Localisation of intrahepatic interleukin 6 in patients with acute and chronic liver disease

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Abstract

Aim: To evaluate the role of local interleukin 6 (IL-6) in the pathogenesis of acute and chronic liver disease.

Methods: The cellular site of IL-6 in cryostat sections of liver from 31 patients with liver disease was examined using indirect immunofluorescence with a monoclonal antibody.

Results: IL-6 staining in sinusoidal endothelial cells was very noticeable and diffusely distributed in the lobules of specimens of acute viral hepatitis. IL-6 expression in endothelial cells, particularly in necrotic areas of hepatocytes, was increased and was accompanied by enhanced expression in Kupffer cells. In contrast, IL-6 staining in infiltrating mononuclear cells was prominent in portal tracts, and the numbers of cytokine positive cells were greater in specimens of chronic active hepatitis compared with chronic persistent hepatitis. In non-specific reactive hepatitis intrahepatic expression of IL-6 was minimal, while in alcoholic liver fibrosis the cytokine distribution in the lobules was similar to that of acute viral hepatitis.

Conclusion: These results indicate that locally produced IL-6 contributes to the inflammatory process and immunological response in acute and chronic liver disease.

Interleukin-6 (IL6), also known as B cell stimulatory factor 2 (BSF-2), induces the final maturation of B cells to antibody producing cells.¹ Recent studies have indicated that IL-6 has many biological properties including affecting the immune and inflammatory responses.²⁻⁴ IL-6 production can be induced by a wide variety of agents in many different cells such as monocytes, T cells, B cells, fibroblasts, endothelial cells, epithelial cells, etc.

As IL-6 is mainly released from injured or infected tissue,²⁻⁴ IL-6 may exert its major influence in local tissue like other cytokines, as suggested in certain inflammatory diseases.⁵⁻¹⁰ We therefore decided to evaluate the localisation of IL-6 in liver tissue to understand precisely whether it has a role in acute and chronic hepatitis.¹¹

Methods

Thirty one patients with acute or chronic liver

disease of various aetiologies were studied (table). They had been diagnosed on the basis of appropriate serological, virological, biochemical, and histological criteria.¹² Five patients with acute viral hepatitis underwent liver biopsy two to three weeks after onset of their illness. Three were biopsied when their serum aminotransferase activities had returned to normal, and the histological findings indicated non-specific reactive hepatitis.

Serum HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe were assayed by commercial radioimmunoassay kits (Abbott Laboratories, North Chicago, Illinois, USA). Hepatitis B infection was diagnosed if antibodies to HBsAg were positive. Non-A, non-B hepatitis (type C) was diagnosed by the detection of serum HCV RNA using the polymerase chain reaction¹³; all patients diagnosed as having non-A, non-B hepatitis were positive for HCV RNA except for one with chronic persistent hepatitis.

Liver biopsy samples obtained from all patients were divided into two. One part was fixed in 10% formalin for routine histological examination. The other part was immediately frozen in liquid nitrogen with OLT compound (Miles Inc Elkhart, England) and used for immunofluorescence microscopy.¹⁴ Sections of frozen tissue 2-3 µm thick were cut by cryostat and fixed in acetone for 15 minutes at room temperature. The sections were incubated with normal goat serum diluted 1 in 40. After washing in phosphate buffered saline (PBS), pH 7.4, sections were incubated with properly diluted mouse monoclonal antibody against human recombinant IL-6 for 30 minutes at room temperature. After washing in PBS, they were incubated with fluorescent isothiocyanate (FITC)-labelled second antibody. Non-relevant antibodies of the same class and normal rabbit serum were used for the control study. The slides were then covered with medium containing phenylene diamine and observed with an Olympus BH-2 epifluorescence microscope equipped with proper filters (Tokyo, Japan).

The characterisation and specificity of a monoclonal antibody of IgM class to human recombinant IL-6 has been described previously.¹⁵ As a second antibody an IgG fraction of goat anti-mouse IgM antiserum was used, which had been preabsorbed with normal human serum not to cross-react with the antibodies to human liver tissues. The binding of anti-IL-6 monoclonal antibody to the liver section was completely blocked by preincubation with recombinant IL-6.

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Distribution and prevalence of IL-6 positive cells in liver tissues of 31 patients with various liver diseases

Diagnosis	No	Age (years)	Sex (F/M)	Distribution of IL-6 ⁺ endothelial cells			Prevalence of IL-6 ⁺ infiltrating mononuclear cells		
				Spotty	Focal	Diffuse	+	++	+++
Non-specific reactive hepatitis	3	36–52	1/2	3	0	0	3	0	0
Acute hepatitis									
type A	2	23–30	0/2	0	0	2	0	2	0
type B	2	21–32	1/1	0	0	2	1	1	0
non-A, non-B	3	22–45	1/2	0	0	3	1	2	0
Chronic persistent hepatitis									
type B	5	20–55	2/3	0	4	1	4	1	0
non-A, non-B	2	43–48	1/1	0	2	0	2	0	0
Chronic active hepatitis									
type B	6	19–62	2/4	0	3	3	2	3	1
non-A, non-B	5	28–59	3/2	0	3	2	2	2	1
Alcoholic liver fibrosis	3	49–58	0/3	0	0	3	2	1	0

Lobular and portal tract areas of liver sections were photographed under light microscopy and followed by appropriate magnification to estimate the number of IL-6 positive cells. Patterns of distribution of stained endothelial cells in hepatic lobules were divided into three groups: spotty type, or cells distributed in less than 25% of the lobule; focal type, or between 25% and 50%; and diffuse type, or greater than 50% of the lobule. The numbers of IL-6 positive infiltrating mononuclear cells, seen mainly at portal tracts, was graded on a + to +++ scale, corresponding to positivity in 1–5%, 6–10%, and greater than 10% of total numbers of infiltrating mononuclear cells examined.

