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New systems to understand hepatitis C biology

The development of HCV subgenomic replicons that produce high levels of one or more HCV polypeptides (Lohmann et al., 1999; Blight et al., 2000), in contrast to the very low and inconsistent levels of wild-type virus produced in tissue culture cells infected with HCV (Table 11.1 and Ch. 11), suggests that there are properties of wild-type HCV that normally attenuate virus gene expression and replication, and that when these constraints are removed, much higher levels of expression could be achieved. One of these constraints may be the complex secondary structural features within the 5' and 3' UTRs of the virus. Many laboratories are developing additional self-replicating replicons that are capable of persisting, and some at high copy number, within transfected or infected cells. These include replicons made from alphavirus (Garoff & Li, 1998; Ying et al., 1999), pestivirus (Moser et al., 1999), other flavivirus (Varnavski & Khromykh, 1999), and coronavirus (Thiel, Siddell & Herold, 1998) vectors. Whether the entire HCV polyprotein could be expressed from such constructs remains to be seen. In addition, since the HCV would be produced from artificial templates, it is not clear whether the sensitivity of virus gene expression and replication to putative antiviral agents would be the same or different to that of virus made from native HCV templates in an infected cell. However, this does not limit the use of such recombinant vectors to screen compounds against individual HCV proteins (Lohmann et al., 1999) and, in fact, such efforts are underway for NS5B (Baginski et al., 2000).

In addition to generating new tissue culture systems using subgenomic replicons (Ch. 11), there have been attempts to increase the efficacy of existing treatments. As outlined in Ch. 9, IFN has been used effectively against both HBV and HCV. However, the sustained response rate is low (less than 20% of treated patients) and side effects could be serious. In addition, treatments are expensive and consist of up to three injections per week for 6–12 months. Recently, it has been shown that polyethylene glycol-modified IFN, referred to as pegylated IFN, is more stable in the bloodstream that unmodified IFN, and that this modification does not alter the properties of IFN. Clinical trials with pegylated IFN are now

underway in patients who are chronically infected with HCV. Initial findings have shown that up to 30% of treated patients with cirrhosis treated in this way have a sustained virological response, compared with less than 10% of patients treated with standard IFN therapy. Transaminase levels normalized in twice the number of patients treated with pegylated IFN compared with standard IFN (Heathcote et al., 2000). Similar results were obtained among patients with an entry diagnosis of chronic hepatitis (Zeuzem et al., 2000). In both trials, pegylated IFN was better tolerated and only required one weekly injection instead of three. Clinical trials using pegylated IFN in conjunction with ribavirin are currently underway, and preliminary results suggest that the majority of patients may benefit (Glue et al., 2000). However, it will be very important to document carefully the sustained virological and histological response to this new combination therapy in current and upcoming clinical trials.

Liver disease associated with chronic HCV infection is a major target for the development of future therapeutics, although there is no laboratory-based animal model that develops HCV-associated CLD (Ch. 13). Certainly, the inducible expression of HCV core in vivo has led to the development of a transient or acute hepatitis (Wakita et al., 1998), which strongly suggests that, in a nontolerant host, the pathogenesis of HCV is immune mediated. A model for HBV-associated CLD has recently become available (Larkin et al., 1999), but an analogous model for HCV is yet to be developed (Ch. 13). However, recent work has shown that when a vaccinia virus recombinant containing the HCV IRES sequences cloned just upstream from the luciferase reporter gene was used to infect mice, they became strongly positive for luciferase gene expression in the liver. Treatment of these animals with antisense oligonucleotides against the HCV IRES significantly reduced reporter (luciferase) gene expression (Zhang et al., 1999) (Ch. 17), indicating that such an approach may be important for the development of reagents that block the production of virus gene products in vivo. Alternatively, it has recently been demonstration that human hepatocytes can survive for months in a matrix under the kidney capsule of mice treated with anti-Met. These hepatocytes are susceptible to HBV and hepatitis delta virus infection (Ohashi et al., 2000), suggesting that such a model could readily be used to screen for drugs against HBV and delta. If, as expected, this model is susceptible to HCV, it may also be adaptable for drug screening.