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Conference report

Summary of the first international symposium on viral hepatitis

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1. Introduction

Viral hepatitis is a major global health problem. There are five known viruses that primarily infect the liver and cause hepatitis: hepatitis A; B; C; D and E. Hepatitis B, C, D virus (HBV, HCV, HDV) infections can lead to chronic liver disease with its attendant long term sequelae including cirrhosis and hepatocellular carcinoma (HCC). Rapid developments in molecular techniques during recent years have had a tremendous impact on our ability to diagnosis and understand the pathobiology of these important viral infections. This enhanced understanding has led to the development of improved antiviral therapy and, in some cases, vaccine strategies.

Between November 1 and 3, 1998, a symposium on viral hepatitis was organized and convened by The Macrae Group (New York, NY, USA) to review the recent progress in the rapidly evolving field of viral hepatitis. During this conference, leading basic scientists and clinicians, from the US and abroad, focused on important aspects of viral hepatitis including natural history, epidemiology, diagnosis, and current and future approaches to treatment. The aim of this manuscript is to review the information presented during this conference. The meeting was chaired by T. Poynard, S. Schalm, H.C. Thomas, and T. Wright.

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Other presenters at this symposium were Wolfram Gerlich (Institute of Medical Virology, Klinikum der Justus-Liebig-Universitat, Giessen, Germany), Gabriele Missale (Azienda Ospedaliera di Parma, Italy), David Wong (Harvard Medical School, Boston USA), Lorne Tyrrell (University of Alberta, Canada), John Thompson (Vertex Pharmaceuticals, Boston, MA, USA), Hung Le (Schering-Plough Research Institute, Kenilworth, NJ, USA), Darius Moradpour (University of Freiberg, Germany), Bud Tennant (Cornell University, Ithaca, NY, USA), Patricia Marion (Stanford University, Palo Alto, CA, USA), Patrizia Farci (University di Cagliari, Italy), Peter Karayiannis (St. Mary's Hospital, London, UK), Anna Lok (University of Michigan, Ann Arbor, MI, USA), Geoffrey Dusheiko (Royal Free Hospital, School of Medicine, London, UK), Rajender Reddy (University of Miami, Miami, FL, USA), Phillip Furman (Triangle Pharmaceuticals, Durham, North Carolina, USA), Mark Atkins (GlaxoWellcome, London, UK), Jack Wands (Massachusetts General Hospital, Boston, MA, USA), Stephen Locarnini (Fairfield Hospital, Victoria, Australia), George Drusano (Albany Medical College, Albany, NY, USA), and Johnson YN Lau (Schering-Plough Research Institute, Kenilworth, NJ, USA).

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2. Mechanisms of viral persistence

In general, viruses establish persistent infections by inducing little direct cell damage and/or by evading immunologic surveillance. Indeed, in vivo and in vitro data suggests that hepatitis virus infections generally cause minimal cytopathic damage. Lack of direct cell injury allows the virus the opportunity for continued viral replication and the establishment of chronic infection.

2.1. Hepatitis B virus

Viral persistence in HBV infection is likely related to the failure of the host to respond to viral antigens. The increased chronicity rate observed in immunodeficient individuals such as the young, elderly, and immunosuppressed, support this contention. Animal and human studies clearly indicate that the establishment of chronic HBV infection is inversely proportional to the age at infection. Infection in the perinatal period or during infancy leads to chronic infection in over 90% of cases, while chronic infection occurs in $< 5\%$ of those infected later in life. Newborn transgenic mice that synthesize high levels of hepatitis B 'e' antigen (HBeAg) have been shown to be tolerant to both HBeAg and its precursor HBcAg (Millich et al., 1990). The similarity between the immunologic events in transgenic mice and natural perinatal HBV infection suggests that HBeAg may function to induce immunologic tolerance and result in chronic infection. Perinatally acquired HBV infection is characterized by high-level viremia and absence of liver disease; thus, further supporting immune tolerance in the development of viral persistence.

Viral persistence can also be related to the ability of the HBV to change under specific drug-induced or immune-mediated genetic pressures. Drug resistant HBV 'mutant' viruses arise during antiviral therapy, which results in continued viral replication and chronic infection.

2.2. Hepatitis C virus

The mechanism responsible for viral persistence in HCV infection is not well defined but viral heterogeneity (quasispecies nature) likely plays a major role. The ability of HCV to change under environmental pressure allows the emergence of variants that can evade immunologic surveillance and lead to persistent infection. Such HCV variants capable of escaping cellular immune recognition have been impli-

cated in the establishment of persistent infection in chimpanzees and humans (Weiner et al., 1993, 1995). The development of chronicity has also been directly correlated with degree of viral heterogeneity during acute infection (Enomoto et al., 1993; Sakamoto et al., 1994; Yamagushi et al., 1994). Experimental evidence suggests that HCV may also infect lymphoid cells (Shimizu et al., 1992; Muller et al., 1993). Thus, sequestration into immunologic 'privileged' sites, may be another mechanism that promotes chronic HCV infection.

3. Mechanisms of hepatocellular damage

In general, viral infection leads to cellular damage in vivo by two mechanisms; namely, direct cytopathicity and immune-mediated injury, targeted against either viral or auto-antigens (Fig. 1). Direct cytopathic effects are the result of the toxic actions of viral products on the infected cell and are usually recognized by morphological alterations of cellular architecture (Ginsberg, 1988). On the other hand, immune-mediated mechanisms rely on lysis of viral-infected cells by either direct lymphocyte cytotoxicity, antibody-mediated damage, or viral-induced autoimmunity. In viral-specific lymphocyte cytotoxicity, antigen-presenting cells (APC) recognize and phagocytize viral particles. After processing, viral antigens are presented to helper/inducer T-lymphocytes (CD4 +), which in turn, activate suppressor/cytotoxic T-cells (CD8 +). The cytotoxic T-lymphocytes then attack target cells expressing processed viral peptides, which are usually 8–10 amino acids long, in conjunction with the human leukocyte antigen (HLA) class-I molecules. In antibody-mediated

