PAPERS AND ORIGINALS

Cellular and Humoral Immunity to Hepatitis-B Surface Antigen in Active Chronic Hepatitis

W. M. LEE, W. D. REED, C. G. MITCHELL, R. M. GALBRAITH, A. L. W. F. EDDLESTON, A. J. ZUCKERMAN, ROGER WILLIAMS

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Summary

The hepatitis-B surface antigen (HBsAg) may be persistently present in the serum in a few cases of active chronic hepatitis but the cause of the disease in most patients is unknown. In a study of 39 HBsAg-negative cases cell-mediated immunity to HBsAg was observed in 24 (62%), suggesting a high frequency of previous infection with the hepatitis-B virus. Hepatitis-B surface antibody was detectable by radioimmunoassay in six patients, in all of whom complexes of HBsAg were present in the serum on electron microscopy. Out of 12 patients with HBsAg-positive active chronic hepatitis who were also studied eight, including all those untreated at the time, showed a cellular response to the antigen. Evidence of sensitization to a liver-specific cell surface lipoprotein was found with similar frequency in the two groups.

These results are consistent with the hypothesis that hepatitis-B virus infection is important in initiating the disease in many cases of active chronic hepatitis and that sensitization to the liver cell membrane antigen is the autoimmune process responsible for the perpetuation of the liver injury.

Liver Unit, King's College Hospital and Medical School, London SE5 8RX

W. M. LEE, M.D., Visiting Research Fellow (Present address: Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, N.Y.)

W. D. REED, M.R.C.P., Visiting Research Fellow

C. G. MITCHELL, PH.D., Immunologist

R. M. GALBRAITH, M.R.C.P., Research Fellow

A. L. W. F. EDDLESTON, D.M., M.R.C.P., Senior Lecturer in Medicine and Consultant Physician

ROGER WILLIAMS, M.D., F.R.C.P., Director and Consultant Physician

Hepatitis Research Unit, London School of Hygiene and Tropical Medicine, London W.C.1

A. J. ZUCKERMAN, M.D., D.SC., Professor of Virology

Introduction

A striking feature of hepatitis-B virus infection is the great variability in the host's immune response. In patients who develop typical acute hepatitis, hepatitis-B surface antigen (HBsAg) is cleared from the serum and protective immunity develops, while in others exposure is followed by chronic carriage of the infectious agent with variable amounts of liver damage (Nielson *et al.*, 1971). Examination by radioimmunoassay showed HBsAg to be present in 18% of 94 patients with active chronic hepatitis (Reed *et al.*, 1973); but, apart from an increased frequency of HBsAg in male patients and those born overseas, there were no significant differences in clinical manifestations or autoantibody patterns between the two groups. Since it seemed possible that the condition may have been initiated by a hepatitis-B infection—with the virus subsequently being successfully eliminated in one group—we looked for evidence of this using the leucocyte migration test to detect previous sensitization to the antigen as well as radioimmunoassay for identification of hepatitis-B surface antibody (anti-HBs) and electron microscopy to detect complexes in the serum. These studies were carried out on 39 patients with HBsAg-negative active chronic hepatitis and on 12 patients from the HBsAg-positive group. Both groups of patients were also examined for evidence of sensitization to a liver-specific membrane lipoprotein thought to be important in the perpetuation of an autoimmune reaction in this condition (Miller *et al.*, 1972). The clinical and immunological changes in a patient with acute type B hepatitis who progressed to HBsAg-negative active chronic hepatitis over nine months are also recorded.

Methods

Detection of HBsAg and anti-HBs was carried out by a solid-phase radioimmunoassay technique (Reed *et al.*, 1973), and serum titres of HBAg were determined by complement fixation. For electron microscopy serum was subjected to light centrifugation to remove cell debris and the supernatant centrifuged at 45 000 *g* for one hour at 4°C. The pellet was resuspended in 0·1 ml sterile distilled water and a drop mixed with an equal volume of 4% ammonium molybdate buffered at pH 5·3. The suspension was placed on carbon-formvar Smethurst No. 400 copper grids and, after removal of excess fluid, immediately examined in a AE1 801 electron microscope at a magni-

fication of 63 000. Serum known to contain the three morphological forms of HBAg was included in each test run.

Cell-mediated immunity was investigated using a micromodification of the leucocyte migration test (Mitchell *et al.*, 1972). HBsAg partially purified by column chromatography (Reed *et al.*, 1974) and liver-specific lipoprotein (Eddleston *et al.*, 1973) were used as antigens. Normal ranges for migration indices were taken as 0·80-1·20 for HBAg and 0·75-1·05 for the liver-specific lipoprotein on the basis of previous observations.

