Antibodies to double-stranded (native) DNA in active chronic hepatitis

P. Davis and A. E. Read

From the Department of Medicine, Bristol

Summary Sera from 36 patients with active chronic hepatitis were studied for the presence of antibodies to double-stranded (native) DNA. These antibodies are a specific antinuclear antibody previously shown to have a high degree of specificity for systemic lupus erythematosus. Fifteen of the 36 patients (42%) were found to have levels of antibody usually only reported in systemic lupus erythematosus and higher than those seen in a control population. Anti-DNA antibodies were not found in a group of 22 patients with other forms of liver disease.

It is suggested that these antibodies may prove to have a specific role in the immunopathogenesis of active chronic hepatitis.

Active chronic hepatitis is a chronic inflammatory disease of the liver characterized histologically by a widespread round cell infiltration of the portal tracks, ‘piecemeal’ necrosis of the liver parenchyma, and eventual cirrhosis. Although the aetiology of this disease is obscure in many patients, immunological and genetic factors and drugs are known to be involved in the pathogenesis.

The similarities in the serological changes of active chronic hepatitis and systemic lupus erythematosus (SLE) have in the past provoked comment because of the high incidence of antinuclear antibodies and Le cells in both diseases.

Clinically, in some patients with active chronic hepatitis many systems are involved, producing lupus-like skin eruptions, arthralgia, renal disease, ulcerative colitis, and neuropsychiatric complications similar to those in SLE (Read, Sherlock, and Harrison, 1963; Golding and Mason, 1971).

In SLE there has been considerable interest in the nature of the antinuclear antibody and its clinical and pathological role in this disease. A large number of antibodies have been detected to a number of nuclear antigens, including single-stranded RNA, polynucleotides, single-stranded DNA, nucleoprotein, ribosomes, and nucleoli. Of importance, however, has been the demonstration of the specificity and sensitivity of double-stranded (native) DNA for SLE and of the findings of antibodies to double-stranded viral RNA. Hughes, Cohen, and Christian (1971), and Hughes (1971) have demonstrated that antibodies to double-stranded DNA are found almost exclusively in active SLE and that levels of DNA antibody fall as the activity of the disease regresses. In addition, Koffler, Carr, Agnello, Thorburn, and Kunkel (1971), in a series of elution experiments, have been able to demonstrate that DNA/anti-DNA complexes are responsible for lupus nephritis. Antibodies to double-stranded viral RNA have also been demonstrated in lupus sera and their presence has caused speculation as to the possible role of a virus in the pathogenesis of SLE (Davis, Cunnington, and Hughes, 1975).

In view of the known benefit of the characterization of antinuclear antibody in SLE, and the known similarity serologically and, in some cases clinically, between SLE and active chronic hepatitis the following study was undertaken to determine whether similar antinuclear antibodies were found in the two diseases.

Cases Studied

Thirty-six cases of active chronic hepatitis, diagnosed on clinical, serological, and histological grounds, have been included in this study. The serological features of the group are shown in the table and did not differ significantly from the changes reported in larger series. One case had active chronic hepatitis thought to be due to methyldopa and only two had persistent Australia antigenaemia.

Thirty-four patients were receiving therapy which
consisted of varying doses of steroids. In only two patients in this study was it possible to study DNA antibodies before therapy.

Normal sera were used as controls and sera from a group of SLE patients were tested and used for comparison.

In addition sera were tested from a group of patients with a variety of other liver diseases, including primary biliary cirrhosis, alcoholic cirrhosis, and both Australia antigen-positive and -negative acute viral hepatitis.

Results

The maximum results for DNA binding recorded in each patient have been plotted (see fig). Seventeen of 36 patients with active chronic hepatitis (47%) had levels of DNA binding in the range previously only reported in active cases of SLE. There were no clinical features to distinguish those patients with DNA antibodies from those without.

None of the patients with other liver diseases were found to have DNA antibody levels outside the normal range (range 0-30%, mean 12%).

All patients with significantly elevated DNA antibodies had positive antinuclear antibody and all five in whom it was measured had smooth muscle antibody. The majority of the patients without DNA antibodies also had antinuclear antibody but less than half had smooth muscle antibodies. There was no significant correlation between the presence of LE cells and DNA antibodies.

The results for patients with active and inactive SLE are recorded in the table (range 0-93%, mean 40%) and also for normal controls (range 0-31%, mean 11%).

Method (Wold, Young, Tan, and Farr, 1968)

A wide number of techniques are available for the detection of antibodies to nuclear antigens. The most widely used giving good reproducible and quantitative results is the modification of the Farr test which has been used in this study.