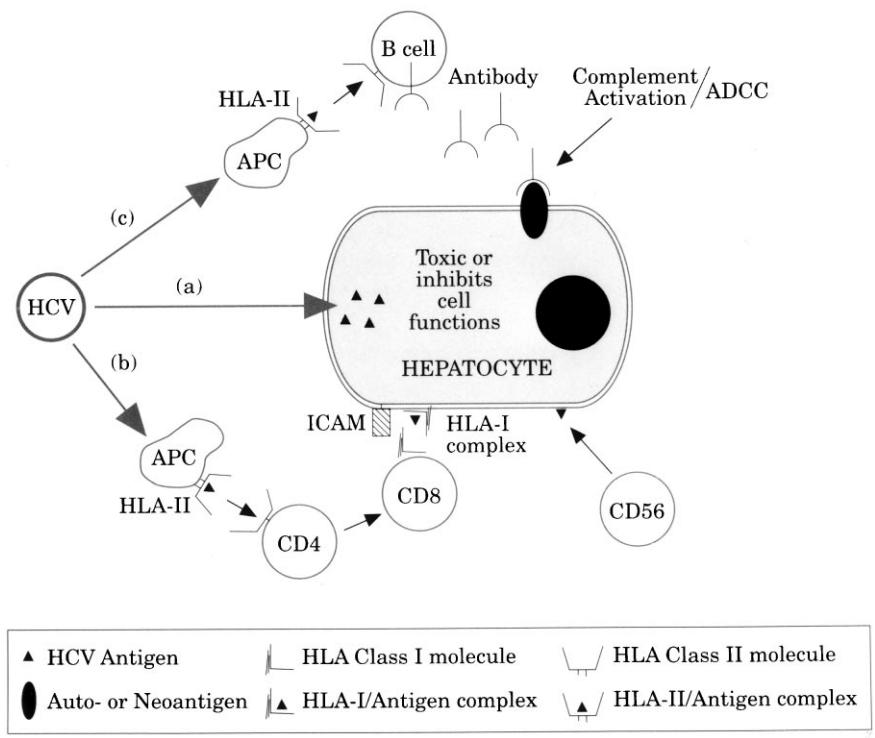

Fig. 1. Possible pathogenetic mechanisms responsible for hepatocellular damage in viral hepatitis: (1) direct cytopathicity; (2) immunemediated damage; (3) autoimmune mechanism. ICAM, intercellular adhesion molecule; ADCC, antibody dependent cellular cytotoxicity; APC, antigen presenting cells. (Reprinted with permission from González-Peralta, R.P., Davis, G.L., Lau, J.Y.N., 1994. Pathogenetic mechanism of hepatocellular damage in chronic HCV infection. J. Hepatol., 21, 255–259).

injury, APC present the viral antigen(s) to B-cells and induce the production of specific anti-viral antibodies. These antibodies then mediate the elimination of viral infected cells either through its direct cytotoxic effect, complement cascade activation and/or antibody-dependent cellular cytotoxicity.

HBV and HCV may be directly cytopathic in situations that allow unusually high levels of viral replication and protein expression such as in immunosuppressed patients. However, accumulating experimental evidence suggests that immune-mediated mechanisms play a critical role in mediating liver cell damage injury in chronic infections. The pathogenesis of hepatocellular damage in HBV and HCV infections has been the focus of recent reviews (Koziel, 1998; Nelson and Lau, 1998).

3.1. Hepatitis B virus

That immune-mediated mechanisms play a critical role in the pathogenesis of hepatocellular damage and viral clearance in HBV infection is supported by the absence of overt liver disease in immunosuppressed patients, the predominance of CD8+ (cytotoxic T-lymphocytes, CTL) in areas of histologic inflammation and by the enhanced hepatocyte necrosis noted with stimulation of the immune system. Several CTL immunodominant epitopes have been detected within the viral nucleocapsid protein (HBcAg) indicating that this protein is likely a major target for immune activation (Missale et al., 1993). The induction of vigorous CTL activity during acute HBV infection, which lasts for up to 10 years, results in the characteristic manifestation of liver inflammation. A strong CTL immune response is necessary to induce efficient HBV clearance and prevent the development of chronic infection. In recent experiments, lamivudine treatment resulted in the detection of significant HBcAg-specific CD4+ proliferative responses in chronic HBV infection (Boni et al., 1998). Interestingly, lamivudine also enhanced the responses of peripheral blood mononuclear cells to mitogens and recall antigens, showing that its effect was not limited to HBV-specific T cells. The immune recovery during lamivudine therapy was seen early, within four weeks after initiation of therapy. Although the proliferative response in the treated patients was similar to that seen in those with acute HBV infection who subsequently recover, it was not sufficient to induce durable and complete remission in chronically infected individuals. The exact mechanism for this interesting finding is unknown but it is likely that the findings suggestive of immunologic recovery in this population were related to the lamivudine induced reduction of viremia.

3.2. Hepatitis C virus

Accumulating evidence indicates that immune-mediated mechanisms also play a critical role in the pathogenesis of liver damage in HCV infection. First, lymphoid aggregates are commonly noted in liver biopsy specimens of patients with chronic HCV infection. The lymphoid aggregates, which suggest immunologic stimulation, consist of a germinal center, comprised of activated dendritic cells and B-lymphocytes, surrounded by a layer of CD4+ and CD8+ lymphocytes (Mosnier et al., 1993). Second, essential immunologic 'players', including activated CD8+, CD4+ lymphocytes, HLA-I and HLA-II are present and highly expressed in areas of liver inflammation (Onji et al., 1992; Botarelli et al., 1993; González-Peralta et al., 1993; Khakoo et al., 1997). Third, resolution of acute hepatitis C has been correlated with vigorous CD4+ proliferative responses and efficient CTL activity (Lechman et al., 1996; Missale et al., 1996; Cooper et al., 1998). Fourth, HCV-specific CTL activity has been demonstrated in peripheral blood mononuclear cells (PBMC) and in liver-derived lymphocytes from patients with chronic hepatitis C as well as in experimental chimpanzee infections (Koziel et al., 1992; Erickson et al., 1993; Nelson et al., 1997). In one study, HCV-specific CTL activity has been associated with disease activity, less viremia and sustained response to interferon-α therapy (Nelson et al., 1998); thus, providing indirect evidence that immune mechanisms are important in modulating viral replication and in mediating hepatocellular damage.

Multiple viral-specific dominant CTL epitopes (directed against structural and nonstructural viral proteins) have been identified within individuals. The presence of multiple immunodominant epitopes may hinder the development of specific immune-based therapy such as peptide and DNA vaccines.

In the model commonly used to study bulk CTL activity, patient-derived Epstein–Barr virus (EBV) immortalized B cells are used as targets. The B-lymphocytes are infected with HCV-containing vaccinia virus and labeled with radioactive chromium. The radiolabeled B cells are then cultured in the presence of serial dilutions of autologous CD8+ lymphocytes (to satisfy HLA-I restriction) isolated from peripheral blood or liver. Recognition of viral antigens by the CD8+ lymphocytes results in B-cell lysis with release of chromium into the supernatant. The amount of chromium released can be quantitated and provides a measure of CTL activity to the particular viral peptide tested. CTL activity is usually expressed as a percentage of lysis with respect to 'wild type' (nonHCV containing) vaccinia virus. To accomplish fine epitope mapping, HLA-I binding sites on chromium-labeled B-cells are saturated by incubation in the presence of excess overlapping peptides. The 'peptide-primed' B-cells are then used as targets in CD8+ cytotoxicity assays.

