Section of Clinical Immunology & Allergy

President Deborah Doniach MD

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President's Address

The Concept of an 'Autoallergic' Hepatitis

by Deborah Doniach MD MRCP
(*The Department of Immunology, Middlesex Hospital Medical School, London W1*)

A number of human diseases are now accepted as 'primary' autoimmune disorders; i.e. the immune processes directed against tissue constituents are held responsible for the pathogenesis of the lesions, the word 'primary' implying that no known organism or triggering external agent can be implicated at the present time for the perpetuation of the observed autoallergic phenomena (Table 1). In the organ-specific group, autoimmune thyroid disease is clinically manifest as three separate syndromes: Hashimoto goitre, primary myxœdema and Graves' disease (cf. Doniach & Roitt 1969). Autoimmune gastritis may be 'simple' or proceed to pernicious anæmia if antibodies to intrinsic factor are also present (cf. Roitt & Doniach 1969). Idiopathic adrenal atrophy may present as Addison's disease (Anderson *et al.* 1968) or be accompanied by ovarian failure according to the antigens involved (Irvine *et al.* 1969). All these conditions are characterized by serum antibodies directed specifically against the affected organ and lesions can be obtained in animals by injection of organ extracts with Freund's adjuvant. In the autoimmune hæmocytopenias antibodies are specific and pathogenetic in themselves. In the non-organ-specific group of disorders, e.g. systemic lupus erythematosus and allied connective tissue disorders, the immune phenomena are also held responsible for the lesions although these cannot be easily produced by autoimmunization in animals. All these disorders in man probably have a significant genetic component and in animals this has been clearly accentuated by inbreeding (Bielschowsky *et al.* 1959). The ultimate defect appears to reside in the immune responses and the cause is unknown.

Table 1
Features of 'primary' autoimmune diseases

(1) No known cause or precipitating factors
(2) Female preponderance
(3) Protracted course, exacerbations and remissions; not always progressive
(4) Appropriate serum antibodies
(5) Histological evidence of immune hyperactivity
(6) Familial aggregation
(7) Association with other autoimmune conditions
(8) Animal models
(9) Autoimmunity to an organ can result in more than one clinical syndrome

In the case of hepatic disease, the concept of a primary autoallergic disorder is more difficult to uphold. No liver-specific antibodies have yet been demonstrated and immunization of animals with liver extracts has not given rise to a convincing form of hepatitis or to progressive cirrhosis (Paronetto & Popper 1969). In spite of these objections, increasing evidence is accumulating that three chronic liver syndromes, i.e. primary biliary cirrhosis (PBC), active chronic hepatitis (ACH, 'lupoid' hepatitis, 'juvenile cirrhosis') and certain cases of cryptogenic cirrhosis, particularly in women, represent expressions of an underlying disease process closely associated with autoimmune phenomena and which can in many ways be compared with the other more easily accepted autoallergic disorders (Doniach & Walker 1969).

The clinical and histological features of primary biliary, 'juvenile' and cryptogenic cirrhosis have been extensively described. In primary biliary cirrhosis the characteristic lesion is a necrosis of bile ducts (Baggenstoss *et al.* 1964, Rubin *et al.* 1965) while in active chronic hepatitis ('aggressive' hepatitis) (Read *et al.* 1963, de Groote *et al.* 1968) the brunt of the tissue destruction falls on hepato-

Table 2
Histological overlap between hepatitis and primary biliary cirrhosis

	Hepatitis – 83 cases including viral and ACH (Poulsen & Christoffersen 1969)	*Primary biliary cirrhosis – 31 cases (Hadziyannis et al. 1970)*
Bile duct lesions	17%	81%
'Piece-meal' necrosis of hepatocytes	29%	90%