Results

IMMUNE RESPONSES TO HBSAG

Antigen-positive Group

In all 12 patients in whom HBsAg was detected in the serum serial sampling showed it to be persistent. At the time of study the antigen had been present for 4-18 months. The complement fixation titre ranged from 1/8 to 1/1024 in nine patients, but in the remaining three HBsAg was detectable only by radioimmunoassay. In one of the latter patients anti-HBs was also found on radioimmunoassay, but in all four in whom serum was studied by electron microscopy aggregates of HBsAg particles indicative of immune complexes were observed.

Results of the leucocyte migration test using HBsAg as the antigen showed inhibition of migration when first tested in five of the 12 patients. Five others, who initially had normal migration indices, were retested two to six months later, and in three there was significant inhibition of migration. These variations in the cellular response to HBsAg were examined in more detail in two patients in whom it was possible to carry out repeated tests over two weeks. Changes in the migration index were observed which were clearly related to changes in the titre of HBsAg in the serum. Migration inhibition was initially present in both, but shortly after the titre of HBsAg in serum rose to 1/512 by complement fixation, which is higher than that used in the migration test chamber, it could no longer be seen. Inhibition was once more recorded when the serum antigen titre fell to lower levels (fig. 1).

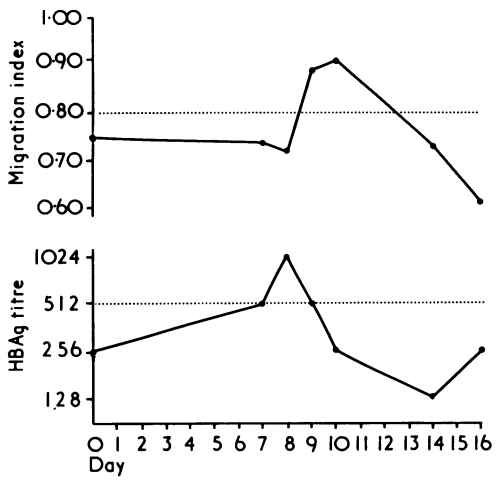

FIG. 1—Results of leucocyte migration test using HBsAg as antigen related to changes in titre of HBsAg in serum over 16 days; patient had HBsAg-positive active chronic hepatitis.

The results of the leucocyte migration test also appeared to be influenced by immunosuppressive therapy. Four patients in this group were untreated at the time of study and all showed sensitization to HBsAg. The remaining eight were taking prednisone, with or without azathioprine, and inhibition of migration was recorded in only four.

Antigen-negative Group

Though HBsAg was not detected in these 39 patients six were found to be positive for anti-HBs, and, in these, aggregates of HBsAg were also observed in the serum on electron microscopy (fig. 2). The finding of aggregates, consistent with antigen-antibody complexes, appeared at least in some cases to be a transient phenomenon. Neither antigen nor antibody was detected in later serum samples from two of the three patients from whom they could be obtained. In the third case aggregates of HBsAg were again observed three months later and antibody was also detected by radioimmunoassay, but by five months there was no longer any evidence of the presence of antigen or antibody. Leucocyte migration inhibition with HBsAg was present in five of the six patients positive for anti-HBs (table).

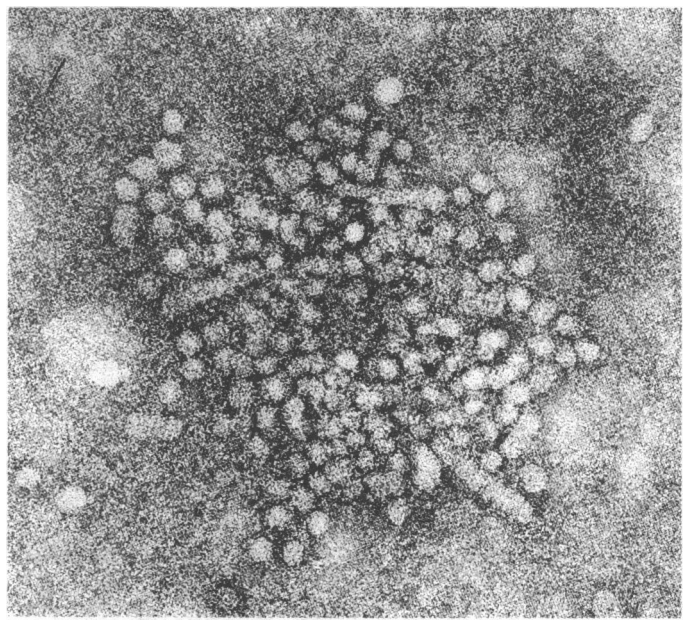

FIG. 2—Electronmicrograph of serum from patient with HBsAg-negative active chronic hepatitis in whom antibody to HBsAg was detected. An aggregate of small pleomorphic particles of approximately 20-22 nm and some tubular forms are present (× 126 000.)