Although this system has been widely used to study host-CTL responses in viral hepatitis, it is labor intensive and relatively insensitive. To overcome these limitations, innovative methods have recently been developed including the ELISPOT (McCutcheon et al., 1997; Schmittel et al., 1997) and tetramer assays (Altman et al., 1996). In the ELISPOT assay, patient-derived T-lymphocytes (from blood or liver) are activated by incubating with viral-specific peptides. Recognition of viral antigen by CTL results in the production of specific cytokines, which are captured by antibodies bound to a solid phase (microwell plate). The cytokines released from the activated CTL can be detected by conventional enzyme-linked immunoassays as 'spots' on the microwell plate. The ELISPOT assay is a simpler and more sensitive technique to assess CTL frequency. However, in the presence of elevated CTL precursor frequency, counting large numbers of spots can be relatively subjective and lead to undesired variability. In the tetramer assay, fluorescent-labeled HLA-I molecules complexed with viral-specific peptides are incubated with patient-derived CTL (CD8+ lymphocytes). If CD8+ cells that recognize the viral peptide are present, they will bind the multimeric HLA/peptide complexes. The binding can then be easily detected by fluorescent activated cell sorting (FACS) analysis. Thus, reliable CTL frequency can be rapidly accomplished with the tetramer method, without relying on the more labor-intensive cytotoxicity assays. The application of these novel techniques to HCV will facilitate the study of CTL in this infection.

4. Models of viral hepatitis

4.1. Hepatitis B virus

Animal models have been important in advancing our understanding of HBV infection, particularly in defining the mechanisms of viral replication. Several HBV animal models including woodchuck, squirrel, duck, and transgenic mice have been described and widely used.

4.1.1. Woodchuck model

Hepadna virus infection leads to chronic hepatitis and cirrhosis in woodchucks, as in humans. Higher chronicity rates are associated with young age at infection, higher infectious doses and animal-host factors. Animals who are infected early in life become chronic viral carriers. As in humans, infection is associated with active HBV replication (high-level viremia and hepatic viral antigen expression and mild inflammatory activity). In addition, nearly all chronically HBV-infected woodchucks develop HCC, the major cause of death in these animals. It appears then, that the biology of woodchuck HBV is similar to that of its human counterpart; thus, suggesting that pathobiological information from this model may be applicable to humans.

Toxicology studies have demonstrated similar safety profiles for several antiviral compounds in woodchucks and humans. Thus, these animals have been used in preclinical efficacy trials of recently developed antivirals including lamivudine, lobucavir and adefovir. Woodchucks have also played an important role as a model to study HBV kinetics. Mathematical analyses have indicated that as in humans (see below), HBV processing is a very dynamic process. Up to 10^{11} HBV particles are produced daily, so that each infected hepatocyte produces between 10 and 100 virions daily.

4.1.2. Duck model

Peking ducks are also susceptible to hepadna virus infection. The avian model of infection offers several important advantages. First, the mechanisms of hepadna virus replication in ducks are similar to that of human HBV infection. Second, Peking ducks are available worldwide and they are easy to house and handle. Third, all animals develop chronic infection after viral inoculation with rapid onset of high-level viremia. Finally, reliable avian in vitro hepatocyte-culture systems are available that can be used to complement in vivo data. Potential limitations of the duck hepadna model include differences in viral protein production; the HB-X gene in human HBV is not present in the avian hepadna virus. Although chronic infection occurs in all inoculated animals, the development of hepatitis is unpredictable. This finding is likely, in part, related to differences between the host-immune response to viral infections among infected animals. HBV induced immune response, important in the pathobiology of human HBV infection, in poorly understood in ducks. Finally, infected ducks do not develop liver cancer, which underlies important oncogenic differences between avian and human HBV.

Peking ducks have also been used to test antiviral agents in development. One of these compounds, BMS200475, has induced effective suppression of hepadna replication in cell cultures (Innaimo et al., 1997) as well as in ducks (unpublished data).

4.1.3. Transgenic mouse model

The transgenic mouse model has been particularly important in defining mechanisms responsible for HBV persistence and hepatocellular damage. The major advantage of the mice model is that the virus produced is the human HBV. In addition, mice are small-inbred animals whose immune system is well characterized. One shortfall of this animal model is that viral re-infection does not occur; thus, replication in this system likely represents only a partial life cycle of HBV. Moreover, viremia noted in transgenic mice is much lower than that observed in human HBV infection, highlighting another potential limitation of this model.

4.2. Models of hepatitis C virus infection

4.2.1. Chimpanzee

Besides humans, the natural host of HCV, chimpanzees are the only animals that have been shown to be permissive to HCV infection. In fact, chimpanzee experiments confirmed the infectious nature of HCV, many years before the virus was discovered (Feinstone et al., 1975; Dienstag et al., 1977). In addition, chimpanzee serum recovered from experimental infections was used as source from which physico-chemical properties of HCV were initially determined (Bradley, et al., 1985).

In the absence of an efficient in vitro HCV replication system, the chimpanzee has continued to play a major role in HCV research. The persistence of infection in a proportion of chimpanzees, despite the presence of HCV-directed antibodies suggests that such antibodies fail to induce viral clearance. It has been demonstrated that chimpanzees that effectively cleared initial HCV infection could be re-infected by both heterologous and autologous inoculation (Farci et al., 1992). Chimpanzees challenged with in vitro neutralized inocula indicated that serum obtained 11 years before infection had no neutralizing effect, whereas serum sample collected 2 years after infection was able to neutralize the infecting strain. Cloning and sequencing analysis of viral isolates suggested that the emergence viral diversity (quasispecies) was responsible for the failure of neutralization (Farci et al., 1994a,b). Finally,

chimpanzees have been used in the initial development of HCV vaccines. In preliminary experiments, 5 of 7 chimpanzees immunized with HCV envelope 1 and 2 glycoproteins were protected from intravenous challenge of a homologous viral strain (Choo et al., 1994).

4.2.2. Tetracycline-regulated in vitro replication model

While the chimpanzee model has contributed to our understanding of HCV infection, this animal model has several limitations including expense, availability, and different host-immune response. Several groups have shown in vitro HCV replication in lymphoblastoid and liver-derived cells. However, only low level viral replication have been demonstrated in these replication systems, based on the intermittent detection of HCV RNA and replicative intermediates. A novel in vitro replication system that overcomes these limitations has been recently developed (Moradpour et al., 1998a,b). In this system, the entire HCV genome is cloned into a plasmid, which contains a tetracycline-responsive repressor element. Thus, in the presence of tetracycline, HCV transcription does not occur but in its absence the complete viral genome is precisely reproduced. Of importance, the viral proteins produced in this system are faithfully processed, indicating that the cellular and viral proteolytic machinery and post-translational modification pathways are fully functional in these cell lines. Recent studies have demonstrated the utility of this system. In one series of experiments, shortening of the HCV protease (created by site-specific mutations) resulted in reduction of the half-life from 24 h for the intact protease to 3 h for the shortened protein. Moreover, only intact viral protease was localized to the endoplasmic reticulum, the presumed site of HCV processing. Thus, these results suggest that the intact HCV protease is more stable and that its stability may be critical to its function. Ongoing experiments with this system have focused on elucidating the effects of the interactions of HCV proteins with interferon-α induced cellular responses.