cytes. However, it is becoming increasingly recognized that overlap of these distinct syndromes occurs not infrequently. It is known that both primary biliary disease and active chronic hepatitis may progress to an advanced cirrhosis in which the histology is indistinguishable from 'cryptogenic' cirrhosis (Scheuer 1967, Mistilis 1968). Cholestatic forms of juvenile and of cryptogenic cirrhosis (Datta *et al.* 1963) are also well documented while typical primary biliary cirrhosis can be associated with all the histological features of aggressive hepatitis (Hadziyannis *et al.* 1969) or present clinically as cirrhosis with portal hypertension (Zeegen *et al.* 1969). Furthermore, the bile duct cell necrosis typical of PBC has now been reported in some cases of ACH (Poulsen & Christoffersen 1969) (Table 2). The close relationship between these three hepatic syndromes has become further clarified by the application of the immunofluorescence technique to the study of liver diseases. A number of serum antibodies giving different fluorescence patterns have proved to be useful markers. The most important of these include antinuclear (ANA) (Bouchier *et al.* 1964, Doniach *et al.* 1966) smooth muscle (Johnson *et al.* 1965) and mitochondrial antibodies (Walker *et al.* 1965, Goudie *et al.* 1966, Whittingham *et al.* 1966, Kantor & Klatskin 1967, Paronetto *et al.* 1967), but bile ductular (Paronetto *et al.* 1964), bile canalicular (Johnson *et al.* 1966) and renal glomerular (Whittingham *et al.* 1966) immunofluorescence patterns have also been described. The latter three reactions have not been fully evaluated in this context but the other antibodies are well characterized, and are demonstrable regularly, persistently and in high titres only in the three syndromes discussed. Extensive studies in other forms of hepatitis and jaundice, and in the cirrhoses of known etiology have produced almost uniformly negative results in different parts of the world (Doniach *et al.* 1968). Cryptogenic cirrhosis undoubtedly represents a heterogeneous group of conditions resulting from a number of unrelated liver injuries and is numerically much more frequently encountered than either juvenile or primary biliary cirrhosis, the estimated relative frequencies being of the order of 100:10:1 respectively. Nevertheless a proportion of patients with unexplained cirrhosis show strong evidence of autoimmunity along with antibodies of similar range and titre to those found in the other two conditions. Nuclear antibodies of 'diffuse,' 'speckled' and 'nucleolar' varieties are detected in 75% of juvenile cirrhosis cases, in 46% of primary biliary cirrhosis patients and in 38% of patients with cryptogenic cirrhosis (Doniach *et al.* 1966). Smooth muscle antibodies are particularly prominent in 'lupoid' hepatitis where LE-cells are demonstrable (Whittingham *et al.* 1966) but are also found in 30–50% of cryptogenic and of biliary cirrhosis cases (Doniach *et al.* 1966). So far the most diagnostically useful has proved to be the mitochondrial fluorescence test. This non-organ- and non-species-specific antibody is directed against a lipoprotein constituent of the mitochrondrial inner membranes (Berg *et al.* 1967, Berg, Muscaletto, Horne, Roitt & Doniach 1969, Berg, Roitt, Doniach & Horne 1969, Berg, Roitt, Doniach & Cooper 1969) and occurs in 79–94% of patients with primary biliary cirrhosis and in only 0–5% of cases of main bile duct obstruction (Table 3). The small variations in incidence reported by different laboratories can be accounted for by differences in the techniques used. Tests done on 1:10 dilutions of the patient's serum using specific anti-IgG conjugates are the most discriminating (Kantor & Klatskin 1967) but will fail to detect about 10% of primary biliary cirrhosis cases. Since this antibody is found only infrequently and in low titres in other noncirrhotic forms of cholestasis, e.g. virus hepatitis (3%), cholestatic drug jaundice, pericholangitis associated with colitis and benign recurrent cholestatis, a strongly positive reaction is of great help in distinguishing primary biliary cirrhosis from these disorders which may closely simulate it (Walker *et al.* 1967). In patients with 'aggressive' hepatitis and cryptogenic cirrhosis the reported incidence of mitochondrial antibodies has varied in different laboratories probably owing to the widely different criteria employed in the classification of case material. The 'post-necrotic' histological picture is known to result from a number of causes and in some series (Goudie *et al.* 1966, Kantor & Klatskin 1967) a low incidence of mitochondrial antibodies can be

Table 3
Incidence of mitochondrial antibodies in liver diseases

Diagnosis	*No. tested*	*Percentage positive*
Primary biliary cirrhosis	139	93
Active chronic hepatitis	102	24
Cryptogenic cirrhosis	84	26
Extrahepatic biliary obstruction	81	5
Viral hepatitis	79	4
Alcoholic cirrhosis	34	0
Hæmochromatosis	26	0
Chlorpromazine jaundice	17	(1/17)
Halothane jaundice	3	(2/3)
Hyperbilirubinæmias	29	(1/29)

attributed to the inclusion of a high proportion of cases of alcoholic etiology since there is a notable absence of antibodies in alcoholic cirrhosis. In one series (Doniach *et al.* 1966), about 50% of females and 30% of males classified as cryptogenic cirrhosis had high titres of autoantibodies. Active chronic hepatitis cases showed an incidence of about 25% mitochondrial fluorescence.