In the remaining 33 patients antibody was not detected by radioimmunoassay, and in none of the 25 in whom sera were also examined by electron microscopy were aggregates or single particles of HBAg seen. The results of the leucocyte migration test with HBsAg as antigen (table), however, showed significant migration inhibition in 19 (58%).

Cellular Immunity to HBsAg and Liver-specific Lipoprotein (LSP) in Relation to Different Subgroups of Disease and Immunosuppressive Therapy

	No. of Patients	No. with Leucocyte Migration Test Positive for:	
		HBsAg	LSP
HBAg-positive group	12	8 (67%)	8 (67%)
HBAg-negative group: With anti-HBs	6	5 (83%)	4 (67%)
HBAg-negative group: Without anti-HBs	33	19 (58%)	14 (42%)
Effect of immunosuppressive therapy:			
Untreated cases	10	8 (80%)	9 (90%)
Treated cases	41	24 (59%)	17 (41%)

EVIDENCE OF SENSITIZATION TO LIVER-SPECIFIC LIPOPROTEIN

Migration inhibition in the leucocyte migration test, using liver-specific lipoprotein as antigen, was present in 26 (51%) of all 51 patients. The incidence of sensitization did not differ significantly between the antigen-positive and antigen-negative groups (table) but was related to whether immunosuppressive treatment had been instituted. Ten patients were untreated at the time of study, and in nine of these inhibition of migration was present. These included four in whom serial measurements were also carried out after institution of prednisone treatment together with azathioprine in some cases. In three, migration inhibition was no longer detectable after one to five months of treatment. The frequency of sensitization in the treated group as a whole was significantly lower than that in the untreated

group (41% and 90% respectively; $P < 0.02$). In those treated patients in whom sensitization to the liver-specific lipoprotein was still detectable the serum γ-globulin remained persistently raised. The mean level was 23.4 ± 7.8 g/l, which was significantly higher than that for the group with normal migration indices (mean 15.1 ± 7.0 g/l; $P < 0.05$).

CASE ILLUSTRATING PROGRESSION

The patient was a 51-year-old man who, after a typical prodromal illness, presented with jaundice, hepatomegaly, and a serum aspartate aminotransferase level of 500 IU/l. Serum was positive for HBsAg by immunoelectrophoresis, and liver biopsy showed the histological changes of acute viral hepatitis (fig. 3, above). The results of the leucocyte migration test indicated cellular immunity to HBsAg (migration index 0·69) but not to the liver-specific lipoprotein (migration index 0·86). During the next four months, however, in addition to continued evidence of cellular immunity to HBAg, sensitization to the liver-specific antigen also became detectable, with migration indices of 0·72, 0·59, and 0·58. At that stage HBsAg could no longer be detected in the serum even by radioimmunoassay. The serum aspartate aminotransferase level remained high and a further liver biopsy, nine months after presentation, showed the histological features of chronic aggressive hepatitis (fig. 3, below).

