PAPERS AND ORIGINALS

Cell-mediated Immune Responses in Chronic Liver Diseases

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Summary

Studies of the cell-mediated response to liver antigens, using the leucocyte migration test, in 163 patients with various liver disorders showed that abnormal responses were almost confined to active chronic hepatitis (53% abnormal), primary biliary cirrhosis (64%), and cryptogenic cirrhosis (29%). The test was also abnormal in five out of seven patients with jaundice due to drug hypersensitivity and in one patient with acute infectious hepatitis at a time when mitochondrial antibodies were present in the serum. More of those with active chronic hepatitis on prednisone or azathioprine had normal tests than of those who were untreated, and in 8 out of 10 examined serially during therapy there was an accompanying improvement in leucocyte migration. Abnormal responses to salivary gland or kidney antigens were also found in nearly half of those with features of Sjögren's syndrome or renal tubular acidosis as part of a multisystem involvement—this, though occurring in cryptogenic cirrhosis, was found with greater frequency in active chronic hepatitis and primary biliary cirrhosis. These cell-mediated immune responses, perhaps triggered by the initial damage to the liver from viral or other agents, may be responsible both for the perpetuation of the liver disease and, because of common surface antigens, for the damage to other organs.

Introduction

The pathogenesis of active chronic hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis remains controversial despite accumulating evidence for an autoimmune basis. The circulating autoantibodies to smooth muscle, nuclei, and mitochondria which are so often present in the serum are not organ-specific and cannot be correlated with the activity of the underlying liver disease or its duration (Doniach *et al.*, 1966). So far little attention has been paid to the role of cell-mediated immune processes in the pathogenesis of the liver damage. Whatever the mechanism involved, it must also satisfactorily account for the high frequency with which other organs are involved, recent studies showing the presence of Sjögren's syndrome or renal tubular acidosis in up to 40% of these patients (Golding *et al.*, 1970; Golding and Mason, 1971).

Using the leucocyte migration test as a measure of cell-mediated immune response (Søborg and Bendixen, 1967; Mookerjee *et al.*, 1969; Rosenberg and David, 1970; Federlin *et al.*, 1971), we have now examined 100 patients with these disorders as well as 63 patients with various other chronic and acute liver conditions. The relation of the abnormal responses found with liver antigen to liver function tests and to therapy with prednisone or azathioprine was investigated, and in 66 patients found to have either Sjögren's syndrome or renal tubular acidosis the response to antigens from salivary gland and kidney was also determined.

Patients and Methods

The group of 100 patients with possible autoimmune liver disease consisted of 58 patients with active chronic hepatitis, 28 with primary biliary cirrhosis, and 14 with cryptogenic cirrhosis. In addition, 28 patients with cirrhosis of known aetiology (alcoholic 19, haemochromatosis 9) were investigated. The group with acute liver disease was made up of 15 patients with acute infectious or serum hepatitis, 8 with drug jaundice, and 12 with jaundice due to extrahepatic biliary obstruction. Most of those with cirrhosis were systematically examined for evidence of Sjögren's syndrome and renal tubular acidosis by standard methods (Golding *et al.*, 1970; Golding and Mason, 1971).

LEUCOCYTE MIGRATION TEST

Our modification of the method of Søborg and Bendixen is described in detail elsewhere (Smith *et al.*, 1969). Leucocytes

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harvested from heparinized venous blood were allowed to migrate from capillary tubes into chambers containing tissue culture medium 199 with 10% horse serum. After 20 hours' incubation at 37°C the areas of migration were measured, and an index was calculated by dividing the mean of three to six measurements in the chambers to which the antigen had been added by the mean of a similar number of measurements for the control chambers. A migration index of less than one indicates inhibition of migration and greater than one stimulation. The latter is found with weak sensitization to the antigen, whereas inhibition of migration is indicative of strong sensitization (Søborg, 1967). To identify a transition value which could thus fall within the normal range, it is necessary in addition to measure migration against a lower concentration of antigen. An abnormal response—namely, stimulation of migration—will be then found, and in the present studies migration was always determined with 200 μg as well as with the standard concentration of 400 μg of protein per ml of tissue culture medium.

