Rosette inhibition test in chronic liver disease

F. SALERNO, S. R. FARGION, M. D. CAPPELLINI, G. FIORELLI, AND N. DIOGUARDI

From the Istituto di Clinica Medica III dell’Università degli Studi di Milano, via Pace 15, 20122 Milano, Italy

SYNOPSIS A role has recently been assigned to cell-mediated immunity in chronic liver diseases in addition to the well-known alterations of humoral immunity. We now report the results of the rosette inhibition test for the evaluation of T-lymphocyte ‘sensitization’ in patients with different chronic liver disorders. A cell-mediated immune reaction was found in 81% of patients with chronic active hepatitis and in 71% with primary biliary cirrhosis, whereas patients with chronic persistent hepatitis showed no reaction. The correlation with the incidence of hepatitis B antigen showed that T-lymphocyte sensitization was more frequent in the group of HB-positive patients. Finally, improvement of the test was observed in four out of nine patients given immunosuppressive treatment.

Changes of cell-mediated immunity have recently been proposed in the pathogenesis of some chronic liver diseases (Dudley et al., 1972b; Popper and Mackay, 1972). On the basis of knowledge of the different populations of peripheral lymphocytes we attempted to demonstrate a specific sensitization of T-cells in chronic liver disease by means of the rosette inhibition test (RIT). T-lymphocytes are able to form spontaneous rosettes with sheep red blood cells in vitro, and this property is a sensitive physiological marker of this subpopulation (Jondal et al., 1972). Bach and Antoine (1968) have reported that antilymphocyte serum markedly reduces spontaneous rosette formation; on this property is based the RIT (Bach et al., 1969). In the beginning the RIT was used to evaluate the degree of immunosuppression obtained with various cytotoxic drugs in patients with renal or lung allografts (Munro et al., 1971; Cullum et al., 1972). Recently it was reported that the RIT might also be useful in assessing the degree of T-lymphocyte sensitization in subjects with autoimmune diseases (Farid et al., 1973).

In the present study the RIT was used in patients with chronic liver diseases to investigate T-lymphocyte sensitization and to evaluate the effect of immunosuppressive therapy. The distribution of T and B cells in the peripheral blood, serum autoantibodies, and hepatitis B antigen (HBsAg) has also been evaluated.

Patients and methods

The group of 64 cases of chronic liver disease consisted of 12 patients with chronic persistent hepatitis (CPH), 37 with chronic active hepatitis (CAH), 7 with primary biliary cirrhosis (PBC), and 8 with alcoholic cirrhosis (AC). The diagnosis based on clinical and biochemical criteria were confirmed in all cases by histological examination of liver biopsies. Patients were not previously treated with corticosteroid or immunosuppressive drugs. Twenty-four healthy subjects were tested as a control group. Nine patients (6 CAH and 3 PBC) were followed for periods ranging from 3 to 12 months after starting the immunosuppressive therapy. In these patients the treatment began with 25 mg of prednisone per day, the dose being tapered gradually; in two cases azathioprine, 100 mg daily, was added.

ROSETTE INHIBITION TEST

The method used was that described by Bach et al. (1969) as adapted by Munro et al. (1971) with the following modifications: lymphocytes were separated from peripheral blood, with the addition of preservative-free heparin, by a technique of sedimentation in Ficoll-Isopaque density gradients (Jondal et al., 1972). The cells were washed three times in Hank’s BSS and adjusted to 4 × 10⁶/ml. 0.1 ml of this suspension was incubated with 0.25 ml of serial dilutions of antilymphocyte globulin (ALG) for 90 min at 37°C. The dilutions of ALG ranged from 1:4000 to 1:128 000. A tube in which Hank’s BSS was substituted for ALG was also set up as a control. Sheep
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Rosette inhibition compared to the control was plotted against ALG concentration. The concentration at which 75% rosettes was observed was termed the MIC.

Fig 1  Rosette inhibition test. Per cent rosette formation compared to the control was plotted against ALG concentration. The concentration at which 75% rosettes was observed was termed the MIC.

