Review article

Alcohol induced liver disease

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SUMMARY Alcohol induces a variety of changes in the liver: fatty change, hepatitis, fibrosis, and cirrhosis. The histopathological appearances of these conditions are discussed, with special attention to differential diagnosis.

Many forms of alcoholic liver disease are associated with Mallory body formation and fibrosis. Mallory bodies are formed, at least in part, from intermediate filaments. Associated changes in intermediate filament organisation in alcoholic liver disease also occur. Their significance in the pathogenesis of hepatocyte death may be related to abnormalities in messenger RNA function. The mechanisms underlying hepatic fibrogenesis are also discussed.

Although alcohol has many effects on the liver, all except cirrhosis are potentially reversible on cessation of alcohol ingestion. Cirrhosis is irreversible and usually ultimately fatal. It is therefore important to determine what factors are responsible for development of alcohol induced cirrhosis, especially since only 17-30% of all alcoholics become cirrhotic.¹ This is of some urgency now, since there has been an explosive increase in alcohol consumption in the Western World, particularly affecting young people, resulting in a dramatic increase in the incidence of alcoholic liver disease and cirrhosis. For example, in New York City, alcoholic cirrhosis is the third commonest cause of death in people aged between 25 and 64.² Our experience in Oxford shows that 20% of all liver biopsies and 50% of liver biopsies performed by a physician with a special interest in hepatology, show alcoholic liver damage.

This review is in two parts. In the first part the pathological features of alcohol induced liver disease will be described, with emphasis on their diagnostic, prognostic, or pathogenic importance. Although the usual convention is to divide these features into three broad categories—namely, fatty change, alcoholic hepatitis with or without fibrosis, and cirrhosis—this division may be artificial for three reasons. Firstly, these categories may well represent a continuum of disease. Secondly, at any given time, one or any combination may be present in the same liver. Thirdly, if a liver biopsy is per-

formed after some period of alcohol abstinence, alcohol related changes may not be seen. Accordingly, we shall consider the morphological changes associated with alcohol abuse under the headings in Table 1.

In the second part, the pathogenesis of alcohol induced liver disease will be discussed, but this will deal only with the induction of alcoholic hepatitis, fibrosis, and cirrhosis—that is, chronic alcoholic liver disease—and not with fatty change, for two reasons. Firstly, the role of ethanol in the pathogenesis of fatty liver is well documented, while its role in inducing the other features is much less clear. Secondly, the consensus of available evidence is that fatty change alone probably has no role in the production of cirrhosis, which for the reasons outlined in the first paragraph is the most important result of excess alcohol ingestion on the liver. Several reviews of ethanol metabolism and its role in pathogenesis of fatty change have been published.¹⁻³

Pathology of alcohol induced liver disease

Alcohol induces a number of changes in the liver (Table 1). No one feature is diagnostic, but some are relatively specific. For example, Mallory bodies are present in hepatocytes in 94-100% of cases of alcoholic hepatitis and cirrhosis. They are also seen, however, in 66-100% of cases of Wilson's disease, 17-48% of cases of primary biliary cirrhosis, 11-84% of cases of Indian childhood cirrhosis,⁴ and

Table 1 *Pathological changes in alcohol induced liver disease*

<i>Lesion</i>	<i>Specificity*</i>
Fatty liver	
Fatty change	—
Fatty cysts	—
Lipogranuloma	—
Megamitochondria	+
Hepatocyte swelling	+
Alcoholic hepatitis	+
Fibrosis	
Pericellular	+
Periportal	—
Lipogranuloma	—
Central sclerosing hyaline necrosis	+
Pericentral venous	+
Cirrhosis	
Micronodular	—
Macronodular	—
Mixed	—
Miscellaneous	
Cholestasis	—
α_1 -antitrypsin granules	—
Haemosiderosis	—
Chronic active hepatitis	—
Hepatocellular cancer	—

*+ = yes, — = no.

1.5% of cases of non-alcoholic cirrhosis.⁵ In contrast, fatty change is not specific for alcoholic liver disease, being found in a multitude of other diseases (see below). Although it may appear that non-specific changes are unimportant, their presence in

association with other more specific changes often leads to a correct diagnosis of alcoholic liver disease.

