IgA deposition in alcoholic liver disease

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SUMMARY Immunoglobulin deposition in alcoholic and non-alcoholic liver disease was studied using an indirect immunoperoxidase technique. A continuous pattern of IgA deposition, with IgA outlining the sinusoids, was shown to be a specific and sensitive marker for liver disease caused by alcohol in both cirrhotic and non-cirrhotic livers. The sensitivity was lowest in cases of alcoholic disease showing fatty change alone. In one case it was possible to show the absence of IgA in liver disease caused by a drug, which was histologically indistinguishable from alcoholic hepatitis.

Alcohol induced liver disease poses a problem for the histopathologist both in terms of its increasing incidence in the western world¹ and in making a diagnosis on morphological grounds.² This is because although the overall histological picture may be characteristic, no single feature is pathognomonic. Relatively specific features include: megamitochondria, hepatocyte swelling, alcoholic hepatitis associated with Mallory's hyaline, pericellular fibrosis, central sclerosing hyaline necrosis and pericentral fibrosis.

Immunofluorescence⁴ and immunoperoxidase⁵ techniques have been applied to renal biopsy specimens for some years. They have added both diagnostic precision and an improved understanding of the pathogenesis of several conditions.⁶ Studies, in which immunoglobulin deposition shown by immunofluorescence in liver disease, have been described.⁷⁻¹⁰ These suggested that a continuous pattern of IgA is relatively specific for alcoholic liver disease. In this paper the results of a study using an immunoperoxidase technique on formalin fixed paraffin embedded material are described.

Material and methods

The material used in this study was derived from two sources. In the first part 30 cirrhotic livers derived from recipients in a liver transplantation programme were examined for their patterns of IgG, IgA, IgM and C3 deposition. The diagnosis of an alcoholic aetiology was based on clinical and histological criteria. In the second part 60 livers seen in a routine histopathology department were studied retrospectively in a similar way. They were stained using an indirect

immunoperoxidase technique. The method used was as follows:

- 1 Dewax sections and take to 74 over proof alcohol.
- 2 Inhibit endogenous peroxidase. Immerse sections in freshly prepared 1% hydrogen peroxide in methanol for 15 minutes.
- 3 Trypsinise sections. Drain slides and transfer to pre-heated distilled water (37°C) for five minutes. Transfer slides to preheated trypsin solution (37°C). The time for which sections were left varied according to the nature of the specimens. This ranged from five minutes for needle biopsy specimens to 30 minutes for the cirrhotic livers that had been fixed in formalin for years.
- 4 Wash in Tris buffered saline (TBS) for 15 minutes. A stock solution (1000 cc 0.1 M Tris and 800 cc 0.1 M hydrochloric acid (with the pH adjusted to 7.6) was diluted 1/10 with 0.9% sodium chloride.
- 5 Incubate with 1/40 (rabbit) antihuman immunoglobulin (Dako) for 30 minutes.
- 6 Wash in TBS for 15 minutes.
- 7 Incubate with peroxidase labelled (swine) anti-(rabbit) immunoglobulin (Dako) at 1/40 for 30 minutes.
- 8 Wash in TBS for 15 minutes.
- 9 Develop with diaminobenzidine (DAB). This was made up fresh: DAB was added to TBS until the pH

Table 1 *Livers removed before transplantation*

Diagnosis	Pattern of IgA deposition		
	Total	Continuous	Discontinuous
Alcoholic cirrhosis	15	11	4
Non-alcoholic cirrhosis	15	3	12
Post-viral	5	1	4
Idiopathic	5	2	3
Metabolic	3	0	3
Biliary atresia	2	0	2

Fig 1 (a) Alcoholic hepatitis with area of hepatocyte necrosis associated with neutrophilic infiltrate; (b) section from same biopsy specimen stained by indirect immunoperoxidase technique for IgA, illustrating continuous, sinusoidal pattern of immunoglobulin deposition.

- was 5.5, together with a drop of 100 volumes hydrogen peroxide.
- 10 Wash in tap water for five minutes.
 - 11 Counterstain in haematoxylin.
 - 12 Dehydrate, clean, and mount sections.

Results

In the first part of the study 30 cirrhotic livers obtained from recipients in a liver transplantation programme were examined for patterns of immunoglobulin deposition. Table 1 shows that in 11 of 15 cases of alcohol induced disease a continuous pattern

of IgA deposition was seen. A sinusoidal pattern of deposition, with IgA outlining the sinusoids, was the predominant pattern in all these cases (fig 1). A pericellular pattern was only seen as a minor component. Continuous IgA deposition was only seen in three of 15 cases of cirrhosis due to various other causes ($0.01 > p > 0.001$). No significant differences were seen in the patterns of IgG, IgM, and C3 deposition between alcoholic and non-alcoholic cases (results not shown).