The antigens were obtained initially from freshly pooled human fetal tissue and more recently from necropsies of two adult patients carried out within 40 minutes of death, the liver, salivary gland, and kidney tissue removed being immediately frozen in liquid nitrogen. The pooled 30% homogenates in medium 199 were then prepared and centrifuged at 2,000 *g* for 15 minutes, the supernatants being transferred in 0·1-ml aliquots to sterile containers and stored at −70°C until use.

The measurements were carried out without knowledge of the patients' diagnoses and some of the control subjects were tested at regular intervals so as to detect any alteration of the antigen with storage which could produce non-specific inhibition of migration. This was never found.

Results

An analysis of 100 determinations of migration index selected at random gave a mean coefficient of variation of 10% (range 1-17%) for the three to six measurements in each result. Variation between observers was also acceptably low, and in 50 tests, each measured independently by three experienced observers, the mean coefficient of variation was 3·8% (range 0-7·6%). Studies on a control series of 22 normal individuals and 20 hospital inpatients with unrelated disease showed that responses to liver, kidney, and salivary antigens were very similar, with mean values of 0·93, 0·94, and 0·94 respectively. The results for all three antigens were therefore used in the calculation of a normal range, the upper and lower limits (mean ± 2 S.D.) of which were 1·10 and 0·78.

RESPONSE TO LIVER ANTIGEN

In chronic liver disease significant stimulation or inhibition of migration was found, with few exceptions, only in active chronic hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis. More than half the patients in the first two groups had abnormal results (Table I). Of those with acute liver disease, one patient with infectious hepatitis showed a markedly abnormal response, with inhibition of migration on three occasions. Low titres of mitochondrial antibody were present in the serum but the course of the hepatitis was not in any way atypical, and with recovery all detectable immunological abnormalities disappeared. The frequency with which abnormal results were encountered in the eight patients with drug jaundice was striking. Two of the three patients with halothane hepatitis, both of those with jaundice attributed to methyldopa, one of the two with hepatic necrosis due to amine oxidase inhibitors, and the one patient with paracetamol as the cause had abnormal tests.

TABLE I—*Results of Leucocyte Migration Tests with Liver Antigen*

	Leucocyte Migration Test			
	Normal (No.)	Stimulation (No.)	Inhibition (No.)	Abnormal
Active chronic hepatitis	27	8	23	53%
Primary biliary cirrhosis	10	5	13	64%
Cryptogenic cirrhosis	10	2	2	29%
Other types of cirrhosis	25	2	1	11%
Drug jaundice	2	4	2	75%
Acute infectious or serum hepatitis	13	1	1	13%
Extrahepatic biliary obstruction	12	0	0	—

Relation to Treatment and Liver Function in Active Chronic Hepatitis.—Thirty-seven of the 58 patients with active chronic hepatitis were on azathioprine or prednisone, and a greater number of these (60%) had migration indices within the normal range than of those who were untreated (40%). The difference was not statistically significant ($\chi^2=1·69$, $P>0·1$). However, in eight of the ten selected patients with abnormal responses to liver antigen in whom a second reading was obtained from a few weeks to three months after starting therapy there was a coincident improvement in migration index, which had returned to normal in all but one of these cases (Fig. 1), and in four this

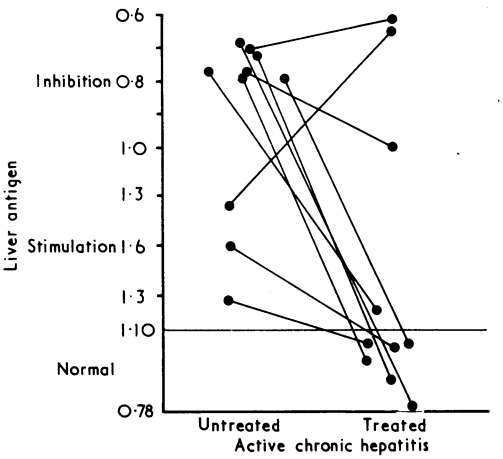

FIG. 1—Serial measurements of migration index in 10 patients before and during prednisone or azathioprine therapy. The values for the migration index are plotted on a diagrammatic scale representing increasing in-vivo sensitization derived as described in text.