FATTY CHANGE (Fig. 1)

Fatty change in hepatocytes is not a diagnostic feature of alcohol induced liver disease. It occurs in many other conditions, including obesity, diabetes mellitus, drugs such as steroids and methotrexate, congestive cardiac failure, alimentary disorders, abetalipoproteinaemia, and Kwashiorkor.⁶ However, it invariably appears after excessive alcohol ingestion, usually being visible within three to seven days.⁷ On cessation of drinking, fatty change may disappear within a few days, but in severe cases it takes four to six weeks to clear.⁸ Accordingly, it is unusual not to see some fatty change in a biopsy from an alcoholic unless a reliable history of prolonged alcohol abstinence is obtained. In established alcoholic cirrhosis, however, fatty change may be absent. Thus the presence of fatty change, even in minimal amounts, should suggest the diagnosis, if only for exclusion.

Fatty change typically occurs focally in the centrilobular zone, but in severe cases it can affect virtually the whole liver lobule, mimicking Kwashiorkor. Usually the cytoplasm is almost totally replaced by a single vacuole, with displacement of the nucleus to the periphery of the hepatocyte producing "large

Fig. 1 *Fatty change and fat cysts. Hepatocytes show both large and small droplet fatty change.*

droplet" fatty change. Occasionally the fat is in the form of many small cytoplasmic vacuoles with no nuclear displacement ("small droplet" fatty change), mimicking the appearances seen in tetracycline toxicity and fatty liver of pregnancy. It has been suggested that the "small droplet" change reflects recent rapid accumulation or mobilisation of lipid.⁹

FAT CYSTS (Fig. 1)

These are not infrequent and consist of small lipid filled spaces in the liver lobule, apparently formed by the rupture of contiguous fat filled hepatocytes. They are apparently of no importance, except perhaps indicating severity of fatty change.

LIPOGRANULOMA

These are small foci of mononuclear cells surrounding a central extracellular fat filled space. The cells present can vary from a small collection of eosinophil polymorphs and mononuclear cells, to well formed epithelioid granulomata with multinucleate giant cells, although this latter type is rather uncommon. Lipogranulomata are normally found in a centrilobular location but can occur anywhere, including portal tracts. They are reported to occur in 30–50% of cases of alcohol induced liver disease.¹⁰ In our experience in the UK, however, the incidence of lipogranulomata is much lower.

Although the recent convention has been to regard fatty change and its sequelae (for example, lipogranuloma) as totally reversible and of no long term significance, results of baboon experiments have suggested that fatty change may progress to cirrhosis without a detectable intervening stage of alcoholic hepatitis. In addition, lipogranulomata can occasionally apparently coalesce and be associated with surrounding fibrosis.¹¹ The significance of these findings is unknown, but will be discussed in more detail later (see section on fibrosis and pathogenesis).

MEGAMITOCHONDRIA (Fig. 2)

Megamitochondria are not infrequently found in alcohol induced liver disease. They are similar in size and eosinophilia to red blood cells, but their presence in hepatocytes and their perfect symmetry allows their positive identification. Some authors claim that they are more readily detected in trichrome stains (as red cytoplasmic globules), but in our experience haematoxylin and eosin staining is adequate for their detection. They probably arise as a result of the alcohol induced alteration in mitochondrial membrane redox potential and the resultant disturbance in the citric acid cycle. Their occurrence in relatively large numbers (that is, 3–4

Fig. 2 Megamitochondria. Swollen hepatocytes contain megamitochondria in cytoplasm (arrows).

per high power field) is said to indicate heavy alcohol ingestion within the last 30 days.¹² They are occasionally found in morphologically normal livers and in non-alcohol induced disorders, but with much lower frequency.¹³ Although they are not of any known prognostic significance, the presence of large numbers of megamitochondria is highly suggestive of recent heavy alcohol consumption.