4.2.3. Hybrid hepatitis C virus-GBV chimera

Another in vitro system that is currently being developed is the use of hybrid HCV-GBV viruses. GB viruses (GBV-A, GBV-B, and GBV-C) are a newly discovered group of infectious agents that are phylogenetically related to HCV (Karayiannis and McGarvey, 1995; Simons et al., 1995; Linnen et al., 1996). GBV-A and GBV-B are naturally occurring viruses in tamarins, while humans are the natural host for GBV-C. Initial studies suggested that GBV-C virus was associated with chronic liver disease, hence it was also named hepatitis G virus (HGV). However, numerous subsequent studies have failed to confirm this association. The observed similarities in genetic organization, replication strategy and polyprotein processing between HCV and GBV viruses would suggest that it may be possible to use hybrid chimeric viruses to infect tamarins with HCV-containing GB viruses. Tamarins are smaller and easier to handle than chimpanzees, which make them more attractive model animals. Importantly, GB viruses are less heterogeneous than HCV and do not display quasispecies nature in either the core, envelope or protease regions. The decreased genomic heterogeneity facilitates the generation of chimeric infectious clones, which is currently in progress.

5. Treatment of viral hepatitis

Significant advances have been made in the treatment of chronic viral hepatitis during the past few years. In general, the goals of antiviral therapy include prevention of progression from acute to chronic hepatitis, viral eradication (from serum and possibly liver), improvement of the signs and symptoms of chronic liver disease, preventing histologic inflammation, and prevention of the long-term consequences of chronic hepatitis such as cirrhosis and HCC.

5.1. Hepatitis B virus

The natural history and clinical course of HBV infection depends on the age at which infection occurs. HBV infection during the perinatal period

and infancy leads to the development of a 'carrier-state' in over 90% of patients characterized by persistence of HBsAg, high-level HBV DNA and minimal (if any) liver disease. In contrast, HBV infection in adults results in an acute 'icteric' hepatitis with active inflammation that resolves in 90% of those infected. In HBV-infected adults who develop chronic hepatitis, there is persistent liver dysfunction and risk of progression to cirrhosis and HCC. The prevention of these long-term consequences forms the rationale for antiviral therapy.

5.1.1. Interferon-α

Interferon-α is an established treatment for chronic hepatitis B. Numerous prospective, randomized placebo-controlled clinical trials have shown that a four to six month course of interferon-α is effective in inducing viral remission (HBV DNA and HBeAg seroconversion) in 25–40% of patients with chronic hepatitis B (Perrillo, 1993; Hoofnagle, 1998 and references contained within). Longitudinal follow-up of treated patients has demonstrated that the response rate in these individuals is sustained and that a significant proportion of them becomes HBsAg negative over time. These studies have also indicated that, in comparison to control patients, signs and symptoms of chronic liver disease resolve and the risks of decompensated liver disease and HCC decrease in interferon-treated patients.

Several reports have suggested that American and European patients have a higher response rate to interferon therapy than Asians. While host factors have been implicated in the reduced response rate, studies have indicated that the poor response to interferon in Asians is related to degree of liver disease (serum transaminases and histologic activity)(Lok, 1991; Wu et al., 1992). A strategy to increase response rates, which would be particularly applicable for patients with minimal liver disease, has been to use short courses of prednisone before interferon. 'Prednisone priming' is based on the fact that glucocorticoids increase HBV replication and hence, enhanced hepatocyte viral antigen expression. Upon prednisone withdrawal, initiation of interferon therapy results in a stronger immune response and would theoretically lead to more effective viral clearance. However, attempts to improve response rates by pre-treating with prednisone have been generally disappointing (Perrillo et al., 1990; Perez et al., 1993; Zarski et al., 1994). Prednisone can also cause severe decompensation of liver disease, and must be, therefore, used with caution.

Factors that have been associated with a sustained response to interferon therapy include elevated serum transaminases, low HBV-DNA levels, short duration of disease, adult-acquired infection, active histologic liver inflammation, female gender, and absence of HDV, HIV or other immunodeficiency disease (Perrillo, 1993; Hoofnagle, 1998 and references contained within). Overall, interferon has been well tolerated in most patients. The most common adverse event described is a flu-like syndrome characterized by general malaise, fever, headaches, chills, and myalgias, that generally resolves after the initial 2–3 doses of interferon. Other important side effects include bone marrow depression, neuropsychiatric disorders (anxiety and depression) and fatigue.

5.1.2. Lamivudine

Nucleoside analogues are compounds that serve as substrates for HBV polymerase (reverse transcriptase). The incorporation of an analogue lacking a 3′-hydroxy group into a growing HBV DNA chain results in the termination of elongation and inhibition of viral replication. These compounds are generally well absorbed from the gastrointestinal tract and have excellent bioavailability.

Lamivudine (3′-thiacytidine, 3TC), a cytosine analogue, inhibits the synthesis of negative strand HBV DNA from pre-genomic viral RNA. Initial studies indicated that lamivudine effectively reduced active viral replication as measured by rapid and marked reduction of HBV DNA during therapy (Dienstag et al., 1995; Nevens et al., 1997). However, viral relapse was common following cessation of treatment. A recent controlled trial has confirmed these preliminary results (Lai et al., 1998). In this Asian study, a 12-month course of lamivudine (at least 100 mg daily) resulted in normalization of transaminases and clearance of HBV DNA in 100% of treated pa-

tients (compared to 10% of those given placebos). HBeAg and HBsAg seroconversion rates (the conventional parameters that define sustained response) at the end of therapy, were 17 and 3%, respectively (Lai et al., 1998). In an attempt to enhance therapeutic efficacy, combination lamivudine-interferon was recently studied. Combination lamivudine-interferon resulted in improved, but not statistically significant HBeAg seroconversion rates (29%) compared to either interferon (19%) or lamivudine monotherapy (18%). In addition, histologic improvement was noted in similar number of patients receiving either lamivudine or interferon alone or combination therapy (Heathcote et al., 1998). Importantly, lamivudine has been very well tolerated in these clinical trials.

5.1.3. Emergence of lamivudine resistance in chronic hepatitis B

The use of potent inhibitors of viral replication have resulted in the emergence of drug-resistant viruses; a problem that is not unique to viral hepatitis. Herpes virus strains resistant to ganciclovir, aciclovir and famciclovir and HIV strains resistant to reverse transcriptase, protease and integrase inhibitors have been reported. Accumulating experimental in vitro data suggest that drug-resistant mutants are less replication-efficient than their wild-type counterparts. In HIV infection, lamivudine resistance occurs early and rapidly, so that by 6 months after initiation of therapy, nearly all patients had evidence of mutants. Interestingly, HIV viral load remained below baseline pre-treatment levels despite the emergence of drug-resistant variants suggesting that mutant viruses were also less replication-efficient in vivo.