In the next stage several needle biopsy specimens processed as routine surgical specimens showed IgA (table 2). Overall, a continuous pattern was seen in 19

Table 2 Needle biopsy specimens

Diagnosis	Total	Pattern of IgA deposition	
		Continuous	Discontinuous
Alcoholic:	28	19	9
Fatty change only	15	6	9
Alcoholic hepatitis or cirrhosis, or both	8	8	0
Cirrhosis	5	5	0
Non-alcoholic:	32	2	30
Fatty change only	14	1	13
Acute hepatitis (viral)	2	0	2
Drug induced cholestasis or hepatitis, or both	3	0	3
Chronic active hepatitis (viral)	6	0	6
Chronic active hepatitis (autoimmune)	4	1	3
Primary biliary cirrhosis	3	0	3

Fig 2 (a) Hepatitis associated with ingestion of antianginal drug, perhexilene maleate, closely resembling that seen in alcohol induced liver disease; (b) section from same biopsy specimen stained by indirect immunoperoxidase technique for IgA, illustrating absence of immunoglobulin deposition.

Fig 3 (a) Fatty change associated with ingestion of alcohol. There was no evidence of fibrosis in this case; (b) section from same biopsy specimen stained by indirect immunoperoxidase technique for IgA, illustrating absence of immunoglobulin deposition.

of 28 cases from the alcoholic group and in two of 32 cases in the non-alcoholic group ($0.01 > p > 0.001$).

In the group with liver disease induced by drugs the drugs (and the associated histological findings) were as follows: perhexilene maleate (fatty change and a neutrophilic infiltrate associated with hepatocyte degeneration), prednisolone (fatty change), and the combined oral contraceptive pill (bile thrombi). Interestingly, particularly in the first case which was histologically indistinguishable from alcoholic hepatitis (fig 2), there was no IgA deposition in any of these cases.

In cases of fatty change induced by alcohol continuous IgA deposition was seen in six of 15 cases, while in non-alcoholic induced fatty change it was seen in one of 14 cases ($0.01 > p > 0.001$) (fig 3).

Discussion

Several abnormalities have been observed in alcohol induced liver disease entailing both the cell mediated and humoral arms of immunity.¹¹ One of the earliest changes discovered was an increase in serum IgA,¹² which may be observed in the presence of fatty change alone.¹³ This finding, however, is too variable to be of diagnostic value. In several studies using immunofluorescence techniques it has been claimed that a continuous pattern of IgA deposition in the liver is specific for alcoholic liver.⁷⁻¹⁰ This pattern may be either pericellular or sinusoidal. IgA, together with secretory component, has also been shown within 50% of normal hepatocytes.¹⁴

A body of experimental evidence, derived mainly from work in rats, has shown that the IgA found in bile is derived from the serum and that it probably reaches the bile by active transport through hepatocytes.¹⁵⁻¹⁷ In one study radiolabelled IgA injected into rats was found bound to the plasma membrane of hepatocytes and from there was transported through the cytoplasm into the bile canaliculi. In the case of alcoholic liver disease IgA may reach the liver via the portal circulation in increased amounts due to inflammation of the bowel wall caused by the ingested alcohol, leading to increased secretion by plasma cells.¹⁸ It has been suggested that the immunoglobulin, both IgG and IgA, found bound to the membranes of hepatocytes in alcoholic liver disease may be due to antigen modification of the cell membrane by alcohol and that the antibodies may have a role in the production of hepatocyte damage.¹⁹

The results of this study confirm the observations, previously made using an immunofluorescence technique, of the specificity of a continuous pattern of IgA deposition in alcoholic liver disease. The study extends the specificity to also include cases of liver disease morphologically indistinguishable from

alcoholic hepatitis but of different aetiology, such as those caused by perhexilene maleate.

Similar results were found both in cirrhotic livers, taken from a transplantation series, and routine needle biopsy specimens. When the cases were broken down, however, according to the histological pattern of alcoholic liver disease, differences were observed. The most striking of these was that liver biopsy specimens taken from alcoholics whose livers showed fatty change only displayed a much lower incidence of continuous IgA staining than that seen in other forms of the disease. ($0.01 > p > 0.001$).

In a study of prognostic factors in alcoholic liver disease it was shown that the presence of fatty change alone did not carry any adverse significance.²⁰ It is known that such changes can be induced over a period of days and can disappear when drinking is stopped within weeks.²¹ The pathogenesis of fatty change induced by alcohol has been extensively studied and has been shown to be due to metabolic changes within the cell.²² Bearing these findings in mind, it is perhaps not surprising to find that there was poor correlation between fatty change due to alcohol and liver biopsy specimens showing a continuous pattern of IgA deposition (six of 15). On the other hand, only a single positive was seen in 14 cases of fatty change due to other causes. Thus it may be seen that even in these circumstances the pattern of immunoglobulin deposition may be useful as a negative screen.

IgA deposition may have a role in the pathogenesis of liver cell damage due to alcohol, or reflect its effects. Despite advances in immunofluorescence techniques immunoperoxidase methods retain several advantages. These include the fact that it is possible to visualise tissue structure relatively easily and that it is not necessary to use a special microscope. Thus such a technique is well suited to correlating morphological appearances with patterns of immunoglobulin deposition.

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