HEPATOCYTE SWELLING (Fig. 3)

Hepatocyte swelling is a characteristic and well documented feature of alcoholic liver disease.¹⁴ Its presence and significance, however, is not always realised by the non-specialist. It involves hepatocytes mainly in the centrilobular region and affects many hepatocytes, but to varying extents. The hepatocyte may be massively swollen, often being three times normal size. The cytoplasm does not show the changes of cloudy swelling, hydropic or fatty change, but clumps either into fine eosinophilic granules or into a fine web like pattern. These cells also often contain typical Mallory bodies or finer structures of Mallory body type which react with antibodies to Mallory bodies.¹⁵ This resembles the ballooning degeneration of viral hepatitis, but there are rarely any acidophil bodies or other features of viral disease. This change is characteristic of alcoholic liver disease, and accordingly its presence, either alone or in conjunction with fatty change, is evidence of an alcohol aetiology. Since it has not previously been given great emphasis, the

Fig. 3 *Ballooned hepatocytes. Hepatocytes are enlarged (arrows) and have web like filamentous and granular cytoplasm.*

pathogenesis and prognostic importance of this hepatocyte lesion is not clear.

ALCOHOLIC HEPATITIS AND MALLORY BODIES (Figs. 4, 5)

The characteristic feature of alcoholic hepatitis is the presence of Mallory bodies—alcoholic hyaline—in hepatocytes, with a surrounding neutrophil and mononuclear cellular infiltrate. Alcoholic hepatitis can be diagnosed, however, in the absence of Mallory bodies if other features of alcoholic liver disease are present—for example, hepatocyte swelling, inflammatory cell infiltration, etc. Mallory bodies are fairly discrete collections of eosinophilic hyaline intracytoplasmic material, commonly situated around the nucleus. The hepatocyte is almost invariably swollen, and in alcoholic hepatitis the affected hepatocytes are usually found in the region of the central vein. In cirrhotic livers, however, hepatocytes containing Mallory bodies are most frequently found at the interface between regenerating

Fig. 4 *Ballooned hepatocytes and Mallory bodies. Mallory bodies are predominantly perinuclear. These hepatocytes do not have a discernable intermediate filament network. By contrast, hepatocytes which do not contain Mallory bodies sometimes also lack this network (small arrow) while others have a filamentous cytoplasmic network (large arrow). This is an indirect immunoperoxidase preparation with antibody to Mallory's hyaline.*

Fig. 5 *Alcoholic hepatitis. Ballooned hepatocytes with Mallory bodies (arrows) are surrounded by neutrophil polymorphs and mononuclear cells.*

Fig. 6 *Pericellular fibrosis. The reticulin framework around individual hepatocytes is increased and there is formation of scars as individual hepatocytes are removed (Gordon and Sweet's reticulin).*

nodules and fibrous septa. Often the cells containing Mallory bodies rupture. Although hepatocytes containing Mallory bodies may be surrounded by polymorphs or mononuclear cells, or both, associated inflammatory cells are frequently absent. The mononuclear cells are presumably lymphocytes, macrophages, and perhaps fibroblasts, but the future use of monoclonal antibodies specific for lymphocytes and macrophages may definitely identify these cells.

Mallory bodies are not exclusive to alcoholic liver disease; they are found in hepatocytes in a variety of other diseases—namely, primary biliary cirrhosis, Wilson's disease, Indian childhood cirrhosis, intestinal bypass, diabetes mellitus,¹⁶ longstanding bile duct obstruction,⁴ perhexiline maleate ingestion,¹⁷ and abetalipoproteinaemia. They have also been reported in obese patients, but this may be because they are covert alcohol abusers.¹⁸ Structures with similar light and ultrastructural morphology are found in the lung in asbestosis,¹⁹ in pituitary tumours (Morton JA, Esiri MM, McGee JO'D, unpublished observations), and also in the parathyroid.²⁰ The nature of Mallory bodies and their role in the pathogenesis of chronic alcoholic liver disease will be discussed later.

FIBROSIS (Figs. 6, 7 and 8)

Fibrosis in alcohol induced liver disease can vary from minimal amounts detectable only with special stains to the massive fibrosis of cirrhosis. In whatever quantity, however, its appearance is, by definition, indicative of chronicity. There are five patterns. These will be described in order of frequency, the most common first.

