vaccination is well tolerated with only 30% experiencing local discomfort and erythema at the injection site.

HBV vaccination in dialysis patients

In dialysis patients, the percentage of seroconversion after HBV vaccination by the conventional i.m. route with the production of sufficient anti-hepatitis B surface (anti-HBs) antibodies (50-73%) has long been unsatisfactory. This figure is significantly lower than the seroconversion rate observed in healthy individuals (>90%). This suboptimal seroconversion rate is probably related to the progressive impairment in cellular immune response associated with deteriorated renal function. Because of the apparent suboptimal response to the conventional regimen, it is recommended that for uremic patients, a four-dose schedule (40 mg/dose given at 0, 1, 2 and 6 months) instead of the conventional three dose schedule (20 mg/dose given at 0, 1 and 6 months) should be given. Alternatively, some reports have shown that by activating specific epidermal cells, i.d. administration of the vaccine might improve lymphocyte responses and increase the seroconversion rate, despite using lower individual and cumulative doses. Apart from seroconversion rate, another important consideration is the durability of the induced immunity [40].

It is considered unnecessary to maintain anti-HBs greater than 10 mIU/mL for a low-risk, healthy population after successful vaccination and initial seroconversion because of the presence of an immunological memory; however, a booster dose of vaccine is generally recommended for dialysis patients because of their immunosuppressive state, poor responses to vaccination and environmental risks for cross-infection. In this context, the shorter durability of immunity for i.d. administration

compared with i.m. administration of the vaccine is particularly relevant. Despite the promising seroconversion rate for high-dose i.d. vaccination, further studies are needed to clarify the costs of vaccination using i.d. versus i.m. administration before its widespread application can be recommended.

Future retrospect

There are an estimated 150 million patients infected with HCV worldwide. The rates of HCV positivity among cadaveric donors vary from 1% to 11.8% in various parts of the world. Post-transplant immunosuppression leads to enhanced viral replication and accelerates the natural history of liver disease. The most important predictor of post-transplant mortality is pre-transplant cirrhosis. Therefore, liver biopsy should be considered in all potential renal transplant candidates who lack clinical or radiologic evidence of cirrhosis. Unfortunately, interferon-α has poor efficacy and tolerability in both HBsAg positive and HCV positive renal transplant recipients and may precipitate renal allograft rejection. In contrast, lamivudine therapy produces safe and effective suppression of chronic HBV infection in HBsAg positive transplant recipients. Routine lamivudine prophylaxis begun at the time of transplantation may prevent severe HBV reactivation. In the future, molecular antiviral therapies against HCV should allow similarly safe and effective post-transplant suppression of HCV. A preliminary study by Weinstein *et al.* has showed on the improved immunogenicity of a novel third-generation recombinant HBV vaccine in patients with end-stage renal disease, in which a seroconversion rate of 86% was achieved after three standard i.m. doses [40]. However, further study would be required to confirm its enhanced efficacy in dialysis patients before it could be applied and recommended in daily clinical practice. Future approaches in patients with ESRD may include the new immunogenic recombinant preS1, preS2 vaccines, therapeutic T cell peptide vaccines, and DNA vaccines [41].

Obstacles in developing an HCV vaccine

The development of an effective vaccine against HCV faces many challenges. First, substantial sequence diversity exists among HCV strains isolated within and between geographic areas. There are at least 6 HCV genotypes and more than 50 subtypes. This makes the development of a global HCV vaccine rather complex. Second, even within an infected person, HCV isolates with rather divergent sequences in certain region of viral genome (quasispecies) are present and mutations occur frequently during the course of infection [42]. In particular, the N-terminus of the E2 protein contains a hypervariable region of about 30 amino acids (HVR1), which shows extensive variation among all known isolates. The genetic variability within this region is thought to allow the virus to escape immune surveillance. Third, immunologic correlates that are associated with protection or disease progression are still being defined. The knowledge of immunogenic epitopes and their relevance to viral clearance and the existence of conserved cross-reacting epitopes are still unclear [43]. These problems are further complicated by the lack of a reliable infectious tissue culture system for testing neutralizing antibodies or passage and expanding of the virus. The availability of such tissue culture systems has been invaluable in the successful development of other vaccines. For HCV, a surrogate assay for the determination of possible neutralizing antibodies has been developed. In this assay, antibodies are tested for their ability to neutralize the binding of highly purified recombinant E2 protein (NOB assay) [44] or antibody-captured HCV derived from high-titer sera 54 onto susceptible cells such as MOL-4 cells. This assay measures only inhibition of binding to target cells, which does not necessarily reflect neutralization of infectious virus in vivo.

The only reliable model for HCV infection is the chimpanzee, which as an endangered species is not only costly but also difficult to study. Furthermore, the course of HCV infection in chimpanzee may not necessarily represent that in humans. Earlier experiments in chimpanzees in which challenge of apparently recovered chimpanzees with a homologous or heterologous strain of HCV resulted in reinfection suggest an absence of protective immunity from natural infection. In addition, HCV manages to persist in chronically infected persons despite the presence of broad antibody and T cell responses. The viral and host factors that lead to persistence are not fully understood and remain to be elucidated in the future. Because the availability of small animal models would have greatly facilitated the development of HCV vaccine, intense effort has been under way to search for such models. Tupaia belangeri, a small primate-like animal, has been shown to be infectable by HBV [45] and is now being evaluated as a small animal model for HCV. However, the robustness and reproducibility of this

model remain to be fully confirmed. Alternatively, mouse models for HCV have been developed by either establishing HCV transgenic mice or transplanting human hepatocytes into immunodeficient mice. These models may prove to be useful in certain aspects of HCV vaccine development. The eventual goal, of course, should be prevention of both HBV and HCV infection before renal transplantation through universal HBV vaccination and the adoption of infection control practices in patients with end-stage renal disease.

A new generation of vaccines are liposomal peptide vaccines which are efficiently tested in the preclinical mouse model and now are investigated the immunogenicity of a liposomal or virosomal vaccine in clinical trails. Among the different strategies to deliver peptides through the immunogenic route, liposomes are favorable in many aspects: firstly, they can be produced inexpensively from completely synthetic compounds, secondly they protect peptides from extracellular degradation and thirdly they have been safely used in humans for many years in different clinical applications [10]. Future experiments will be investigated the use of HCV protein sequences integrated or cross-linked to the surface of liposomes. This may allow simultaneously stimulating the $CD4^+$ and $CD8^+$ T cell responses against HCV epitopes. Thereby the rather broad immune response induced by recombinant viral vectors or plasmid DNA may be combined with the potent and strongly focused $CD8^+$ T cell response stimulated by liposomal formulations.

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