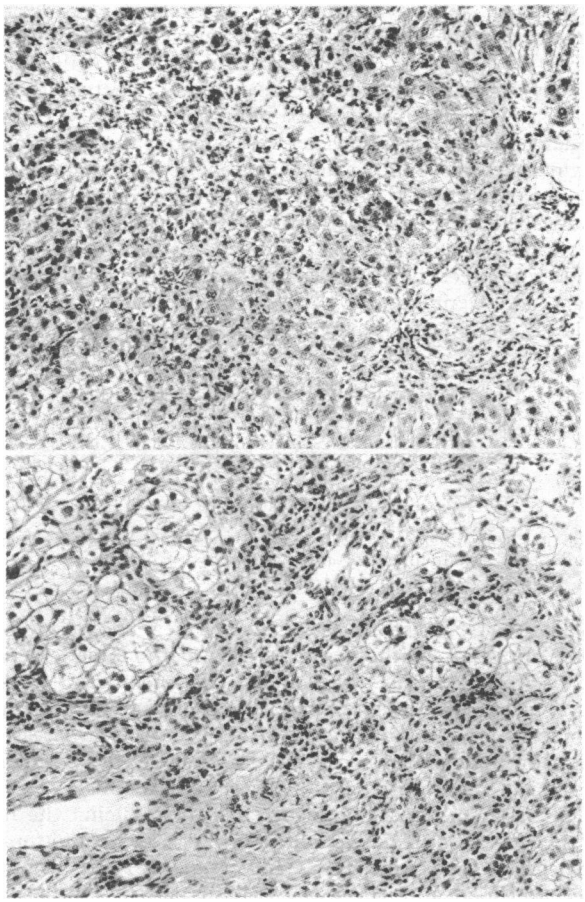

FIG. 3—Liver biopsy specimens. Appearances early in above course of acute HBsAg-positive hepatitis showing characteristic parenchymal changes with mild monocuclear cell infiltrate in portal areas. Below: appearances nine months later, when HBsAg was no longer detectable in serum, showing changes of chronic aggressive hepatitis with dense mononuclear cell infiltrate in portal areas, piecemeal necrosis, and rosette formation.

Discussion

The persistence of HBsAg in the serum in antigen-positive active chronic hepatitis has been attributed to an inadequate cell-mediated immune response to the antigen (Dudley *et al.*, 1972). In the present series, however, 67% of those with antigenaemia showed a positive response, and if all the cases had been studied before starting treatment with immunosuppressive drugs the frequency may well have been considerably higher. When serum levels of HBsAg are high the response to the leucocyte migration test is masked, and this also interferes with attempts to estimate the true frequency of sensitization. It seems most unlikely, however, that persistence of the antigenaemia in these patients can be accounted for by a specific defect in cellular immunity to HBsAg. A defect in the humoral antibody response, which is more directly involved in the clearance of circulating antigen, is much more likely. This could be a qualitative abnormality or a quantitatively inadequate response.

In view of the frequency of cellular immunity to HBsAg in the antigen-negative patients the finding of anti-HBs in six of these was not unexpected, but we were surprised to find HBsAg particles aggregated in the serum. This emphasizes the difficulty of excluding HBsAg when the antibody is also present. The presence of excess antibody may prevent the detection of HBsAg even when a sensitive radioimmunoassay technique is used. Aggregated HBsAg particles in the presence of circulating antibody can probably be taken as equalling immune complexes. Though the role of immune complexes in the pathogenesis of active chronic hepatitis in not clear, the lack of correlation with disease activity suggests that they may be of minor importance. They appeared to be present only transiently, at least in the three patients studied serially. Intercurrent infection with the hepatitis-B virus seemed unlikely, as there was only one patient in this group in whom a history of transfusion or other exposure during the previous six months could be elicited.

The frequency of sensitization to HBsAg in our patients with active chronic hepatitis who had neither HBsAg nor anti-HBs in the serum approached that found in patients recovering from acute serum hepatitis. This suggests a link between the hepatitis-B virus and active chronic hepatitis whether or not HBsAg is detectable in the circulation at the time of presentation. Direct evidence in favour of this is the clinical course of the patient who progressed from acute type B hepatitis to antigen-negative active chronic hepatitis. Three similar cases were described by Nielsen *et al.* (1971).

Though the mechanisms underlying such a progression are poorly understood, increasing evidence suggests that the chronic liver cell damage is the result of a cell-mediated immunological response to the liver-specific lipoprotein. Sensitization to this normal component of the hepatocyte membrane was present in all but one of the patients with untreated active chronic hepatitis. In addition, we have recently shown that lymphocytes from the peripheral blood of patients with active chronic hepatitis will kill isolated hepatocytes and that this reaction is mediated specifically by cells reacting with the lipoprotein antigen (Thomson *et al.*, 1974). Such an autoimmune reaction might be induced by hepatitis-B infection because of a change in antigenicity of the hepatocyte cell membranes due to alteration of existing antigens or the appearance of viral determinants. Allison *et al.* (1971) indicated how T-lymphocytes responsive to such new antigenic determinants could promote a B-cell response to unaltered self-antigens. The synthesis and release of the resulting autoantibody is in turn subject to control by suppressor T-cells. These complex interactions between T- and B-cells, initiated by viral infection, could be of fundamental importance in the pathogenesis of active chronic hepatitis (Eddleston and Williams, 1974). Thus in the HBsAg-positive cases persistence of the virus infection could, by promoting continuous T- and B-cell co-operation, result in prolonged activation of an autoimmune response to the liver-specific membrane lipoprotein. Whereas, in the antigen-negative form of the disease there may be an inherited defect in suppressor T-cell function, thus allowing the autoimmune response to continue well after recovery from the initial viral hepatitis.