Alcoholic hepatitis is often accompanied by, or results in, fibrosis. In some definitions of alcoholic hepatitis, fibrosis is part of the lesion. This fibrosis typically occurs as fine strands surrounding individual hepatocytes giving the picture of *pericellular fibrosis* (Fig. 6). Since alcoholic hepatitis is commonly found near central veins, pericellular fibrosis usually affects centrilobular hepatocytes. Individual hepatocytes gradually disappear and larger foci of fibrosis are formed, eventually resulting in solid, often stellate, septa of fibrosis, radiating from the central vein. There is usually pericellular fibrosis at the periphery of these septa. This form of fibrosis is characteristic of alcohol, and has considerable diagnostic importance. Sometimes these scars can extend to link up with portal tract fibrous tissue, thus dissecting the normal liver architecture. It does not necessarily indicate a poor prognosis, however, as it

Fig. 7 Central sclerosing hyaline necrosis. A hepatic vein is surrounded by extensive fibrosis, which is extending out into the lobule. Many central hepatocytes have been lost.

Fig. 8 Central sclerosing hyaline necrosis. This liver shows the fine granular scarring of central sclerosing hyaline necrosis, particularly at the anterior edge.

can totally regress.

The second type of fibrosis is that found around portal tracts. This non-specific, *periportal* fibrosis shows stellate extension of fibrous tissue into the lobule with occasional linkage to other portal tracts or to the other fibrous scars mentioned above. Like the fibrosis of lipogranulomata (see below), it is neither specific for alcoholic liver disease, occurring in many other diseases, nor is it apparently necessarily indicative of future progression to cirrhosis.

The third type of fibrosis is that found around *lipogranulomata*. As mentioned in the section on fatty change, lipogranulomata can coalesce to form larger multinodular structures. These are characterised by the presence of collagen fibres in and around the individual component granulomata, and on occasions the amount of fibrosis can become considerable, forming stellate lesions. Since lipogranulomata are most often found in a centrilobular location, these lesions are usually centrilobular. While they usually lie separately in the lobule, they can link with fibrosis extending from the central vein or from the portal tract, thus again dissecting up the normal liver lobular architecture.

The fourth type of fibrosis found in alcoholic liver disease is "*central sclerosing hyaline necrosis*."²¹ This is a form of fibrosis characteristic of alcoholic

liver disease. The fibrosis ("sclerosis") forms around the central veins ("central") and extends out into the surrounding hepatocytes. These are lost ("necrosis") and the fibrosis gradually extends. Occasionally, pericellular fibrosis is seen around surviving hepatocytes. These often contain Mallory bodies ("alcoholic hyaline") and may show changes of alcoholic hepatitis. Eventually massive fibrôsis may result with total replacement of lobules. The characteristic feature of the latter is the presence of dilated central veins in the middle of massive amounts of fibrous tissue, in which all liver components have been lost (Fig. 7); surviving portal tracts can be found, however, on careful examination. It produces a characteristic macroscopic appearance of a very fine, granular, rock hard fibrosis of the liver, totally unlike micronodular cirrhosis (Fig. 8). In its full blown form it is not common in the UK and is associated with a poor prognosis producing portal hypertension and ascites, even in the absence of cirrhosis. It may represent a particularly severe form of either the first type of fibrosis described above—namely, pericellular fibrosis—or the fifth pattern of fibrosis—pericentral venous fibrosis—described next.

In pericentral venous fibrosis there is fibrosis around the central vein only. Other abnormalities

alone or in combination are fatty change, megamitochondria, Mallory bodies, and hepatitis. On the basis of human and animal work, it has been suggested that this lesion is characteristic of alcohol damage and, more importantly, is a possible indicator of ultimate progression to cirrhosis.²² At present, the accuracy of this suggestion is still being assessed.

The principal component of the fibrous tissue is collagen. To date five types of collagen have been described, differing in small, but important, respects in their amino acid sequence. This produces specific biochemical and biophysical differences. Normally the types of collagen present in the portal tracts and sinusoids are types I and III collagen, but the collagen laid down in alcohol induced liver disease is type III.²³ As the fibrous tissue matures, this is replaced by type I collagen.²³ This variation in collagen type is not exclusive to alcohol induced liver disease, being a general characteristic of all fibrotic responses. Collagen typing can only be done using specific antisera on frozen sections of liver and is not a routine procedure.