Analysis of patients with chronic hepatitis B who have received lamivudine therapy in several large clinical trials has shown that drug-resistant mutants occur in up to 40% of patients by the end of 2 years of therapy (Atkins et al., 1998; Leung et al., 1998). Lamivudine resistance is usually conferred by mutations within the HBV polymerase gene ('YMDD' mutation). The emergence of resistance has been associated with several pre-treatment variables, including histologic activity, high serum HBV DNA levels and body mass. Of importance, the emergence of resistant mutants has been usually associated with elevation of serum transaminases and HBV DNA. However, as occurs in HIV infection, both of these variables remain below pre-treatment baseline levels despite the emergence of lamivudine-resistant strains. Moreover, histologic inflammatory activity has remained stable in the majority of patients who developed drug-resistant strains. Of note, the emergence of lamivudine resistance has not resulted in lower virologic responses; HBeAg seroconversion rates are similar among patients with and without mutants. Thus, these data support the hypothesis that lamivudine-resistant mutants are less replication-efficient than 'wild type' HBV in vivo. Longitudinal follow-up studies to assess carefully the long-term impact of lamivudine resistance in chronic hepatitis B are ongoing.

The clinical management of patients who develop lamivudine-resistant HBV mutants is not well defined. Increasing lamivudine doses have not led to effective clearance of drug-resistant variants and drug withdrawal has resulted in elevations of liver enzymes in a proportion of patients. As liver disease remains stable in most patients who develop resistant viruses, continued long-term antiviral therapy seems a reasonable option. Controlled trials are needed to address this important clinical issue.

The emergence of drug-resistant viruses has also become an important problem in patients with recurrent hepatitis B after liver transplantation. In these patients, the emergence of drug-resistant mutants has been associated with a rapidly accelerating, severe hepatitis that has resulted in allograft failure and death (de Man et al., 1998; Yoshida et al., 1998). The detection of HBV variants that are resistant to more than one antiviral agent further complicates treatment in the immunosuppressed population. Ideally, the therapeutic goal in these patients should be to optimize antiviral activity while minimizing the emergence of viral resistance. This can be accomplished by avoiding sequential antiviral therapy and using synergistic potent antiviral combinations that avoid the emergence of multi-drug resistance.

5.1.4. Other nucleoside analogues

In addition to lamivudine, several other nucleoside analogues are in clinical development including dideoxyfluorothiacytidine (FTC), D-diaminopurindixolane (DAPD) and L-fluromethylarabinosyluracil (L-FMAU). As reported in the meeting, all of these nucleoside analogues share in common potent and selective antiviral activity against HBV, which makes them attractive therapeutic agents. Upon phosphorylation, FTC and L-FAMU are potent inhibitors of HBV reverse trinscriptase (polymerase) and result in marked reductions in HBV levels in woodchucks. DAPD is first deaminated to an intermediate metabolite (DXG), which upon phosphorylation actively inhibits HBV polymerase. In contrast to D-FMAU, which was associated with multi-organ failure in human clinical trials, the active L-FMAU phophorylated intermediate is not a substrate for human DNA polymerase and is not accumulated intracellularly. Preliminary pre-clinical toxicity profiles of these compounds is excellent; safety studies are ongoing. In particular, mitochondrial damage, as noted with D-FMAU, has not been observed with L-FAMU.

5.1.5. Targeted nucleotides

The use of targeted nucleotides is another evolving approach to treat chronic viral hepatitis. This approach relies on identifying essential and accessible viral targets and on specifically delivering antiviral elements to hepatocytes, the primary replication site of hepatitis viruses. As reported in the conference, several potential classes of compounds have been identified that can be used to disrupt viruses including antisense oligonucleotides, ribozymes, and intracellular monoclonal antibody. Developing efficient hepatocyte-specific delivery systems has been a more difficult task. Although a number of delivery systems have been developed including adenoviruses, adeno-associated viruses, liposomes, retroviruses and asialoglycoprotein receptor-mediated endocytosis, none are ideal. A novel delivery system currently being developed includes the use of antibodies linked to cholesterol-spermine molecules that assist in the transmembrane transport into cells. The overall negative charge on the covalently-bound linker spermine results in accumulation of nucleic acid-specific antiviral targets while the antibody confers specificity. Another innovative mechanism involves the use intracellular monoclonal antibodies designed to react against specific viral targets. In one such system in development, a single chain monoclonal anti-HBs antibody has been directed to the endoplasmic reticulum. In the endoplasmic reticulum the anti-HBs antibody has been shown to inhibit HBsAg processing, thereby providing 'proof of principle' that this approach can effectively disrupt the life-cycle of HBV and can, therefore, potentially be exploited as antiviral therapy.

5.2. Hepatitis C virus

5.2.1. Interferon-α

Initial pilot studies, conducted even before the identification of HCV, demonstrated that interferon-α results in normalization of serum transaminases in a significant proportion of treated patients. Since then, multiple prospective randomized trials have confirmed that a 12 month course of interferon results in normalization of transaminases and clearance of HCV RNA in approximately 40% of patients by the end of therapy (end of treatment response). However, up to 60–70% of patients relapse within 6 months after discontinuation of therapy (elevated transaminases and reappearance of viral RNA). Thus, only a minority of interferon-treated patients achieves a sustained response (Poynard et al., 1996). Clinically relevant factors that have been associated with sustained interferon responses include low viremia level, HCV genotype other than 1, and pretreatment histologic activity (Davis and Lau, 1997).

Ideally, patients could be selected for therapy based on the risk of disease progression. In such an approach, patients whose liver disease is more likely to progress would be treated, while those expected to have a benign course would be spared treatment. However, the natural history of chronic hepatitis C is complex and may depend on many variables, including both host and viral factors. Viral factors that have been suggested to influence positively the rapidity of progression of

the liver disease include high serum HCV levels, viral genotype 1b, and a high degree of viral genetic diversity. Host factors such as the age at the time of infection, immune deficiency, alcohol consumption, and coinfection with HIV and/or HBV may also influence the rate of disease progression. Thus, it is reasonable to expect that the minimal requirements for therapy should include the presence of anti-HCV antibodies, HCV-RNA, elevated serum transaminases and liver biopsy compatible with chronic hepatitis C.

There have been several groups of patients for whom interferon therapy remains problematic. One of these groups includes those with minimally elevated or normal serum transaminases. Several studies have suggested that this group has a more benign course of infection with minimal risk of progression to cirrhosis if alcohol is not a co-factor. In addition, interferon monotherapy has been associated with infrequent sustained response and 'flair' of the transaminases, raising concerns that interferon may be changing the natural history of this disease. These data underlie the recent NIH recommendations against the use of interferon for chronic HCV patients with normal liver enzymes (Marcellin et al., 1997).

A difficult group to treat has been those with cirrhosis. These patients have the greatest potential benefit from antiviral therapy (high risk for decompensation and hepatocellular carcinoma), but the lowest treatment response rates. There is also an increased adverse events profile, particularly in those with decompensated cirrhosis. Therefore, antiviral therapy with an interferon based regimen is not currently recommended in the decompensated patient, but can be entertained in the well compensated patient with cirrhosis.