was accompanied by a reduction in both plasma bilirubin and serum aspartate aminotransferase levels. Furthermore, among the group as a whole, although the migration results could not be correlated with any one liver function test, those patients with normal plasma bilirubin and serum aspartate aminotransferase levels were significantly more likely to have a normal migration test ($\chi^2=3·87$, $P<0·05$). Among those with abnormal responses no correlation was found between degree of sensitization detected and abnormality of liver function.

FINDINGS WITH SALIVARY GLAND AND KIDNEY ANTIGEN

The frequency of Sjögren's syndrome was highest in primary biliary cirrhosis, with 53% affected, and lowest in cryptogenic cirrhosis (Table II). The abnormalities found were largely confined to the lachrymal and salivary components, only three of the affected patients having rheumatoid arthritis. Sjögren's syndrome was not found in the 28 patients with alcoholic cirrhosis or haemochromatosis examined.

Leucocyte migration against salivary gland antigen was determined in 26 of the 38 patients found to have Sjögren's syndrome (Table II). Abnormal results, with stimulation or inhibition of migration, were obtained in 11 (42%), the frequency being little different in the various diagnostic groups. In 35 patients with these diseases but without Sjögren's syndrome, only one positive test was found, a highly significant difference ($\chi^2=11·9$, $P<0·001$).

TABLE II—*Number of Patients Found to have Sjögren's Syndrome and Renal Tubular Acidosis in the Various Types of Cirrhosis together with the Results of the Leucocyte Migration Tests, Carried out with Salivary Gland and Kidney Antigen Respectively, in some of those Found to be Affected*

	Sjögren's Syndrome					Renal Tubular Acidosis				
	Total Examined	Total Affected	Leucocyte Migration Test			Total Examined	Total Affected	Leucocyte Migration Test		
			No. Tested	Stimulation	Inhibition			No. Tested	Stimulation	Inhibition
Active chronic hepatitis ..	58	20 (35%)	12	2	3	45	12 (27%)	9	1	4
Primary biliary cirrhosis	28	15 (53%)	12	3	2	27	14 (52%)	11	4	3
Cryptogenic cirrhosis ..	14	3 (21%)	2	1	0	14	2 (14%)	1	1	0

Similarly, abnormal leucocyte response to kidney antigen was found to be closely correlated with the presence of renal tubular acidosis (Table II). Of the 21 cases examined, 13 (62%) showed stimulation or inhibition of migration, whereas this was found in only 2 of the 34 patients with active chronic hepatitis, primary biliary cirrhosis, or cryptogenic cirrhosis but without a demonstrable renal lesion ($\chi^2 = 18{\cdot}5$, $P < 0{\cdot}001$).

To determine whether the responses to kidney or salivary antigens were a reflection of cross-antigenicity with the liver antigen, the results were analysed further in those patients in whom the tests with all three antigens were carried out on the same occasion. The result of comparing the responses to kidney and liver antigens in 49 patients is shown in Fig. 2. In 29 (59%) of these there was agreement, both antigens producing either

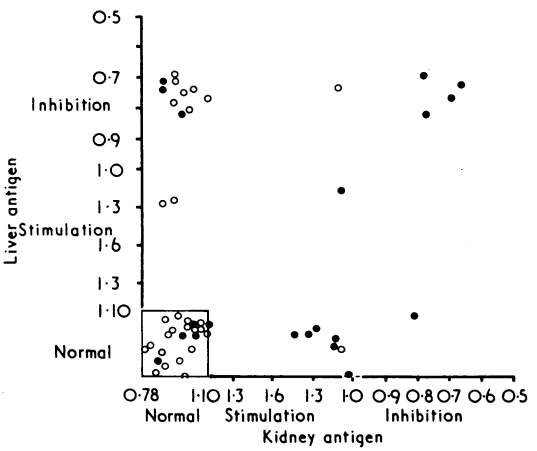

FIG. 2—Comparison of results obtained with liver antigen and those found with kidney antigen in the same patients, values for the migration indices being shown in the same way as in Fig. 1. Closed circles signify those patients with renal tubular acidosis.