The cell of origin of the fibrous tissue of the lobule is a mesenchymal cell found in the perisinusoidal space. It has been given several names—for example, the cell of Ito, the lipocyte, the vitamin A containing lipocyte, the myofibroblast, and perisinusoidal cell.^{23,24} This cell is a facultative fibroblast, apparently having the ability, under appropriate stimuli, to change into a fibroblast and secrete collagen and other components necessary for fibrogenesis. The term perisinusoidal cell is preferred since it leaves open the various functional attributes of this cell type.

CIRRHOSIS

Alcohol induced cirrhosis usually has a micronodular pattern. With prolonged survival, however, particularly after abstinence from alcohol, the pattern changes through a mixed micro- and macronodular one, to a macronodular end stage.²⁵ There are three components necessary for the diagnosis of cirrhosis: namely, fibrosis, hepatocyte nodule formation, often with evidence of regeneration, and distortion of the lobular and vascular architecture, all of which occur diffusely throughout the liver. Any or all of the features described in the preceding sections may be present, thus indicating an alcoholic aetiology. In a few cases of end stage alcoholic liver disease (cirrhosis), however, all of the morphological features suggesting an alcoholic aetiology may have disappeared, especially when alcohol has not been consumed in the recent past. On the other hand Mallory bodies, fat, etc may persist in hepatocytes in a cir-

rhotic liver for many weeks, even when there is reasonable certainty that the patient has not recently consumed alcohol. The reasons for these anomalies are unknown. Since cirrhosis itself has no morphological features specific to alcohol, it will not be described further.

MISCELLANEOUS

Cholestasis

Mild centrilobular cholestasis is occasionally seen in association with alcoholic hepatitis. It presumably reflects considerable severity, but has no other significance. A rare occurrence is the presence of cholestasis with fatty change alone.²⁶ Occasionally, cholestasis may result from alcoholic pancreatitis.

Siderosis

There is great variation in the amount of stainable iron found in the liver. It is said to be common in alcoholic cirrhosis, but in the UK most livers, whether cirrhotic or not, usually show little iron. Occasional cases occur, however, in which there is marked siderosis for no obvious reasons. In our experience, the type of alcoholic beverage consumed does not correlate with the presence or absence of haemosiderosis, and its occurrence does not seem to have any prognostic significance.

In the South Africa Bantu siderosis is common. The reasons for this include high iron content of their diet, genetic predisposition, and increased intestinal absorption of iron.²⁴ The resultant iron overload may play some part in the induction of alcoholic fibrosis and cirrhosis in that country.

α_1 -Antitrypsin accumulation

Accumulation of α_1 -antitrypsin in the cytoplasm of hepatocytes is common in alcoholic liver disease (Fleming KA, McGee J O'D, unpublished observations). Unlike the accumulations seen in true hereditary α_1 -antitrypsin deficiency, however, the affected hepatocytes occur throughout the lobule, rather than the peripheral zone, and the material is often "dust like," rather than in discrete granules (Fig.9). It is difficult to visualise this "dust like" material on diastase-periodic acid Schiff preparations, but it is readily detected by immunohistochemistry. Whether it represents induction of a latent true hereditary α_1 -antitrypsin deficiency or a non-specific accumulation of the protein as a result of general disturbance in hepatocyte metabolism is not clear. Furthermore, these fine accumulations of α_1 -antitrypsin are also found with similar frequency in non-alcoholic liver disease (Fleming KA, McGee J O'D, unpublished observations). Its role in induction of fibrosis or cirrhosis is also unclear.

Fig. 9 α_1 -Antitrypsin in alcoholic liver disease. Diffusely positive hepatocytes are scattered throughout the lobule. Indirect immunoperoxidase with antibody to α_1 -antitrypsin.

Chronic active hepatitis

Occasionally, a picture identical to chronic active hepatitis, with piecemeal necrosis and inflammatory mononuclear cells, is seen in the portal tracts in alcohol induced liver disease. In these cases other features of alcohol damage are present, which allow the diagnosis to be made. Alcohol is thus one of the causes of the histological picture of chronic active hepatitis. Again its prognostic significance is not clear, but in some cases the chronic active component of the disease regresses after alcohol abstinence and without steroid treatment.