5.2.2. Adjunctive Therapy: ribavirin

From the above discussion it can be summarized that interferon therapy, although effective, is only so in a minority of patients. Attempts to increase the efficacy by increasing the dose and duration of therapy have met with limited success. Other strategies to expand and improve the antiviral options available include combining interferon with other agents (such as ribavirin), using a long-acting interferon preparation, and the development of HCV protease/helicase inhibitors and immunomodulatory strategies.

Ribavirin, a guanosine analogue, has a wide spectrum antiviral activity against RNA and DNA viruses. As monotherapy, ribavirin lowers serum transaminases in many patients with chronic hepatitis C, but has little effect on serum HCV RNA levels (Bodenheimer et al., 1997). Thus, as a single agent, ribavirin has little efficacy in the treatment of chronic HCV. However, when used in combination with interferon, it reduces post treatment relapse. Recent randomized placebo-controlled trials in the US and abroad have confirmed the enhanced efficacy of combination interferon-ribavirin therapy in patients who have either relapsed after interferon monotherapy (Brillanti et al., 1994; Davis et al., 1998) or in untreated ('naïve') patients (McHutchinson et al., 1998; Poynard et al., 1998a,b; Reichard et al., 1998). In a recent international study, 832 previously untreated adults with chronic hepatitis C were randomly allocated to receive interferon or interferon with ribavirin for either 24 or 48 weeks. Sustained virological response at 24 weeks after treatment was found in a significantly greater proportion of patients treated with combination therapy (43 and 35% of patients treated for 48 and 24 weeks, respectively) than in patients treated with interferon alone (19%) (Poynard et al., 1998a,b). Improved ribavirin-interferon sustained response rates were also recently reported in a large multi-center control trial in the US (McHutchinson et al., 1998).

These recent trials have also identified variables that influence response to combination interferon-ribavirin therapy. Logistic regression analysis of the large international study of naïve patients identified five independent pre-treatment factors associated with better response rates: HCV genotype 2 or 3 infection; viremia level less than 2 million copies/milliliter, age < 40 years, minimal fibrosis, and female gender (Poynard et al., 1998a,b). Multivariate analysis indicated that sustained virologic response was significantly higher among patients with fewer than three of these factors. Moreover, histologic assessment of these patients indicated that progression of histologic disease was retarded most in those who had a

sustained response, but was also observed in a significant proportion of nonresponders (see below). The side effects of combination therapy were similar to interferon monotherapy except for the increased incidence of ribavirin-induced hemolytic anemia. The anemia was dose-dependent and required dose-reductions in a small proportion of treated patients. Close monitoring of the hemoglobin is therefore required during the first two months of therapy. Ribavirin has also been suspected of having teratogenic potential and needs to be used with caution in patients of childbearing age.

Although combination therapy represents a marked improvement in our ability to treat chronic HCV infection, there are still approximately 50% of patients who will not respond to combination therapy. Thus, improved therapies are needed for the treatment of this chronic viral infection, particularly for those patients that do not achieve adequate responses to current available therapies.

5.2.3. Pegylated interferon.

Thrice weekly administration of interferon, as used in current clinical practice, results in significant variability between plasma peak and trough drug levels. Theoretically, more continuous and sustained plasma interferon concentrations would result in enhanced antiviral effect. To achieve more stable drug levels, interferon has been chemically linked to polyethylene glycol (PEG). Pharmacokinetic studies have indicated that such 'pegylation' results in slower interferon clearance and more sustained drug concentrations. Although the mechanisms are unknown, pegylated interferon also appears to be less immunogenic. An added benefit of its prolonged steady state is that pegylated interferon can be given once weekly. In a recent controlled phase II trial, escalating doses of pegylated interferon were administered to randomly allocated patients. The patients were also stratified by HCV RNA level and genotype. End of treatment response (normal serum transaminases and undetectable HCV RNA) was achieved in approximately 60% of patients treated for 12 months; better response rates were noted in those administered higher doses of pegylated interferon (unpublished data). Interestingly, a proportion of patients who cleared viral RNA had persistently elevated transaminases, suggesting that pegylated interferon induces an earlier antiviral effect than its unmodified form or that the drug itself is associated with elevations in liver enzymes. Efficacy analyses to assess the sustained response rate of pegylated interferon are in progress. Large multi-center controlled trials of combination pegylated interferon-ribavirin are now underway in the United States.

5.2.4. Helicase and protease inhibitors

Another antiviral approach that has been successfully used to treat viral infections is to inhibit viral-specific proteins crucial for replication. HCV protease is one such important protein that has attracted attention as antiviral target. The HCV protease is a serine protease that is critically important in the post-translational processing of the viral polypeptide (Bartenschlager et al., 1993; Eckart et al., 1993; Grakoui et al., 1993). The HCV protease is only distantly related to human serine proteases. Thus, theoretically inhibition of viral protease may be specifically accomplished without interfering in host-cellular processes. As presented at the conference, three potential areas within the HCV protease complex have been identified as candidates for inhibition; namely, the transmembrane NS4 cofactor region, the zinc-binding site and the catalytic domain. Detailed structure analysis has indicated that the NS4 cofactor region is embedded in highly hydrophobic regions of the protein, which hampers the design of inhibitory compounds directed against this area. Moreover, effective inhibition of the zinc-binding site would potentially require biologically toxic compounds. Therefore, the catalytic domain of the HCV has emerged as the most attractive antiviral target and has been the focus of intense research. The catalytic domain of the HCV protease is similar to those of other serine proteases. The HCV catalytic domain is 'shallower' than the catalytic domain of the HIV protease (against which effective inhibitors have been successfully designed). This structural conformation hampers the ability to design antiviral compounds that can effectively bind to this area.

To overcome this obstacle, experiments are underway to link potential antiviral compounds to specific 'binding-enhancing' molecules. This strategy has resulted in effective inhibition of interleukin converting enzyme, a serine protease with a 'shallow' catalytic domain. Another potential problem with the development of HCV protease inhibitors is the complex interactions between the protease and helicase domains of the viral protein. These differences must be clearly taken into account when designing inhibitors against this area. With advances in drug design, protease inhibitors will likely become available for clinical use in the very near future.

Because of its importance in viral replication, HCV helicase has been proposed as another antiviral target. HCV helicase is a multifunctional transmembrane protein essential for unwinding of viral RNA-RNA complexes during HCV replication. Current helicase research has focused on developing appropriate assays to test the efficacy and selectivity of potential helicase inhibitors. A number of chemical assays have been developed to assess the enzymatic activity of HCV helicase. In the scintillation proximity assay, double strand nucleic acid hybrids are incubated with HCV helicase. After incubation, unwound processed nucleic acid hybrids are captured onto magnetic beads, using radiolabeled probes. The amount of recovered radioactivity, measured by scintillation, reflects the amount of unwound nucleic acid in the sample and provides a measure of helicase-induced unwinding. In fluorometric assays, nucleic acid hybrids are treated with HCV helicase and unwinding activity is measured by fluorescent quenching or fluorescent dye displacement. Reliable selectivity assays have also been developed. Recent screening analyses have identified several potential HCV helicase inhibitors. Experiments are underway to test their efficacy and selectivity. Progress in this area is needed to develop effective HCV helicase inhibitors to use in patients.