In primary biliary cirrhosis the level of mitochondrial antibodies in the serum varied from trace amounts to titres of 1:6,000 in the fluorescent test. The antibodies are present in the three main immunoglobulin classes as shown by tests carried out with specific antisera; anti-IgG, anti-IgM and anti-β_{1c} usually react to the same titre, but with anti-IgA conjugates lower titres were obtained. Parallel results are seen in the conventional complement-fixation test (CFT) using rat liver mitochondria as antigen, although this is less sensitive. No antibodies could be recovered in the bile even when serum titres were high. In patients followed over several years, antibody levels usually remained constant on repeated testing and bore no relationship to the duration or stage of the disease. Neither did they show any decrease after treatment with corticosteroids or azathioprine. About 10% of cases had low mitochondrial fluorescence of less than 1:16 with a negative CFT and a small percentage failed to react in either test (Doniach *et al.* 1968). Clinically and histologically the negative reactors could not be distinguished from those with high titres (Hadziyannis *et al.* 1969). However, at least half the patients with negative mitochondrial fluorescence had high levels of antinuclear antibodies, suggesting that they belong to the same autoimmune spectrum. Primary biliary cirrhosis patients frequently show a raised serum IgM level (Hobbs 1967, Feizi 1968) but this could not be correlated with the mitochondrial or nuclear fluorescence titre in individual cases (Walker & Doniach 1968).

Mitochondrial ('M') fluorescence is extremely uncommon in healthy subjects and is detected in less than 1% of mixed hospital patients excluding those with overt liver disease (Table 4). Most of the positive reactors suffered from collagen disorders or other autoimmune diseases and a proportion gave false positive (BFP) reactions for syphilis (Doniach *et al.* 1970). Of 35 'M' positive patients studied in detail (Walker *et al.* 1970), 10 showed raised serum alkaline phosphatase values and impaired bromsulphalein retention in the absence of significant changes in serum bilirubin or transaminase levels. Liver biopsies in 8 of the patients showed mononuclear cell infiltration in the portal tracts and spotty necrosis within the liver lobules in every instance. Several biopsies also showed bile duct proliferation, piecemeal necrosis (Popper 1966) and increased reticulin formation, suggestive of an aggressive hepatitis. Three have since developed more obvious features of clinical liver disease with hepatomegaly and pruritus 2–4 years after initial detection of the antibodies. On the other hand, 25 patients with positive mitochondrial tests have now been followed for periods of up to 8 years without developing any abnormality of the biochemical parameters and it is not known in the absence of biopsy material whether any of them have mild hepatitis lesions by analogy with the focal thyroiditis found in association with thyroid antibodies in symptomless relatives of Hashimoto patients. Family studies in primary biliary cirrhosis have shown an incidence of mitochondrial fluorescence in mothers and female sibs of 7%, i.e. about 10 times the normal incidence, and in two families out of 27 studied there were 2 members suffering from cirrhosis (Doniach *et al.* in preparation). This parallels the findings in familial studies of Hashimoto's disease and pernicious anæmia.

Table 4
Incidence of mitochondrial antibodies in various nonhepatic disorders

Diagnosis	*No. tested*	*Percentage positive*
Lupus erythematosus	59	7
Other 'collagen' disorders	153	8
Rheumatoid arthritis	331	1·5
Pernicious anæmia	142	0·7
Autoimmune thyroiditis	296	0·7
Graves' disease	183	2
Addison's disease	29	3
Myasthenia gravis	17	0
Colloid goitre	91	0
Other diseases	746	0·8

In liver disease the tissue antibodies so far detected are probably not directly responsible for the necrosis of hepatocytes or bile duct cells but represent markers of a continuing autoimmunization. In the spectrum of autoimmune liver disease, it is envisaged that the lesion may evolve towards either juvenile, primary biliary or cryptogenic cirrhosis depending on the relative degree of involvement of these two cell types as targets of the process (Fig 1). The role, if any, of the tissue antibodies in the pathogenesis of the lesions is difficult to evaluate particularly in view of their lack of organ specificity. The same considerations apply to any postulated cell-mediated immune mechanisms. However, the possibility of a reaction involving liver specific cell surface antigens cannot be excluded and warrants further study with sensitive new methods. Evidence of a more circumstantial nature for the existence of an autoallergic hepatitis are the

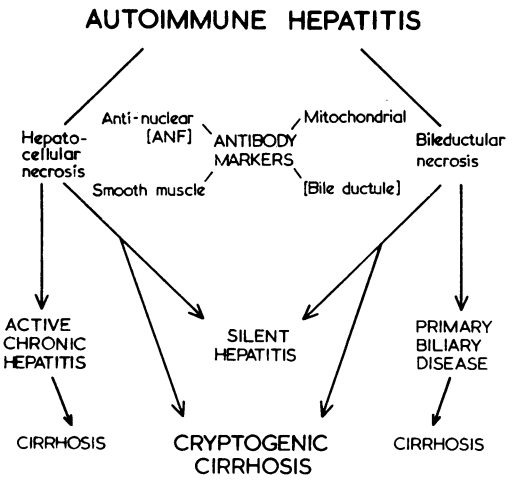

Fig 1 *Autoimmune hepatitis. (Reproduced from Doniach & Walker 1969 by kind permission)*

strong female preponderance particularly in juvenile and primary biliary cirrhosis, the variable clinical course with exacerbations and remissions and the frequent clinical coexistence in the same patient, particularly in juvenile cirrhosis, of thyroiditis, Sjögren's syndrome, systemic lupus erythematosus and autoimmune hæmolytic anæmia (Read *et al.* 1963, Mackay *et al.* 1965).