Hepatocellular cancer

This is a late complication of cirrhosis. Since alcohol is increasingly a cause of cirrhosis the incidence of liver cell carcinoma resulting from alcoholic cirrhosis is rising. In addition, perhaps due to better treatment and increasing longevity the incidence of hepatocellular cancers in alcoholic cirrhotics is also increasing. About 15% of alcoholic cirrhotics develop liver cell carcinoma and some of these cases also have evidence of hepatitis B infection.

Pathogenesis of chronic alcoholic liver disease

The pathogenesis of cirrhosis can be divided into two steps; these stages may overlap. Firstly, the aetiological agent causes hepatocyte damage and death, which then stimulates a repair process, including inflammation and fibrosis. If the cell death and repair process is of sufficient severity and duration, and is more or less continuous, it results in diffuse fibrosis, hepatocyte nodule formation, and distortion of liver architecture—that is, end stage liver disease (cirrhosis). The second stage of the process now occurs. The fibrosis, nodule formation, and distorted liver architecture produce, by themselves, further hepatocyte damage and death. This in turn stimulates further reparative fibrosis and inflammation, exacerbating the cirrhotic process, which in turn causes further hepatocyte death, and so on. Thus once cirrhosis is established it is self perpetuating, irreversible, and ultimately fatal.

Accordingly the most important problem in the pathogenesis of alcohol induced cirrhosis is to determine how alcohol produces that initial hepatocyte damage which leads to alcoholic hepatitis. We shall concentrate here on those factors and discuss the fibrotic repair process only briefly.

PATHOGENESIS OF ALCOHOL INDUCED HEPATOCYTE INJURY

In view of the morphological features described in the first section, a possible sequence of events in the pathogenesis of alcoholic hepatitis is as follows. Alcohol produces biochemical changes, which result in hepatocyte swelling with granular or web like clumping of cytoplasm, ultimately producing Mallory bodies. Liver-cell death and rupture eventually result. These damaged liver cells provoke an inflammatory response causing the appearance of neutrophil polymorphs and mononuclear inflammatory and mesenchymal cells. Thus the genesis of Mallory bodies may be of fundamental importance in the pathogenesis of alcoholic hepatitis.

Mallory first described the hyaline eosinophilic intracytoplasmic inclusions which bear his name in 1911.²⁸ Electron microscopy shows that they are composed of 12–20 nm diameter filaments and sometimes also contain an electron dense amorphous component. Because of their filamentous appearance it has been tempting to assume that Mallory bodies are derived from one of the cytoskeletal elements of normal hepatocytes, of which there are three—namely, microfilaments, microtubules, and intermediate filaments.²⁹

Microfilaments have a diameter of 4–6 nm and contain actin. It has been reported that Mallory

bodies react with human serum containing antiactin antibodies,³⁰ suggesting that they derive from microfilaments, but others have not confirmed their reaction with antiactin.^{15,31} In view of the latter and the large difference in diameter of microfilaments and Mallory body filaments (4–6 nm v 14–20 nm), it would appear unlikely that Mallory bodies derive from this filament class.

Microtubules have a mean diameter of 22 nm and their main polypeptide subunit is tubulin. Since Mallory bodies can be induced in mice with griseofulvin (an antimicrotubular reagent) it was surmised the Mallory body formation may be related to a defect in microtubular function.³² Disruption of microtubular function may be important in Mallory body formation, but it is unlikely that Mallory body filaments are actually composed of microtubule components, since the physical diameter of both are widely different and, furthermore, Mallory bodies do not contain tubulin.³¹