5.2.5. Immune modulation

The host immune response to HCV is made up of both nonspecific and viral specific immune mechanisms (Fig. 1). Nonspecific responses include interferon production and natural killer cell activity. HCV-specific immune responses include humoral and cellular components. Eradication of HCV is probably dependent upon classical CD4+ and CD8+ CTL responses. As already noted, quantitatively, individuals who have a more vigorous CD4+ proliferative and CD8+ CTL response to HCV antigens are more likely to clear HCV after acute infection and clear viremia during interferon-α therapy (Lechman et al., 1996; Missale et al., 1996; Nelson et al., 1997; Cooper et al., 1998). When infection persists, CD4+ and CD8+ cells contribute to the inflammatory infiltrate within the liver and may help mediate ongoing hepatocellular injury.

An integral part of the CD4+ response to HCV is the regulatory molecules that are released by activated immune cells, which are called cytokines. CD4+ T-cells are classified into Th1 and Th2 subtypes, and an inactive Th0 type, based on the profile of their cytokine production. Th1 cells secrete interleukin 2 (IL-2) and interferon-gamma, which are required for host antiviral immune responses including the generation of CTL and natural killer (NK) cell activation. Th2 cells produce IL-4 and IL-10, which help augment antibody production and exert a negative regulatory effect on the Th1 response. Recent immunologic studies indicate that the Th1 response is activated in the liver in response to HCV infection (Napoli et al., 1996). Recruitment and activation of Th1 T-cells to the liver likely represents a host attempt to achieve efficient control and eradication of viral infection. This immune response to control viral replication is ineffective in eliminating infection in most patients with HCV infection and results in liver cell damage with the establishment of persistent infection. The Th2 cellular cascade likely represents an auto-regulatory response, which originates from outside the liver and attempts to confine the Th1 response to the liver, the primary site of HCV replication.

Since immune-mediated mechanisms likely play a major role in the control and pathogenesis of hepatocellular damage in chronic HCV infection, the system can potentially be manipulated to favor viral clearance. One such strategy is to enhance Th1 response, which would lead to enhanced cell-mediated immunity and increased

antiviral immune activation. Therapies with IL-12 (Th1 stimulatory) are currently in progress. In contrast, one could augment the Th2 response and suppress the Th1 response in hope of preventing further liver injury. Trials using IL-10 are underway. On a theoretical basis, another innovative mechanism would be to repress viral immunotolerance, presumably present in most patients with chronic hepatitis C who are unable to clear the virus. Recent work in the HBV transgenic mouse model has suggested that this can be accomplished by the infusion of stimulated dendritic cells, which are 'specialized' antigen presenting cells (Shimizu et al., 1998). Another immune modulating option is the development of 'CTL vaccines' which would help stimulate HCV-specific CTL by exposing CD8+ cells to HCV peptides that correspond to immunodominant epitopes. The identification of many immunodominant CTL epitopes in chronic HCV infection may hamper these attempts.

5.2.6. Impact of antiviral therapy on histologic progression in chronic hepatitis C

The importance of HCV infection lies in its propensity to cause insidious and progressive liver damage with the development of chronic active hepatitis, cirrhosis and HCC. However, progression of liver disease is not a uniform process. Based on histologic changes over time, 3 groups of patients with chronic hepatitis C have been identified: those who have an accelerated course, rapidly developing liver fibrosis, those in whom fibrosis progresses slowly, and those who have an intermediate progression rate (Poynard et al., 1998a).

Recent analysis of paired liver biopsies from several large controlled clinical trials have demonstrated significant reductions in fibrosis (using the validated METAVIR score) in patients who received either combination ribavirin-interferon or interferon monotherapy but not in control patients (Poynard et al., 1998a). Histologic improvement was greater in patients who received a 12 month course of combination therapy than in those who were given 6 month course of combination therapy or interferon monotherapy. Although the overall reduction in fibrosis was greater in patients who achieved sustained response to therapy, as measured by normalization of serum transaminases and disappearance of HCV RNA below detectable levels, it was noted to be significant in a proportion of those who were non-responders. These data suggest that antiviral therapy can result in histologic improvement even in patients who do not achieve conventional sustained responses. A clinical study has demonstrated a decreased incidence of cirrhosis with long-term interferon therapy (Poynard et al., 1995). These observations were recently confirmed in a controlled randomized trial (Sobesky et al., 1998). In this trial, significant reduction of histologic progression was noted in patients with HCV-related cirrhosis treated with long-term maintenance interferon therapy, which further supports the use of antiviral therapy in individuals with advanced HCV-related liver disease. Taken together, these data support the use of long-term antiviral therapy even in those who fail to achieve sustained response and challenge the current clinical practice to discontinue interferon early in patients with persistent detection of viral RNA.

5.3. Hepatitis D virus (delta agent)

HDV is a small single strand, negative sense RNA virus that is phylogenetically related to plant virions (Branch and Robertson, 1984). HDV is a rare cause of chronic hepatitis in the US but it is endemic in various parts of Europe (Rizzetto et al., 1992) It is a defective virus that requires HBsAg for complete virus production. As HDV is dependent on HBV, HDV infection occurs only in individuals with HBV infection. HDV infection can be acquired at the same time as HBV (coinfection) or more commonly, can occur in an individual with chronic HBV infection (superinfection). Most patients who are coinfected do not have active HBV replication (undetectable HBV DNA and HBeAg negative). Although the molecular mechanisms are unknown, this intriguing finding suggests that HDV may inhibit HBV replication. Although HDV-HBV coinfection generally does not lead to chronic hepatitis, HDV superinfection commonly results in chronic liver disease, which progresses to cirrhosis in a signifi-

cant proportion of patients. Spontaneous remission of liver disease rarely occurs in these patients.

Prospective, randomized controlled clinical trials have shown that a 4–12 month course of interferon is effective in inducing remission of hepatitis in 30–50% of patients with chronic HDV infection (Rosina et al., 1991; Farci et al., 1994a,b). Higher interferon doses (9 million units thrice weekly) have been generally associated with enhanced biochemical and virologic response rates and histologic improvement. However, virologic relapses invariably occur upon discontinuation of therapy so that only a few patients derive long-term benefit from interferon in this infection. Ribavirin inhibits HDV replication in vitro, but has not been effective in vivo as monotherapy (Rosina and Cozzolongo, 1994). Clinical trials are needed to determine whether combination interferon–ribavirin therapy would induce improved sustained response rates in chronic hepatitis D.