Results

The main cell elements staining positive for IL-6 in hepatic lobules were the sinusoidal endothelial cells. IL-6 positive endothelial cells were more numerous and diffusely distributed in lobules in acute viral hepatitis than in chronic viral hepatitis (table and fig 1). Expression of IL-6 in endothelial cells was particularly increased in focal necrotic areas of hepatocytes and accompanied by IL-6 positive Kupffer cells and infiltrating mononuclear cells. In contrast, IL-6 positive infiltrating mononuclear cells were more common in portal tracts than in lobules (fig 2). The number of mononuclear cells staining for IL-6 was increased in chronic active hepatitis compared with chronic persistent hepatitis, regardless of the aetiology of the disease (table). The staining appeared to be localised within the cytoplasm. Most of the fibroblasts were also IL-6 positive. Vascular walls were also stained, while hepatocytes and bile duct cells were not. In patients with non-specific reactive hepatitis IL-6 positive endothelial cells were found only occasionally in lobules (fig 3). In patients with liver cirrhosis positive staining of endothelial cells was enhanced and diffusely distributed.

Discussion

Most of the hepatic sinusoidal endothelial cells stained positively for IL-6 in patients with

Figure 1 Enhanced expression of IL-6 in sinusoidal endothelial cells (—→) and Kupffer cells (—▶) around focal necrotic area of hepatocytes in a patient with acute non-A, non-B hepatitis.

Figure 2 IL-6 stained infiltrating mononuclear cells (—▶) at a portal area in a patient with HBsAg positive chronic active hepatitis. Fibroblasts (—→) were also stained for IL-6.

Figure 3 Most cells were negative for IL-6 staining except for vascular wall cells (—→) in a patient with non-specific reactive hepatitis.

acute viral hepatitis. IL-6 expression of endothelial cells was enhanced particularly in focal necrotic area of hepatocytes and Kupffer cells, the fixed tissue macrophages of the liver, were also positively stained, along with some IL-6 positive infiltrating mononuclear cells. These findings suggest that locally produced IL-6 may contribute to the acute inflammatory process of the disease.

In contrast, IL-6 staining infiltrating mononuclear cells was more noticeable in portal tracts than in lobules and their prevalence was greater in chronic active hepatitis than in chronic persistent hepatitis, indicating that local expression of IL-6 correlates with disease activity regardless of its aetiology. IL-6 is probably secreted by mononuclear cells in focal inflammatory areas that have spread into surrounding areas, thereby modulating the inflammatory and immune reaction in chronic liver disease. Although most of infiltrating mononuclear cells have been reported to be cytotoxic T cells,¹⁶ the microscopic observation of FITC staining was insufficient to identify the cell type of mononuclear cells responsible.

Although hepatocytes showed negative staining for IL-6 in this study, IL-6 seemed to bind preferentially to periportal hepatocytes¹⁷ and only a small fraction of IL-6 was taken up by the specific receptors of hepatocytes.¹⁸ Previous studies have confirmed that acute phase protein in hepatocytes is regulated by a variety of cytokines including IL-6.^{1-4,11} Lotz *et al.* also showed that human hepatoma cells as well as primary hepatocytes produce IL-6 activity and express IL-6 proteins and mRNA.¹⁹ Furthermore, in thyroiditis epithelial cells were found to express IL-6 using in situ hybridisation and immunohistochemical methods.^{8,9} Therefore, we cannot exclude the possibility that hepatocytes produce IL-6 in patients with acute and chronic liver disease. We may need to use more sensitive methods with molecular biology techniques.

In the steady state IL-6 is usually not produced by normal cells, but its expression is readily induced by viral infections.^{20,21} In vitro exposure to human immunodeficiency virus (HIV) also induces the expression of IL-6 mRNA and the secretion of IL-6 by monocytes or macrophages.²² It has also been noted that the most potent inducers of IL-6 in the Kupffer cells of rats are viruses.²³ Endothelial cells also can produce IL-6 after stimulation by IL-1 and TNF- α .^{24,25} IL-6 shares many of its biological activities with IL-1 and TNF- α ,²⁶ and thus the three cytokines probably act in concert, directing inflammatory and immunological reactions. For example, our previous study disclosed that TNF- α was produced by infiltrating mononuclear cells in focal inflammatory areas of the liver, suggesting that the numbers of TNF positive cells correlated with the degree of inflammatory activity of chronic liver disease.²⁷

Finally, this study has shown that endothelial, Kupffer, and infiltrating mononuclear cells in liver tissue were activated to produce IL-6 by hepatitis virus infection, contributing to the inflammatory and immune responses in acute and chronic hepatitis. Simultaneously,

IL-6 might provide the stimulatory signals for hepatocytes, resulting in their regeneration.²⁸ This is similar to a process in renal mesangial cells for which IL-6 was an autocrine growth factor.²⁹ In alcoholic liver cirrhosis, serum concentrations and production by leucocytes of IL-6 were increased.³⁰ Our study also showed that intrahepatic IL-6 expression was increased, suggesting that excess cytokines could lead to tissue damage and fibrosis.³¹

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