There is evidence that Mallory bodies are formed from intermediate filaments. Intermediate filaments, although physically indistinguishable in all cell types (mean diameter 10 nm), are biochemically and immunologically heterogeneous.²⁹ Five classes of intermediate filaments have been identified on the basis of their cellular distributions and polypeptide subunit constitutions (Table 2). The proposed major polypeptides of epithelial (including hepatocytes) intermediate filaments are prekeratins; those of mesenchymal cells and muscle cells are vimentin and desmin respectively; while the subunits of neuronal and glial cells are different from each other and also from the other polypeptides mentioned. Although Mallory bodies react with antisera to prekeratins, it is not possible to show the presence of epidermal prekeratin containing intermediate filaments in normal hepatocytes.³³ Furthermore, Mallory bodies contain antigens identified by polyclonal antibodies which do not react with epidermal prekeratin.¹⁵ This would appear to suggest that Mallory bodies are not derived from hepatocyte intermediate filaments. However, we have prepared monoclonal antibodies against Mallory bodies and have shown that at least one of these antibodies (Fig. 4) reacts with Mallory bodies and also with an intermediate filament sys-

tem found in hepatocytes (and other cells) which differs from the other classes of intermediate filaments defined by tissue survey studies.³⁴ The hepatocyte intermediate filament subunit defined by this monoclonal antibody to Mallory bodies is a glycoprotein of 45 000 MW.³⁴ In alcoholic liver disease with pronounced fibrosis those hepatocytes which contain Mallory bodies (and many of those without Mallory bodies) do not contain discernable quantities of this intermediate filament glycoprotein in the adjacent cytoplasm. This suggests that in alcoholic liver disease there is widespread disruption in the organisation, and presumably metabolism, of filaments of this intermediate class. How alcohol might disrupt intermediate filament metabolism or assembly is totally unknown.

The way in which disruption of intermediate filament metabolism results in cell death and consequent inflammation in alcoholic hepatitis is not clear, since the function of these filaments has not been defined. It has been suggested, however, that they are integrators of cytoplasmic space,²⁹ and thus one possibility is that disordered intermediate filament organisation may lead to loss of integrity of hepatocyte structure with subsequent death. More recently, it has been shown that mRNA³⁵ and CAP binding protein³⁶ (which facilitates the translation of mRNA) are spatially associated with intermediate filaments. Thus disruption of intermediate filament "assembly" in alcohol damaged hepatocytes may lead to cell degeneration and death, because the cell can no longer translocate or translate mRNA.

Whatever the mechanism of cell injury, it may provoke an inflammatory response, either directly or indirectly, via immune mechanisms. There is evidence that both mechanisms may apply and that Mallory bodies or intermediate filaments, or both, may be involved. Thus Mallory bodies and intermediate filaments are chemotactic for polymorphs (Morton JA, McGee JO'D, unpublished observations). Mallory body antigen and Mallory body antibody have been detected by one group in sera from patients with alcohol induced liver disease,³⁷ but this has not been confirmed by others.³⁸ Exposure of lymphocytes from patients with alcohol induced liver disease to isolated Mallory bodies results in the lymphocytes becoming activated³⁹ and releasing a fibrogenic factor. In addition, we have shown that HLA class I antigens, which are not detectable in normal hepatocyte membranes, are expressed focally at detectable levels in alcoholic hepatitis³⁴ (the presence of HLA class I antigens on a cell surface is necessary for the cell to be the target of a T cell cytotoxicity response). This therefore allows for the possibility that part of the inflammatory response in alcoholic hepatitis may result from T cell responses

Table 2 Intermediate filaments

Cell type	Intermediate filament protein
Epithelial	Cytokeratins (multiple polypeptides 40 000–68 000 daltons)
Neuronal	Neurofilaments (68 000, 165 000, 200 000 daltons)
Glial	Glial fibrillary acidic protein (55 000 daltons)
Muscle	Desmin (53 000 daltons)
Mesenchymal (fibroblast, endothelium, macrophage, etc)	Vimentin (57 000 daltons)

directed against a hepatocyte component, possibly intermediate filament material.

It is of interest that the Mallory bodies found in some of the other liver conditions mentioned above (see histopathology section) are antigenically similar to Mallory bodies of alcoholic liver disease.⁴⁰ This raises the possibility that Mallory body formation in all these conditions may also result from disruption in intermediate filament metabolism.