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Double-blind Comparison of Tolamolol, Propranolol, Practolol, and Placebo in the Treatment of Angina Pectoris

GRAHAM JACKSON, LYNNE ATKINSON, SAMUEL ORAM

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Summary

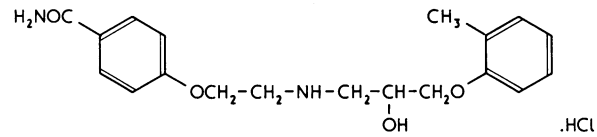
Forty-two patients with angina pectoris have completed a randomized, double-blind trial comparing tolamolol 100 mg and 200 mg with propranolol 80 mg, practolol 100 mg, and placebo, all given three times a day. Tolamolol 200 mg thrice daily was found to be equivalent to propranolol 80 mg thrice daily in anti-anginal efficacy. Anginal attack rates and trinitrin consumption were significantly reduced by all active treatments as compared with the placebo but tolamolol and propranolol were the most effective. Tolamolol 200 mg thrice daily was most effective in reducing blood pressure, while propranolol was most effective in reducing the resting heart rate.

All treatments except the placebo significantly increased the amount of exercise which could be performed before angina appeared (exercise work), while tolamolol 200 mg thrice daily significantly reduced Robinson's index when compared with all other active agents. The degree of S-T segment depression induced by exercise was significantly lessened by both tolamolol and propranolol but not by practolol or placebo. There was no difference in patient preference between tolamolol and propranolol but tolamolol at both dose levels was preferred to practolol.

Both tolamolol and propranolol are potent adrenergic beta-receptor antagonists and equal in anti-anginal efficacy but tolamolol has the advantage of being cardioselective. It is superior to practolol.

Introduction

Adrenergic beta-receptor antagonists are well established in the treatment of angina pectoris. Propranolol was the first of these to be clinically accepted and is probably the standard reference agent used (Gillam and Prichard, 1965; Grant *et al.*, 1966; Wolfson *et al.*, 1966). It is not cardioselective, however, and may predispose to bronchospasm (McNeill, 1964; Stephen, 1966) and reduce myocardial contractility with the risk of cardiac failure (Sowton and Hamer, 1966). A selective action on cardiac beta-adrenergic receptors reduces the incidence of bronchospasm and myocardial depression and is a desirable feature of adrenergic beta-receptor antagonists for angina pectoris (Dollery *et al.*, 1969; Fitzgerald, 1969; Miller *et al.*, 1974). Practolol is a cardioselective adrenergic beta-receptor antagonist with intrinsic sympathomimetic activity (Dunlop and Shanks, 1968). It is, however, less effective than propranolol in angina pectoris (Sandler and Clayton, 1970; Prichard *et al.*, 1971) and though it is less likely to produce bronchospasm and the so-called negative inotropic effect—namely, myocardial depression—these have been induced (Wiseman, 1971). In addition side effects have included systemic lupus erythematosus (Raftery and Denman, 1973), psoriasis-like rashes (Felix and Ive, 1974), and ocular changes (I.C.I., 1974).



Composition of tolamolol (UK-6558-01); 1-[2-(4-carbamoylphenoxy) ethylamino]-3-(2-methylphenoxy) propan-2-ol hydrochloride.

Tolamolol (UK 6558-01) (see fig.) is a new adrenergic beta-receptor antagonist without intrinsic sympathomimetic activity which is cardioselective in animals (Augstein *et al.*, 1973; Adam *et al.*, 1974). In volunteers (Briant *et al.*, 1973) tolamolol and practolol were similar in cardioselectivity, whereas the potency of tolamolol was similar to that of propranolol in antagonizing exercise-induced tachycardias (Adam *et al.*, 1973). In man tolamolol has a dominant effect in reducing the heart rate, the so-called negative chronotropic effect, and only a slight negative inotropic action (Hillis *et al.*, 1974). A preliminary report (Sood and Havard, 1973) in patients with angina pectoris showed an increase in exercise tolerance equivalent to that found with propranolol.

To assess the clinical efficacy of tolamolol a double-blind evaluation comparing it with propranolol and practolol has been undertaken.

Patients and Methods

SELECTION AND ENTRY OF PATIENTS

Patients with exercise-induced angina were selected. They were excluded if the angina was associated with anaemia (haemoglobin less than 13 g/dl), valvular heart disease, cardiac failure, obstructive airways disease, cardiac infarction in the previous three months, hypertension (fifth-point resting diastolic pressure over 100 mm Hg), diabetes, or thyroid disease. In all the patients the angina had been stable for more than three months and all showed electrocardiographic

Cardiac Department, King's College Hospital, London SE5 9RS
GRAHAM JACKSON, M.B., M.R.C.P., Lecturer in Cardiology
LYNNE ATKINSON, B.SC., Research Assistant
SAMUEL ORAM, M.D., F.R.C.P., Director