Red blood cells (SRBC) (0.1 ml, 120 x 10^6/ml) were subsequently added. Cells were incubated at 37°C for 5 min, centrifuged at 200 g for 5 min, incubated on ice for 90 min, and gently resuspended. The percentage of rosette-forming cells was calculated, counting 500 lymphocytes in a Fuchs-Rosenthal chamber. The number of rosettes in the control tube gives the percentage of T-lymphocytes. The dilution of ALG necessary to reduce the rosette formation to 75% of the control was taken as the minimal inhibitory concentration (MIC) (fig 1).

Two different methods were used to identify B-lymphocytes—the EAC rosette test described by Stjernswärd et al (1972) and the immunofluorescence test for surface immunoglobulins (Pernis et al, 1970). ANA, AMA, and SMA were detected by means of indirect immunofluorescence (Coons and Kaplan, 1958), according to Roitt and Doniach (1969). HBsAg was detected in serum by counter-immunoelectrophoresis (Gocke and Howe, 1970), and controlled using the solid-phase Abbott radio-immunoassay.

Results

The percentages of T and B lymphocytes in the different groups of liver diseases are in the same range as in normal subjects (T-cells = 47.2 ± 8.4, B-cells = 21.0 ± 3.2). The results of RIT are shown in figure 2. The MIC of the normal subjects ranged from 1:16000 to 1:48 000 dilutions of ALG and the mean value was 1:25 000. The MIC values of the patients with CPH usually fell within the normal range; increased resistance of the lymphocytes to ALG was demonstrated in 30 patients with CAH (81% of cases) and in five with PBC (71%). The subjects with AC showed variable results: in five cases the MIC fell within the normal range, in two it was higher than normal, and in one lower (1:64 000). The differences between the mean values of MIC in normal subjects and in CAH patients (t = 7.13, p < 0.001) and PBC patients (t = 2.1, p < 0.05) were statistically significant.

In patients with CAH, smooth muscle autoantibodies were more frequently detected in patients with a high value of MIC (36%) than in those with a normal value (15%). In fig 3 the RIT is related

Fig 2  Distribution of values of MIC in different groups of chronic liver diseases.

Fig 3  Correlation between MIC values and incidence of HBsAg in patients with chronic active hepatitis.
During immunosuppressive therapy the reactivity of lymphocytes to liver antigens has been shown to occur in human chronic liver diseases by means of lymphocyte transformation and leucocyte migration tests (Tobias et al, 1967; Miller et al, 1972).

We have used the RIT to investigate the immune reactivity of T-lymphocytes in patients with chronic liver diseases. Although the immunological basis of this test is not clear, recent results obtained in cases of immunosuppression (Morton et al, 1974) support the value of the RIT as a measure of T-lymphocyte function. The marked increase in MIC values which was found in 81% of patients with CAH and in 71% of patients with PBC, compared with the normal MIC found in all patients with CP, supports the view that cellular immunity is a factor in the activity of the liver disease. The same conclusion had been reached by means of the leucocyte migration test, which demonstrated a sensitization of lymphocytes to a liver lipoprotein in patients with CAH (Miller et al, 1972) and to a protein fraction from bile in patients with PBC (Eddleston et al, 1973).

Despite the increase of MIC observed in many patients with chronic liver diseases, an increase in T-lymphocyte count was not found, as reported in other autoimmune disorders. The most probable interpretation is that in these patients only a small subpopulation of T-cells was stimulated, too few to affect the total number but numerous enough to indicate a degree of sensitization.

Concerning the humoral immunity, it was found that smooth muscle autoantibodies are more frequent in patients with abnormal values of MIC than in those with normal values, but the difference between the two groups is not significant. More important is the correlation between the incidence of HBsAg and the change in MIC in the group of CAH patients: subjects with positive HBsAg showed a higher frequency of T-lymphocyte sensitization (100%) than those with negative HBsAg (61%). This finding suggests that HBsAg is associated with an activation of T-lymphocytes: the alteration of cellular immunity in chronic liver diseases could be related to the presence of HBsAg, as already proposed (Dudley et al, 1972a; Popper and Mackay, 1972).

Regarding the effect of immunosuppressive treatment, a return to normal of the MIC was observed in four out of nine cases studied. Therefore patients with chronic liver disease show a different sensitivity of T-cells to immunosuppressive therapy.

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doi: 10.1136/jcp.29.9.778

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