CELLULAR IMMUNE RESPONSE IN LIVER OF PATIENTS WITH CHRONIC HEPATITIS B
—ELECTRON MICROSCOPIC OBSERVATION OF LYMPHOCYTE SUBSETS BY THE IMMUNOPEROXIDASE METHOD USING MONOCLONAL ANTIBODIES—

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Summary

The morphological association between lymphocytes and hepatocytes was studied at the light and electron microscopic levels by the peroxidase-labeled antibody method using mouse monoclonal antibodies against Leu-1, Leu-2a, Leu-3a, Leu-7 and Leu-10 antigens in liver biopsy specimens from patients with chronic hepatitis B. Leu-1 + cells (T cells), especially Leu-2a + cells (cytotoxic/suppressor T cells), infiltrated mostly in periportal areas with piecemeal necrosis and in parenchymal areas with focal necrosis. By double staining techniques, Leu-2a + cells were often seen in contact with hepatocytes containing membranous hepatitis B surface and/or core antigens in patients with chronic active hepatitis. At the ultrastructural level, Leu-2a + cells frequently occupied the sinusoid and also migrated into both the space of Disse and between hepatocytes. Furthermore, they often showed intimate surface-contact with hepatocytes having hepatitis B surface and/or core antigens, and, occasionally, injured hepatocytes were surrounded by several Leu-2a + cells. In contrast, Leu-3a + cells, Leu-7 + cells and Leu-10 + cells sometimes appeared in the sinusoid, but seldom in the space of Disse and between hepatocytes. These findings suggest that cytotoxic T lymphocytes may be associated with the necrosis of hepatocytes in chronic hepatitis B.
Introduction

A number of immunologic mechanisms have been hypothesized to explain the persistence of hepatitis B virus (HBV) and the continued hepatic injury that occurs in chronic hepatitis B\(^1\)\(^-\)\(^5\). An increasing amount of evidence points to the importance of cellular cytotoxicity in the development of chronic hepatitis B\(^4\),\(^5\). The majority of the studies concerning the cytotoxic effector cell responsiveness to surface membrane antigens of hepatocytes determined by the host or HBV have dealt with immunologic events in the peripheral blood\(^6\),\(^7\) rather than the events in the liver itself because it has been difficult to obtain direct evidence of the immune mechanism that occurs in the liver. The intrahepatic infiltration by mononuclear inflammatory cells characteristic of acute and chronic HBV infection and the morphologic association of lymphocytes with hepatocytes suggest that the associated hepatocytolysis may be induced by a cellular immune mechanism\(^8\)-\(^10\). The contact between cytotoxic lymphocytes and hepatocytes bearing HBV has been postulated as an important pathogenic determinant of hepatocellular injury in HBV infection\(^11\),\(^12\). However, morphological analysis at the ultrastructural level of the invasive lymphocytes in chronic hepatitis has not allowed precise identification of the subpopulations of the infiltrating lymphocytes. Using immunohistochemical methods, T cell infiltration (mainly cytotoxic T cells) has been reported at the site of inflammatory lesions in hepatic tissue of chronic hepatitis patients\(^13\),\(^14\). In addition to the role of cytotoxic T cells, the contributions of killer (K) cells\(^15\) and natural killer (NK) cells\(^16\) to the pathogenesis of the disease have also been postulated.

The demonstration of HBV or hepatitis B associated antigens on the surface and within hepatocytes makes it plausible that there is an interaction between the host immune response and the virus. Recently, mouse monoclonal antibodies capable of identifying various functionally discrete lymphocyte subpopulations have been developed\(^17\)-\(^19\). In this paper, the association of lymphocytes and HBV infected hepatocytes was studied by the immuno-peroxidase method using mouse monoclonal antibodies against human lymphocyte subsets in liver biopsy specimens from patients with chronic hepatitis B to clarify the role of the cellular immune reactions in the development of chronic liver injury.

Patients and Methods

Twenty patients with HBsAg-positive sera (determined by the reversed passive hemagglutination method)\(^20\) were studied. Six of them had chronic active hepatitis, activity moderate (CAH 2A), 12 had chronic active hepatitis, activity severe (CAH 2B), and 2 had chronic persistent hepatitis (CPH). Diagnosis was based on histological findings of liver biopsy specimens according to a review by an international group\(^21\). Hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) in the patients' sera were detected by radioimmunoassay (Abbott Laboratories, North Chicago, Ill). Each liver biopsy specimen obtained by peritoneoscopy from all patients was divided into three parts. One part was fixed in Bouin's solution for histological diagnosis by light microscopy, another part was fixed in a periodate-lysine-paraformaldehyde fixative (PLP)\(^22\) for histochemical studies, and the remaining part was fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide for