Immunolocalisation of α interferon in liver disease

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SUMMARY The expression of immunoreactive α interferon was examined in 78 liver biopsy specimens using an indirect immunoperoxidase technique. Biopsy specimens included cases of acute viral hepatitis, chronic active hepatitis, primary biliary cirrhosis, alcoholic hepatitis, large bile duct obstruction and normal liver. Kupffer cells were positive for α interferon in all cases. Hepatocytes were negative for α interferon in normal liver but in acute viral hepatitis were positive in perivenular and necrotic areas. Hepatocytes were positive in periportal areas, associated with piecemeal necrosis, in chronic active hepatitis and primary biliary cirrhosis, and were positive in perivenular areas in alcoholic hepatitis and large bile duct obstruction.

The unexpected finding of α interferon in hepatocytes in non-viral liver disease indicates that the presence of this substance in liver cells cannot be taken as a specific marker of viral infection.

Hepatitis B (HBV) virus is not directly cytopathic and T cell mediated cytolysis is considered to be the means of viral elimination in HBV infection. The recognition of infected cells by cytotoxic T lymphocytes depends on the association between viral determinants and class I major histocompatibility complex (MHC) molecules. The interferons are known to induce an increase of synthesis and display of MHC class I molecules, and it has been proposed that during acute HBV infection, interferon promotes hepatocyte MHC class I display, which increases the possibility of immune attack by cytotoxic T cells. There is indirect evidence to support this hypothesis. In uncomplicated acute HBV infection plasma interferon concentrations rise,¹ and increased hepatocyte expression of β_2 microglobulin (a subunit of HLA class I antigen) has been shown.² Recently a technique for the detection of immunoreactive α interferon (IFN- α) in formalin fixed, paraffin wax embedded tissue has been developed.³ We used this technique on a series of liver biopsy specimens to investigate the expression of α interferon in viral hepatitis and in other liver diseases to ascertain if its expression by hepatocytes is a specific indicator of viral infection.

Material and methods

Seventy eight percutaneous and wedge liver biopsy specimens from nine cases of acute viral hepatitis, 14

of chronic active hepatitis, 14 of primary biliary cirrhosis, 15 of alcoholic hepatitis and 14 of extra-hepatic biliary obstruction and 12 patients without liver disease were selected from our files. Biopsy specimens had been fixed in either buffered formalin or formol-saline.

Sections 4 μ m thick were cut from the paraffin wax blocks and mounted on slides coated with poly-L-lysine. They were stained using an indirect immunoperoxidase technique with a sheep anti- α interferon antiserum (gift from Dr A Meager, National Institute of Biological Standards and Control, Potter's Bar, Hertfordshire). Peroxidase conjugated swine anti-sheep (Serotec, Oxford, England) was used as a bridge and diaminobenzidine was the substrate. The sheep anti- α -interferon antiserum (H51) was raised with human lymphoblastoid interferon (Hu IFN- α Ly Namalwa (Wellferon), Wellcome Research Laboratories, Beckenham, Kent) as antigen which was greater than 80% pure with respect to interferon protein. In a viral culture inhibition assay the antiserum neutralised all preparations containing α interferon⁴ but did not neutralise γ interferon.⁵ The antigen reacted with α interferon but not with recombinant β interferon (Triton Biosciences, California USA) on immunoblot analysis.

Diluted antiserum and normal sheep serum used in control studies were absorbed with a mixture of guinea pig and porcine liver powders (Sigma, Dorset, England) before their use in the indirect immunoperoxidase technique and this reduced non-specific binding to human tissues.

Fig 1 *Kupffer cells positive for IFN- α in normal liver.*

A section of each case was stained with normal sheep serum as a negative control and in addition all positive staining of liver sections was abolished when sections were stained with H51 antiserum which had been preabsorbed with Wellferon. Sections from the cases of chronic active hepatitis and alcoholic hepatitis were also stained using an indirect immunoperoxidase technique with a goat antibody to hepatitis B surface antigen (Dako Ltd, High Wycombe, Buckinghamshire).³

Results

All examples of normal liver showed positive staining of Kupffer cells. Hepatocytes and bile duct epithelium were consistently negative (fig 1).

ACUTE VIRAL HEPATITIS

As patients with viral hepatitis are now biopsied very rarely most of these biopsy specimens were from the era before serological identification of HBsAg became possible. The serological state was unknown, but in each case the history and histological features were strongly indicative of viral hepatitis. All cases showed strong hepatocyte staining for α interferon in the perivenular areas (fig 2). This pattern was particularly

easy to recognise in the cases showing spotty necrosis where bridging and massive necrosis had not disrupted the architecture. There were also scanty positively staining hepatocytes throughout the lobules but only in relation to inflammation or necrosis.

CHRONIC ACTIVE HEPATITIS

Two patients were known to be HBsAg positive while in the remaining 12 there was no history of hepatitis B infection and no immunohistochemical staining for HBsAg in the biopsy specimens, all of which showed the histological features of chronic active hepatitis with portal and periportal inflammation, erosion of the limiting plate, piecemeal necrosis and a greater or lesser degree of fibrosis. Although inflammation was severe in all biopsy specimens, there was a range in severity of piecemeal necrosis from relatively scanty to extremely severe and widespread. All 14 cases showed specific staining for α interferon and this took the form of cytoplasmic staining of hepatocytes related to piecemeal necrosis (fig 3). Where disruption of the limiting plate was identified, associated hepatocytes were always positive. Similarly, isolated hepatocytes were invariably positive. Scanty positively staining hepatocytes within the lobules were also identified in six cases, and these hepatocytes were adjacent to

Fig 2 Zonal distribution of hepatocytes positive for IFN- α in acute hepatitis.

Fig 3 IFN- α positive hepatocytes in an area of piecemeal necrosis in chronic active hepatitis.

Fig 4 Centrilobular distribution of IFN- α positive hepatocytes in alcoholic hepatitis.

inflammatory cells within the lobules. The staining pattern in the two HBsAg positive cases was indistinguishable from the rest. In one case there was positive staining for α interferon in bile duct epithelium.

PRIMARY BILIARY CIRRHOSIS

Fourteen cases of primary biliary cirrhosis with stage 1–4 disease activity were studied. Eleven cases were stage 2 or 3 and showed a variable degree of piecemeal necrosis. In all cases most hepatocytes were negative for α interferon except in the presence of inflammatory disruption of the limiting plates, and all 11 cases with piecemeal necrosis showed a pattern of IFN- α staining similar to that observed in chronic active hepatitis. Hepatocytes distant from areas of piecemeal necrosis were usually negative as were hepatocytes associated with lobular inflammation. Hepatocytes adjacent to portal tracts where there was intense inflammation but no piecemeal necrosis were occasionally positive but the extent of staining was much less conspicuous than that in chronic active hepatitis. Proliferating bile ductules were occasionally positive but not normal bile ducts.

ALCOHOLIC HEPATITIS

Fifteen biopsy specimens of alcoholic liver disease

varying from mild to severe alcoholic hepatitis without cirrhosis were examined. All cases were negative when stained with anti-HBsAg. Hepatocytes showing positive staining for α interferon were present in all specimens. In eight biopsy specimens staining was concentrated maximally in perivenular areas and occurred in hepatocytes which looked essentially normal as well as those which exhibited degenerative features (fig 4). In seven cases positively stained hepatocytes occurred in a pan-lobular distribution or in scanty hepatocytes dispersed throughout the lobule. Steatotic hepatocytes were frequently positive and in these cases the cytoplasmic staining was identified at the periphery of the cell, displaced by the cytoplasmic lipid. Although there was extensive α interferon staining of inflammatory cells, staining of hepatocytes did not seem to be specifically related to inflammation. Bile duct epithelium was negative.

LARGE BILE DUCT OBSTRUCTION

Twelve biopsy specimens contained hepatocytes with positive α interferon staining, and in all of these it was maximal in perivenular zones. In four cases there was also focal hepatocyte staining in the periportal areas associated with intense portal tract inflammation. In 12 biopsy specimens there was also focal positive staining in inflamed bile ducts.

Discussion

The use of this technique has already shown that in normal liver Kupffer cells contain immunoreactive α interferon.⁶ Our study has shown that Kupffer cells were also positive for IFN- α in all other groups of liver disease examined. This disagrees with the findings of a previous study which used immunofluorescence localisation of IFN- α in liver tissue infected with hepatitis B virus and showed its presence only in mononuclear cells of the lymphocyte/plasma cell series, fibroblasts, and polymorphonuclear leucocytes. Kupffer cells and hepatocytes were invariably negative.⁷ In that study the IFN- α anti-sera satisfied usual immunofluorescence specificity controls, but abolition of positive staining by preabsorption of antibody with IFN- α was not attempted. Our own preabsorption control studies strongly support the specificity of the H51 antibody.

Furthermore, there is increasing evidence in animals that macrophages can be induced to produce interferons.⁸ Cells of the mononuclear phagocyte system, including Kupffer cells, have been shown to contain immunoreactive IFN- α using the H51 antibody, and it has been suggested that these cells might be the source of the low concentrations of endogenous IFN- α found in physiological conditions.⁶

We have shown that in all groups of liver disease studied, hepatocytes could be identified which were positive for IFN- α . The distribution of positive hepatocytes generally related to areas of hepatocyte damage. Our finding of IFN- α , mainly in perivenular areas and associated with bridging necrosis in acute viral hepatitis and in periportal zones and areas of lobular inflammation in chronic active hepatitis, is similar to the reported distribution of β_2 microglobulin in HBV infection.² Similarly, in non-viral liver disease the localisation of IFN- α staining was seen in areas of hepatocyte damage or degeneration.

Using the techniques described here, we can only speculate as to whether hepatocytes actually synthesise IFN- α or are engaged in its uptake from surrounding cells. Non-specific uptake of IFN- α by degenerating hepatocytes is obviously a possible explanation for positive hepatocyte staining. In the cases of alcoholic liver disease and large bile duct obstruction, however, positively stained hepatocytes were found in areas quite unrelated to the presence of inflammatory cells. This indicates that hepatocytes containing IFN- α are

not merely a consequence of increased local concentrations of IFN- α in areas of inflammation.

The cuboidal lining epithelium of the choroid plexus has been shown to be strongly positive for immunoreactive IFN- α using the same immunohistochemical technique as that used in the present study,⁶ and recently the presence of IFN- α mRNA has been shown in these cells, indicating that cells outwith the mononuclear phagocyte and leucocyte series have a role in the synthesis of IFN- α (Howatson *et al*, unpublished observations). Application of *in situ* hybridisation techniques to the study of interferon synthesis in the liver would clearly be of interest.

Interferons are known to have a wide range of biological effects and these, in addition to antiviral activity and effects on MHC expression, include antigrowth activity and effects on cellular differentiation.⁹ Interferon may have a role in liver disease beyond that currently proposed in viral hepatitis.

References

- 1 Levin S, Hahn T. Interferon system in acute viral hepatitis. *Lancet* 1982;i:592-4.
- 2 Nagafuchi Y, Scheuer PJ. Expression of B₂ microglobulin on hepatocytes in acute and chronic type B hepatitis. *Hepatology* 1986;6:20-3.
- 3 Foulis AK, Farquharson MA, Meager A. Immunoreactive alpha-interferon in insulin-secreting B cells in type I diabetes mellitus. *Lancet* 1987;ii:1423-7.
- 4 Exley T, Parti S, Barwick S, Meager A. A comparison of the neutralising properties of monoclonal and polyclonal antibodies to human interferon alpha. *J Gen Virol* 1984; 65:2277-80.
- 5 Meager A. Antibodies against interferons: characterisation of interferons and immunoassays. In: Clemens MJ, Morris AG, Gearing AJH, eds. *Lymphokines and interferons—a practical approach*. Oxford: IRL Press, 1987:105-27.
- 6 Khan NU-D, Pulford KAF, Farquharson MA, *et al*. The distribution of immunoreactive interferon-alpha in normal human tissues. *Immunology* 1989;66:201-6.
- 7 Jilbert AR, Burrell CJ, Gowans EJ, Hertzog PJ, Linnane AW, Marmion BP. Cellular localisation of alpha-interferon in hepatitis B virus-infected liver tissue. *Hepatology* 1986;6: 957-61.
- 8 De Maeyer E, De Maeyer-Guignard J. Macrophages as interferon producers and interferons as modulators of macrophage activity. In: De Maeyer E, De Maeyer-Guignard J, eds. *Interferon and other regulatory cytokines*. Chichester: Wiley & Sons, 1987:194-220.
- 9 Pestka S, Langer JA, Zoon KC, Samuel CE. Interferons and their actions. *Ann Rev Biochem* 1987;56:727-77.

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