a normal or both an abnormal response. In the remaining 20 patients the response was different in kind, 12 patients reacting abnormally to liver antigen alone and eight to kidney alone. However, if patients whose cells gave a normal response to both liver and kidney antigen were excluded, only in six (23%) of the remainder was there an abnormal response to both antigens. The findings were very similar when the salivary antigen results were compared with the liver antigen. In 31 (55%) of 57 patients, there was a like response to both antigens, but among the other patients 20 reacted to liver antigen only and six to salivary antigen only. After exclusion of patients with no abnormal response to either antigen, only four (15%) of the remainder reacted abnormally to both antigens.

Discussion

The cellular hypersensitivity to liver antigens which we have demonstrated in active chronic hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis is further evidence in favour of an abnormal immune system in these conditions. Some of our findings are also consistent with the view that cell-mediated responses are directly concerned with the production of liver damage. The improvement of migration index in patients with active chronic hepatitis after prednisone or azathioprine fits the known beneficial effects of such treatment on the mortality of this disease (Cook *et al.*, 1971). Furthermore, there was some correlation with liver function when plasma bilirubin and serum aspartate aminotransferase were considered together. Unfortunately the assessment of disease activity in this condition is particularly difficult and even liver biopsy can be misleading, for histological appearances vary markedly from one area of the liver to another.

The apparently organ-specific nature of the responses detected to liver antigen, abnormal results with kidney or salivary gland antigens being found only with clinical involvement of these organs, is further supporting evidence. In idiopathic Addison's disease, Nerup *et al.* (1970) showed that inhibition of migration is demonstrable only with mitochondria from adrenal tissue. Although mitochondria are present in our antigen extracts, they may be of little importance, for Meyer zum Büschenfelde and Kössling (1971) induced chronic aggressive hepatitis in rabbits by immunization with extracts of human liver containing a liver-specific lipoprotein not of mitochondrial origin. This protein, also present in our extracts, is thought to be sited in the surface membrane of the hepatocyte. This is of considerable interest in relation to allograft rejection, in which the immune response is primarily directed against transplantation antigens on cell surfaces. In patients with a liver transplant we have found significant inhibition of migration to donor histocompatibility antigens during rejection episodes (Eddleston *et al.*, 1971).

Hypersensitivity to liver antigen was also found in primary biliary cirrhosis, which is in keeping with recent studies showing a distinct clinical and histological overlap between this condition and active chronic hepatitis (Hadiziyannis *et al.*, 1970). Nevertheless, attempts to produce primary biliary cirrhosis experimentally by injection of liver extracts have been unconvincing. However, the immune attack may be primarily directed against different antigenic sites—for example, specific bile duct antigens in primary biliary cirrhosis and hepatocyte surface antigens in active chronic hepatitis.

The suggestion that some cases of cryptogenic cirrhosis represent the natural evolution of active chronic hepatitis (Doniach, 1970) receives further support from our finding of abnormal leucocyte migration tests in a significant proportion of those with cryptogenic cirrhosis. The mechanism by which acute hepatitis can occasionally initiate active chronic hepatitis is uncertain and in this context the transient appearance of autoimmune phenomena, both humoral and cellular, in one of the patients with acute infectious hepatitis may be relevant. The abnormal leucocyte migration tests in drug jaundice were also of interest, particularly since hypersensitivity reactions have been implicated on other grounds.