Sera are decomplemented by heating at 56°C for 30 minutes before testing. Then 0-05 ml of serum is diluted 1 in 10 in borate buffer (pH 8-0), and 0-05 ml of diluted serum is mixed with 0-05 ml of 14C DNA (Radiochemical Centre, Amersham) and incubated for one hour at 37°C. Samples are left overnight at 4°C. The samples are kept on ice and 0-1 ml of saturated ammonium sulphate added and left at 4°C after thorough mixing for one hour. Samples are then spun for 45 minutes at 2000 rev/min at 4°C, 0-1 ml of supernatant (S) is removed and added to 0-9 ml of borate buffer, and 10 ml of Bray's fluid is added. Radioactivity in supernatant (S) and precipitate (p) is measured in counts per minute (ct/min) on a B-emitter counter.

DNA binding is calculated by:

\[
\frac{Ct/\text{min}(P) - Ct/\text{min}(S)}{Ct/\text{min}(P) + Ct/\text{min}(S)}
\]

<table>
<thead>
<tr>
<th>Group Studied</th>
<th>ANA</th>
<th>Smooth Muscle Antibodies</th>
<th>Mitochondrial Antibodies</th>
<th>LE Cells</th>
<th>Rheumatoid Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present group (36 cases)</td>
<td>26/36</td>
<td>13/21</td>
<td>0/21</td>
<td>2/36</td>
<td>2/36</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>50%</td>
<td>66%</td>
<td>28%</td>
<td>15%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Table Serology of group studied

Fig Maximum recorded double-stranded DNA antibodies in active chronic hepatitis patients compared with other liver disease, normals and SLE
and expressed as a percentage. The normal range in our laboratory is 0-30%, calculated as the mean ± 2SD in 200 normal controls.

Discussion

The suggestion that active chronic hepatitis is in part due to an immunological abnormality has met with widespread acceptance, although at present its nature is not clearly defined.

Antibodies to specific nuclear antigens have not previously been reported in active chronic hepatitis. The observation that 47% of our patients with active chronic hepatitis had anti-DNA antibodies is of interest, particularly as in SLE complexes of DNA/anti-DNA are known to circulate and produce some of the systemic features also associated with active chronic hepatitis.

It is not clear at present whether immune complexes may be responsible for the histological changes of active chronic hepatitis or whether their formation is only responsible for systemic features associated with it. In the case of the 18% patients with active chronic hepatitis and positive hepatitis B antigen, immune complexes are probably responsible for some of the systemic features recently demonstrated in the synovium of patients with associated arthritis (Schumacher and Gall, 1974). Further support for the role of immune complexes in this disease has been the observation of depressed levels of C3 during disease activity suggesting complement conversion, and anticomplementary activity of active chronic hepatitis serum has been noted (Gitnick et al., 1973). However, neither these studies nor the finding of antibodies to double-stranded DNA in active chronic hepatitis is conclusive evidence that DNA or anti-DNA complexes are responsible for the histological or clinical changes as they are in SLE.

The source of antibodies to double-stranded DNA in active chronic hepatitis is at present unknown. One possibility is that they may be of viral origin. Certainly in SLE the presence of antibodies to double-stranded DNA and viral RNA has led to the suggestion that a virus may be involved in the pathogenesis of this disease (Talal, 1970).

Not all sera from our patients contained anti-DNA antibodies, but were found in 58% of those patients with antinuclear antibody on immunofluorescence. Titres of antinuclear antibody change relatively little with the activity of the disease and are an insensitive method of serially following progress in active chronic hepatitis. Likewise serial studies of liver enzymes may not always reflect the severity or progression of disease activity as assessed by liver histology. Serial studies of DNA antibodies in active chronic hepatitis may, therefore, prove to be useful in monitoring disease activity. In two of our cases studied serially levels of DNA antibody fell to within the normal range when steroid therapy was instituted and this coincided with a return of hepatic enzymes to normal levels. It is of note that in our cases anti-DNA antibodies were found in a significant number of patients despite steroid therapy. As steroid therapy may suppress antibody levels, and the clinical features of SLE, a larger study on patients with active chronic hepatitis who have not received therapy may demonstrate levels of antibody binding in a range more comparable with that in SLE patients.

This study, although preliminary, suggests that the detection of antibodies to double-stranded DNA may be of future value to hepatologists in the study of active chronic hepatitis. Further studies on larger series of patients will be required to determine their specificity for this form of liver disease and also serial studies to elucidate their possible value in monitoring disease activity. This study demonstrates a further similarity in the serological changes noted in active chronic hepatitis and SLE which at present are already known to have in common a number of clinical and immunological similarities. Further studies on the role of anti-DNA antibodies and/or complexes may prove fruitful in the future in elucidating the complicated relationship between these two diseases, if any.

References


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