6. Impact of viral hepatitis in liver transplantation

Although liver transplantation is an aggressive and expensive approach, it is an important option for many patients with chronic viral hepatitis. In fact, HCV-related cirrhosis is the leading indication for liver transplantation in major centers worldwide. Advances in the field of transplantation have resulted in excellent overall long term clinical outcomes.

6.1. Hepatitis B virus

Prophylactic high-dose hepatitis B immunoglobulin (HBIG) led to markedly improved outcomes in post-transplant hepatitis B. Despite HBIG, recurrent post-transplant HBV occurs in a proportion of treated patients. Two clinical patterns of HBIG failure have been identified (Terrault et al., 1998a,b). Early failures generally occur within a few months after initiation of therapy. These patients do not have detectable HBsAg mutations; recurrent viral infection has been attributed to insufficient HBIG doses. Late HBIG treatment failures usually occur one year after transplantation, in the presence of adequate levels of HBIG. Mutations in HBsAg are commonly detected in patients with late failure, and are believed to be the cause of the HBIG inefficacy. A recent pilot study demonstrated that lamivudine in combination with HBIG effectively prevented HBV recurrence following liver transplantation, which resulted in excellent short-term survival rates (Markowitz et al., 1998; Nery et al., 1998). In addition, lamivudine monotherapy has resulted in effective reduction of active HBV replication and improvement of disease in patients awaiting transplantation (Gish et al., 1997; Perrillo et al., 1990; Markowitz et al., 1998; Terrault et al., 1998a,b). Importantly, in one study, preemptive lamivudine therapy led to disease remission or stabilization in nearly 75% of patients. Clinical improvement was impressive in a few patients in whom liver transplantation was avoided (Terrault et al., 1998a,b). It is important to point out that these clinically labile patients with significant liver disease require careful monitoring during therapy; up to 30% of patients require lamivudine withdrawal related to drug-induced adverse events.

Another important concern with lamivudine for the treatment of HBV infection in the transplant population is the emergence of drug-resistant viruses, which have been detected in up to 25% of treated cases. While little is known about the natural history and clinical course of lamivudine-resistant HBV strains, their presence has been associated with the development of severe liver disease, resulting in graft loss and death (de Man et al., 1998; Yoshida et al., 1998). Anecdotal experience using high-dose lamivudine or sequential antiviral therapy has not resulted in significant long-term clinical benefits. Carefully designed studies to define the evolution and clinical impact of drug resistance and the efficacy of combination antiviral therapy are critically needed in this area.

6.2. Hepatitis C virus

Recurrent HCV infection after liver transplantation is nearly universal. Despite the high recurrence rate, HCV infection leads to graft disease in a variable proportion of patients (Chazouilleres et al., 1994; Gane et al., 1996). The degree of clinical and histologic liver dysfunction also varies widely

among patients with recurrent hepatitis C, ranging from mild hepatitis to a rapidly progressive cholestatic syndrome leading to graft failure and death (Dickson et al., 1996). The factors that determine the clinical outcome in these patients are not clear, and likely involve viral (HCV RNA levels, genotype, quasispecies heterogeneity) and host factors (ethnicity, pre-transplant disease severity, HLA type). A recent collaborative study demonstrated a linear rate of progression of fibrosis in recurrent HCV after liver transplantation (Berenguer et al., 1998). The rate of fibrosis progression was only weakly correlated with pre-transplant viremia but not with any other viral or host factors studied. Despite lower immunosuppression, Spanish patients developed fibrosis more rapidly than those from the US did, which underlie the importance of differences in management in determining the clinical course in recurrent HCV infection (Berenguer et al., 1998).

Results of interferon therapy for the treatment of recurrent hepatitis C have been disappointing. Although reductions of viremia have been demonstrated in some clinical trials, effective HCV RNA clearance has been rarely noted. Recent clinical trials using combination ribavirin-interferon therapy have been encouraging. Research to define optimal therapy in these patients is critically needed.

7. Significance of viral dynamics in chronic hepatitis

7.1. Hepatitis B virus

The development of potent inhibitors of viral replication such as lamivudine has allowed the study of HBV dynamics by mathematical modeling (Nowak et al., 1996). The model relies on the rate of change of the uninfected susceptible cell mass, the rate of change of HBV-infected cell mass and the rate of virus production. Differential equations can be derived for each of these variables and solved mathematically, assuming complete inhibition of viral replication by lamivudine. Analysis of the dynamics of HBV infection using this model indicates that the half-life of infected cells during active disease is approximately 10 days. Based on this short half-life, it can be estimated that in case of complete suppression of viral replication by antiviral agents, eradication of HBV would require approximately one year of treatment. In contrast, during chronic persistent hepatitis, the half-life of infected cells has been calculated to be markedly longer, ranging from 10 to 100 days. With this long half-life, viral cure would require many years of treatment. Viral modeling has also suggested that like in animal models, human HBV infection is an exceedingly dynamic process that yields up to an estimated 10^{12} virions daily. Moreover, mathematical calculations indicate that HBV infection results in a rapid turnover of the liver cell mass. This enhanced rate of liver cell regeneration, contrasts with the slow rate of hepatocyte turnover in the normal liver and likely plays a role in the development of HCC in HBV infection.

7.2. Hepatitis C virus

Similar mathematical modeling has been applied to HCV infection. These analyses have indicated that, like in chronic hepatitis B, the half-life of HCV infected liver cells is short (< 3 h) (Neumann et al., 1998). This model has indicated that HCV infection is also a very dynamic process, which results in the production of an estimated 10^{12} viral copies daily. However, numerous studies have indicated that the number of HCV-infected hepatocytes is low, usually $< 20\%$, using sensitive in situ techniques (Lau et al., 1996). Thus, unless viral assembly and cellular export is a very efficient and rapid process, it is unlikely that the observed number of infected cells could produce 10^{12} virions daily, as predicted by the model. If the initial reduction in viral levels is assumed to result from interferon induced immune-mediated clearance, the daily estimated production of virus is lower (median 10^{10}) (unpublished data). The lower viral production rate is biologically more plausible and in better agreement with available in situ detection data. It should be emphasized, however, that the differences in HCV dynamics predicted between these mathematical models are directly related to the

different assumptions that are driving the analyses. Further testing of these hypotheses is needed as this information can be clinically important in estimating the length of effective treatment and in determining maximum antiviral activity during the early phase of therapy.

8. Conclusions

Chronic viral hepatitis is a major cause of liver disease worldwide. Recent technical achievements have led to significant advances in our understanding of the replication and pathobiology of these important human pathogens. Of particular importance has been identifying the critical role played by immune-mediated mechanisms in the pathogenesis of hepatocellular damage in HBV and HCV infections. This enhanced knowledge has led to considerable progress in the development of therapeutic strategies directed against these chronic viral infections. However, currently available antiviral therapies are still ineffective in a large proportion of patients with chronic viral hepatitis. Ongoing research focused on the design of potent and selective antiviral compounds and immunomodulatory agents offer optimism for these patients.

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