4. Other Relevant Data

4.1 Pathology

The putative association between HCV and HCC is based primarily on large epidemiological and serological reviews and not on histological studies of the evolution of carcinoma. The course of HCV includes acute viral hepatitis (usually subclinical) with transition to chronic hepatitis and cirrhosis and to HCC.

4.1.1 *Acute infection*

Many of the histological features of acute viral hepatitis are common to all four hepatitis viruses (HBV, HCV and hepatitis A and D viruses), and hepatic reactions are similar, so that histological features do not distinguish a specific agent. Some patients with acute hepatitis due to HCV, however, have a milder inflammatory reaction than those with disease due to HBV or hepatitis A virus, and this reaction may resemble infectious mononucleosis. The similarity to mononucleosis is due to sinusoidal inflammatory proliferation and portal lymphoid hyperplasia, without hepatocellular cytopathic change. In follow-up biopsy samples from patients with post-transfusion hepatitis, the hepatocellular changes may be very mild, and a diagnosis of acute viral hepatitis is based on clinical and histological correlation. The histological changes of acute viral hepatitis due to HCV are often mild and include the same inflammatory and hepatocellular degenerative changes seen in acute viral hepatitis due to HBV. Most observations of histopathological changes in acute viral hepatitis were restricted to clinically apparent, icteric cases, which constitute a minority of infections in a population. The majority of cases are therefore not confirmed histologically (Alter, 1990).

4.1.2 *Chronic infection*

The transition of acute viral hepatitis due to HCV to a progressive form of chronic hepatitis is usually gradual and not easily recognized by clinical or histological criteria. The same histopathological terminology for chronic hepatitis used for HBV has been widely applied to chronic hepatitis due to HCV. In addition, chronic hepatitis due to HCV often has some features not common to HBV, which include: portal lymphoid hyperplasia with germinal follicles, hepatocellular fatty change, bile-duct damage and multinucleated giant cells (Lefkowitch & Apfelbaum, 1989; Lefkowitch *et al.*, 1993). All of these features cannot

be diagnosed in a single case. In a follow-up study of chronic post-transfusion non-A, non-B hepatitis, 82% of cases were related to HCV, and a spectrum of hepatic lesions was described, which included chronic active hepatitis and cirrhosis; a few patients had minimal histological changes (Di Bisceglie *et al.*, 1991b). In another series of chronic non-A, non-B hepatitis patients followed for a longer period (3–20 years; mean, 8 years), 75% were due to HCV. Multiple biopsy samples from 24 patients with chronic post-transfusion non-A, non-B hepatitis revealed a spectrum of chronic hepatitis, ranging from mild chronic persistent hepatitis (55%) to chronic active hepatitis with cirrhosis (16%); the other cases were sporadic non-A, non-B hepatitis (Hopf *et al.*, 1990). Kiyosawa *et al.* (1990a) examined 231 patients with chronic non-A, non-B hepatitis and correlated the histological features with time after transfusion. The mean time since transfusion was 10 years for 96 patients with chronic hepatitis, 21.2 years for 81 with cirrhosis and and 29 years for 54 with HCC. Histological and serological data in several of these reports showed slow sequential progression of chronic hepatitis to cirrhosis and HCC.

4.1.3 *Cirrhosis and hepatocellular carcinoma*

The pathogenesis of cirrhosis is described in detail in the monograph on HBV (pp. 114–115). In most of the surveys of chronic hepatitis C, HCC developed in cirrhotic patients and not only in those with early chronic active hepatitis. This point has not been widely studied, but the situation appears to be different from that for HBV.

The transition of regenerative nodules to HCC is also described above. Ferrell *et al.* (1992b) examined 110 sequential explant livers with cirrhosis: 19 livers had 40 distinctive nodules measuring 0.8–3.5 cm. After careful histological examination, 28 were categorized as macroregenerative nodules and 12 as small HCCs. Thirty of the 110 cirrhotic livers were from patients with antibodies to HCV, which was associated with a greater risk for distinctive nodule formation (47%) than for all causes of cirrhosis in the series. Furthermore, patients with cirrhosis who were seropositive for HCV markers had an increased risk for incidental HCC; thus, four of the eight patients with HCC were HCV seropositive.

4.2 Molecular biology

The presence of minus-strand HCV RNA (implying replication) has been demonstrated in HCC and non-tumorous tissue (Gerber *et al.*, 1992; Gerber, 1993). There is at present no experimental evidence that HCV sequences are integrated into the host cell genome or for an HCV coded *trans*-activating protein.

Comparison of the nucleotide and predicted encoded amino acid sequences of HCV isolates in chronically infected chimpanzees suggest that the HCV genome is susceptible to mutations, as has been observed frequently for other RNA viruses (Okamoto *et al.*, 1992b). Genomic mutations of HCV in the course of chronic infections might promote persistence of the virus and its pathogenicity. In chimpanzees inoculated sequentially with different HCV strains derived from five unrelated patients with transfusion-associated non-A, non-B hepatitis, infection did not elicit protective immunity against reinfection with homologous or heterologous strains (Farci *et al.*, 1992b).

4.3 Other observations relevant to possible mechanisms of action of HCV in carcinogenesis

Chimpanzees were originally the primary model for studying the biology of HCV. In eight chimpanzees infected experimentally with HCV, three patterns of hepatitis C viraemia were seen: (i) acute resolving hepatitis with transient appearance of HCV RNA, (ii) chronic hepatitis with persistent HCV RNA and (iii) chronic hepatitis with intermittent appearance of HCV RNA (Abe *et al.*, 1992).

Analysis of serial clinical specimens obtained from chimpanzees infected experimentally with HCV revealed a relatively uniform relationship between peak expression of tissue markers of infection, appearance of HCV RNA in serum and elevated levels of serum ALT. Since the cytoplasmic antigen becomes detectable in infected chimpanzees within one week after inoculation, it was speculated that replication of HCV occurred very early in the incubation phase of hepatitis (Shimizu *et al.*, 1990). In another study (Shindo *et al.*, 1992b), the levels of HCV RNA in both serum and liver samples from champanzees paralleled disease activity, as measured by serum ALT levels. Serum ALT rose two days after inoculation, fell to normal levels between 10 and 14 days and rose to peak values at eight weeks. Serum HCV RNA became detectable by cDNA PCR three days after inoculation, persisted during the increase in serum ALT and was maximal at seven weeks. The first antibodies to appear, at nine weeks, were anti-C33c, directed against epitopes coded by the non-structural 3′ region of the genome; anti-C100-3 appeared at 12 weeks and antibody to 5-1-1 antigen shortly thereafter. Approximately 30–75% of the infected chimpanzees developed chronic infections, detected by the presence of genomic and anti-genomic viral sequences in the liver (Abe *et al.*, 1992; Farci *et al.*, 1992a; Hilfenhaus *et al.*, 1992). In the animals that developed chronic infection, both HCV RNA and anti-C100 (as detected in a first-generation test) remained persistently detectable.