The etiological role of the hepatitis virus(es) in the initiation of active chronic hepatitis is still under discussion. It is likely that a prolonged or relapsing virus infection may cause a histological lesion indistinguishable from that of the hypothetical autoimmune disease, leading to irreversible cirrhosis (Gitnick *et al.* 1969) without autoimmunity, and this may account for cases showing no tissue antibodies.

Studies with the Australia or SH antigen (London, Sutnick & Blumberg 1969, Prince 1968) in juvenile cirrhosis (Wright *et al.* 1969, Fox *et al.* 1969, Gitnick *et al.* 1969) (Table 5) in parallel with tissue antibody estimations will help to clarify the problem. This antigen is related to one of the hepatitis viruses (Giles *et al.* 1969, London, DiFiglia, Sutnick & Blumberg 1969, Hirschman *et al.* 1969) and is found in the serum during the prodromal stages of acute hepatitis, but also in persistent or relapsing cases and in silent carriers and blood donors (Okochi & Murakami 1968).

The opposite approach, i.e. the search for tissue antibodies in cases of known bacterial or viral infections of the liver, has shown that the pericholangitis associated with ulcerative colitis (Mistilis 1965), which may simulate primary biliary cirrhosis, does not give rise to autoantibodies while in classical viral hepatitis these markers appear only occasionally and as a temporary phenomenon (Walker *et al.* 1967). In some cases of jaundices due to halothane or chlorpromazine the sensitized individuals develop mitochondrial fluorescence (Doniach *et al.* 1966, Rodriguez *et al.* 1969) but this is of low titre and disappears upon clinical recovery. In the cirrhoses due to known metabolic errors, i.e. Wilson's disease and hæmochromatosis, or extrinisic factors such as alcoholism, and in secondary biliary cirrhosis due to atresia or main bile duct obstruction, no tissue antibodies have been found. Negative results have also been obtained in a group of children with Indian infantile cirrhosis (Chandra 1969, personal communication).

It is clear therefore that release of liver components by itself would not account for the sustained autoimmunization seen in certain patients. The earlier theory that tissue antibodies in liver disease result from release of antigenic material consequent upon injury, whatever its cause, was based upon work with the complement-fixation test using crude tissue extracts, and on animal experiments. Certain laboratory animals, in particular the rat and rabbit, regularly have complement-fixing antibodies almost from birth (Elson & Weir 1969). These antibodies can be further boosted by chemically-induced liver injury or by injection of tissue homogenates (Pinckard & Weir 1966). They are non-organ- and non-species-specific, usually of IgM type, labile on storage, and fail to react in the immunofluorescence test with tissue sections. The human appears to behave differently in this respect in that the CFT with tissue extracts is obtained in only 3% of normal individuals and in low titres (Doniach *et al.* 1966). It is, however, possible that the CFT reactions seem in a proportion of patients with infective hepatitis or toxic liver injury, e.g. due to alcohol, drugs, &c., represent a type of antibody response comparable to that seen

Table 5
Hepatitis virus (Au/SH) antigen in chronic liver disease

	Clinical diagnosis		
	Active chronic hepatitis *No. +ve/ No. tested*	*'Cryptogenic' cirrhosis* *No. +ve/ No. tested*	*Primary biliary cirrhosis* *No. +ve/ No. tested*
Gocke & Kavey (1969)	1/6	0/10	
Wright *et al.* (1969)	6/24	1/26	0/44
Gitnick *et al.* (1969)	3/31	(2/17)●	
Fox *et al.* (1969)	0/32	1/49	0/39
Total	10/93 (9%)	2/85 (2·5%)	0/83

● These 17 cases were included in the 31 cases with ACH but had reached the stage of cirrhosis histologically

in the animals. High and persistent titres of CF antibodies are uncommon in most liver disorders and are found with regularity only in the three particular syndromes which we now postulate represent the spectrum of 'autoimmune hepatitis'. The CFT reactions described earlier in these liver diseases and in the collagenoses (Mackay & Gajdusek 1958, Asherson 1959, Deicher *et al.* 1960, Pasnick *et al.* 1962, Wiedermann & Miescher 1965) were due in part to the mitochondrial antibodies now detected by immunofluorescence, in part to nuclear antibodies, and in part to antibodies directed against phosphate-soluble components of the cell cytoplasm (Deicher *et al.* 1960). It is further possible that certain reactions resulted from nonimmunological protein interactions (Beall 1963).

Although new methods are obviously required to provide further evidence that autoallergy may be one of the causes of hepatic cirrhosis, it is evident that the immunofluorescence technique has done much to segregate a small number of liver syndromes as candidates for this concept.

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