Identification of HCV-associated antigen(s) in hepatocytes

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Summary. HCV-associated antigens (HCAg) were localized morphologically using immunofluorescence methods. Fluorescence was found in the cytoplasm of individual liver cells or in groups of cells. Nuclear fluorescence was not observed. Blocking and absorption studies suggest that HCAg is related to nucleocapsid or envelope proteins.

Attempts to identify liver antigen(s) associated with posttransfusion hepatitis virus infection have not been adequately documented either immunologically or morphologically. In this study, hepatitis C virus (HCV) and disease-associated antigen (HCAg) were detected immunohistochemically in hepatocytes in patients with chronic hepatitis C and in chimpanzees experimentally infected with HCV isolates.

IgG fractions of chimpanzee and human sera were used as fluorescein isothiocyanate (FITC)-labeled probes to identify HCAg in liver. Immunologic studies were carried out to establish reactivity of immunoglobulins (PRC.2126, JEN.A) used for identification of HCAg in hepatocytes with HCV recombinant proteins. Enzyme immunoassays revealed that PRC.2126 and JEN.A immunoglobulins reacted with recombinant proteins expressed by clones F3 and F6 (encoded with NS3 and NS5, respectively). PRC.2126 reacted also with HCV nonstructural protein expressed by the F5 clone (equivalent of C100-3, NS4 [1, 2]).

In HCAg positive liver biopsies, the antigen was found in the entire cytoplasm of individual hepatocytes or in groups of liver cells. Liver cell nuclei never contained HCAg. HCAg fluorescence had a very fine granular, powder-like pattern with superimposed larger granules of distinct and...
brilliant fluorescence. HCAg was detected in nine tested chimpanzees with acute HCV hepatitis before and shortly after ALT elevation.

In control studies, FITC-labeled anti-HCAg did not react with chimpanzee liver biopsy specimens obtained either before HCV inoculation or during convalescence. HCAg-negative liver biopsy specimens were obtained from chimpanzees (n = 11) and from patients (n = 13) with various etiologies of viral hepatitis: type A, B, delta, enterically transmitted non-A, non-B, and non-viral hepatitis liver conditions. Hepatocellular reactivity of HCAg was not host-derived as evidenced by absorptions of anti-HCAg FITC-labeled probes with normal liver homogenates, IgG, fibrin/fibrinogen, or red blood cells.

The reactivity of FITC-labeled antibodies with hepatocellular HCAg was blocked by serum samples from patients and chimpanzees experimentally infected with HCV, but not by preinoculation serum samples from chimpanzees or by control samples from either primates or patients infected with hepatotropic viruses other than HCV.

Fluorescent antibody blocking studies of HCAg in hepatocytes with sera from chimpanzees (n = 11) and patients (n = 6) showed that the HCAg fluorescence, although specific for HCV infection, was unrelated to anti-HCV (C100-3, NS4) reactivity. Serum samples from posttransfusion hepatitis cases used for preparation of FITC-labeled reagents and found reactive with HCAg in hepatocytes were positive for anti-HCAg with titers of 1:100 or higher.

Absorptions of the fluorescein-labeled anti-HCAg immunoglobulins with HCV nonstructural recombinant proteins (F3 (NS3), F5 (NS4), and F6 (NS5)) revealed that HCAg fluorescence in hepatocytes depends on reactivity other than that of nonstructural proteins. Minimal inhibition of HCAg fluorescence was observed only after absorption with E3 (NS3) proteins. These observations suggested that hepatocellular reactivity of anti-HCAg may be related to HCV structural proteins (nucleocapsid or envelope).

A fluorescent antibody blocking assay on liver cryostat sections containing large deposits of HCAg was used to determine the presence of anti-HCAg in serum samples from experimental primates and patients. Profiles of anti-HCAg and anti-HCV (C100-3, NS4) in serum in relation to HCAg in hepatocytes were established in sera obtained from chimpanzees (n = 7) with acute HCV infection.

HCAg was detected in liver biopsy specimens obtained during the chronic phase of the disease from experimentally infected chimpanzees and from patients with various clinico-pathologic forms of chronic non-A, non-B hepatitis seropositive for anti-HCV. The antigen was identified in 5 of 10 chimpanzees with elevated ALT values and/or histopathologic changes in the liver characteristic for chronic HCV infection. HCAg was found in 11 of 12 patients with chronic persistent hepatitis (1/1), chronic active hepatitis (8/9), and active liver cirrhosis (2/2). All the animals with HCAg in hepato-