Alcohol not only injures the liver but has damaging effects in other organs, many of which contain material (presumably intermediate filaments) which cross reacts with monoclonal antibodies to Mallory bodies.³⁴ In particular, it has long been known that cirrhosis is associated in an unknown manner with renal disease, particularly glomerulonephritis, in which IgA deposition occurs. We have recently identified Mallory body antigen (or material cross reacting with a monoclonal antibody to Mallory bodies) and IgA in glomeruli of some cases of alcoholic liver disease; neither of these substances occurs in normal glomeruli.⁴¹ It is tempting to speculate that this represents immune complex deposition, with its attendant effects in the glomerulus. Similar immune complex deposition may occur in other organs. Alternatively, these extrahepatic lesions may result from an autoimmune reaction in those organs which contain intermediate filament subunits which cross react with those of hepatocytes and Mallory bodies.

PATHOGENESIS OF FIBROSIS

Two pathways have been postulated for the appearance of alcohol induced fibrosis in the liver. The two processes appear to be separate: one postulates that fibrosis can follow fatty change only, while the other states that alcoholic hepatitis must be present and that fatty change by itself is not fibrogenic.⁴²⁻⁴³ The latter mechanism is based on the results of human experience, while the former comes from experimental work on baboons. The baboon model is the only animal one available, and there is some evidence, albeit slight, that some cases of human alcohol induced liver fibrosis can occur in the absence of alcoholic hepatitis.⁴² Therefore, it may be that both processes are relevant to man, but that one is predominant and the other (fatty change) has a supplemental and/or restricted role.

Whatever the mechanism, it is easier to understand how fibrosis follows alcoholic hepatitis, where there is inflammation, rather than fatty change, where inflammation is absent, since it is axiomatic that inflammation, in general, induces fibrosis by mechanisms which are poorly understood. Accordingly, we shall describe briefly what is known of the induction of fibrosis following any inflammatory

process, with emphasis on those features which may be of particular relevance to alcoholic hepatitis (for more detailed consideration of post-inflammatory fibrosis see O'Hare *et al*⁴⁴)

The process of induction of fibrosis by inflammation is complex. Since collagen is the principal component of fibrous tissue, regulation of collagen synthesis and deposition is of paramount importance. Theoretically this regulation can occur at several levels, including gene transcription and translation; post-translational modification; and secretion, deposition, and degradation: there is evidence that regulatory factors apply at all these stages.⁴⁵ The best understood concerns the level of activity of the enzyme prolyl hydroxylase, which is involved in post-translational modification of collagen. This enzyme can be regulated by several factors including lactate (which is increased by alcohol ingestion). This enzyme is increased in alcoholic liver disease and may serve as a marker of active collagen formation.⁴⁶

Within the inflammatory response itself, the predominant cells concerned are lymphocytes, macrophages, and fibroblasts. It is presumed that interaction between these cells leads to stimulation of collagen synthesis with resultant fibrosis, and there is some evidence to support this. This is best shown by exposing sensitised lymphocytes to their target antigen. These produce lymphokines, which stimulate fibroblast collagen production directly and which also activate macrophages to produce factors which then stimulate fibroblast collagen synthesis.⁴⁷ Thus, as mentioned in the previous section, Mallory bodies stimulate lymphocytes from patients with alcoholic liver disease to release fibrogenic factors.³⁹ As another example of liver related fibrogenesis, factors have been extracted from damaged livers of mouse and man which are capable of stimulating collagen synthesis in mouse and human fibroblasts in vitro.⁴⁸ Their derivation and mode of action are unknown.

Another example of the link between collagen synthesis, immune activity, and inflammation is in the *in vitro* demonstration that complexing of C1q, by aggregated IgG, results in increased collagen synthesis by fibroblasts.⁴⁹ Since virtually all inflammatory reactions involve antibody aggregation and binding of C1q, and since alcoholic hepatitis may be associated both with the presence of Mallory body antigen and antibody in the serum and with possible immune complex deposition, this particular mechanism may also apply to the fibrosis associated with alcoholic hepatitis.

In summary, there is evidence for the existence of several different mechanisms of fibrogenesis in inflammation. Some of these mechanisms entail

production of fibrogenic factors by the immune response, which then act directly or indirectly through the medium of the macrophage to stimulate collagen synthesis by fibroblasts or perisinusoidal cells. Since there is evidence for the existence of both T and B cell immune reactions involving Mallory bodies in alcoholic hepatitis, it may be that part of the fibrogenic process in alcohol induced liver disease is related to these reactions.

Mrs Diane Smith typed the manuscript.

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