Hans Popper (1971) suggested that the development of multisystem involvement, particularly Sjögren's syndrome and renal tubular acidosis, is due to the deposition of circulating antigen-antibody complexes. If so, why are the renal tubules affected rather than glomeruli as in other forms of immune complex disease? A specific interaction between antigen and antibody may be implicated as the mitochondrial antibodies, found most commonly in primary biliary cirrhosis but also in active chronic hepatitis and cryptogenic cirrhosis, can be shown by an immunofluorescent technique to react principally with the distal tubules of the kidney. This antibody also reacts strongly

with preparations of salivary ducts. Nevertheless, we were unable to find any correlation between the presence of mitochondrial antibodies in the serum and renal or salivary gland involvement. The finding that abnormal responses to kidney and salivary gland antigens were almost confined to patients with clinical involvement of these organs could indicate that cell-mediated mechanisms are more important in their pathogenesis. Furthermore, in certain cases in which biopsy material was available lymphocytic infiltration in the parotid glands and in the kidney was found, similar to that present in the liver.

The presence of cross-reacting antigens in the various tissues could also explain the development of multisystem disease. An initial alteration or release of antigens from the liver after viral or other damage could lead, if the central immune system of that individual is abnormal, to a cell-mediated response involving, in addition to the liver, other organs containing antigens of similar structure. This is supported by recent work of Farrow *et al.* (1971) showing receptors on the chick embryo liver cell which react with an antibody to smooth muscle. However, a few of our patients reacted abnormally only to antigens from salivary gland or kidney, suggesting an organ-specific response, though in almost all of these the test showed stimulation of migration, indicating that the sensitization was slight. To study this problem further it will be necessary to solve the difficult technical problems involved in the isolation and purification of organ-specific antigens.

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Prevalence of Farmer's Lung in Scotland: A Pilot Survey

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Summary

In a survey of the farming population of Orkney, Ayrshire, and East Lothian the prevalence of farmer's lung was estimated at 86 per 1,000 in both Orkney and Ayrshire and 23 per 1,000 in East Lothian. If cases with a negative farmer's lung hay (F.L.H.) precipitin test are excluded these figures are reduced to 43, 36, and nil respectively, but those for Orkney and Ayrshire are still about 20 times higher than any figure previously reported for the prevalence of farmer's lung in Britain.

Regional variations in prevalence are probably related both to climatic conditions and to differences in agricultural methods, the latter often being dictated by economic circumstances. Nevertheless the prevalence of farmer's lung could be reduced considerably by the energetic application of preventive measures, backed by financial incentives. The most important of these are efficient drying of hay and cereals before storage, more extensive use of silage, better ventilation of farm buildings, and the introduction of mechanical feeding systems. Individual farmworkers could be taught how to recognize the early symptoms of the disease and encouraged to wear respirators when handling mouldy fodder.

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Introduction

The disease now called farmer's lung has apparently been known to farmers for over 100 years. Bjornsson (1960) mentioned that his grandfather, an Icelandic farmer born in 1855, suffered from it as a young man, and referred to the disease as "heymaedi" meaning "hay shortness of breath." It was, however, not recognized as a clinical entity until Campbell (1932) published his original account of "acute symptoms following work with hay" affecting farmworkers in Westmorland. The 1931 hay crop had been harvested under unusually wet conditions, and had become very mouldy during storage. When the last part of the stored crop was being fed to cattle between April and June 1932 several farmworkers developed severe dyspnoea, cyanosis, slight dry cough, and mild pyrexia. They were found to have diffuse dry rales and sibilant rhonchi on auscultation and "fine granular stippling" throughout both lung fields on *x*-ray examination.

The term "farmer's lung" was first used by Pickles (1944) when he reported a similar case in Yorkshire, but the nature of the disease remained obscure until Pepys *et al.* (1962) found that precipitins against extracts of mouldy hay ("F.L.H. antigens") were present in sera from patients with farmer's lung. Pepys *et al.* (1963) identified *Thermopolyspora polyspora* (now renamed *Micropolyspora faeni*) as the main source of F.L.H. antigen, and Williams (1963) showed that the inhalation of either F.L.H. antigen or an extract of *M. faeni* reproduced the symp-