Paraproteins with antibody activity in acute viral hepatitis and chronic autoimmune liver diseases

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SUMMARY Of 27 patients with liver disease and cryoglobulinaemia 18 proved to have paraproteins. Six of these monoclonal immunoglobulins were shown to have antibody activity, directed to human gamma globulin, alpha,-fetoprotein, smooth muscle, and mitochondria.

Eight of the patients suffered from acute viral hepatitis, five of whom were HB Ag positive; in all these cases the monoclonal spikes were transient and their antibody activities were directed against IgG in two cases and alpha,-fetoprotein in one.

Seven of the patients had active chronic hepatitis and in these the paraproteinaemia persisted, though remaining quantitatively unchanged over several years. One of them had a cryoprecipitable monoclonal smooth muscle antibody. Three patients had primary biliary cirrhosis and in two of them monoclonal IgM mitochondrial antibodies were demonstrated.

In three out of the 18 cases there was a double M-component.

Since these monoclonal antibodies are directed to autoantigens not unlike the polyclonal ones usually seen in autoimmune hepatic diseases, it is suggested that the factor which triggers the uncontrolled plasma cell proliferation to produce paraproteins must meet cells from an already expanding clone.

Monoclonal proteins are increasingly reported in the absence of overt myeloma or Waldenström's macroglobulinaemia and are considered to result from the uncontrolled clonal proliferation of plasma cells. Antibody activities have been recognized in these homogeneous immunoglobulins and their study is of particular interest.

The purpose of this paper is to report the investigations performed on the sera of patients with acute viral hepatitis and chronic hepatic autoimmune disorders, ie, active chronic hepatitis and primary biliary cirrhosis.

The presence of paraproteins in cirrhosis is well documented (Michaux and Heremans, 1969; Zawadzki and Edwards, 1970, 1972), but their occurrence in acute hepatitis has only been mentioned on a few occasions (Waldenström, 1961; Osserman and Takatsuki, 1963; Seligmann, Danon, and Clauvel, 1971). Furthermore, antibody activities in the isolated M-components, including those directed to self antigens such as smooth muscle or mitochondria and the coexistence of two paraproteins in the same serum, although well recognized in myeloma and Waldenström's macroglobulinaemia, have not been previously reported in hepatic disease.

Materials and Methods

Of a group of 27 patients with acute viral hepatitis and chronic liver diseases selected on the basis of cryoglobulinaemia in a previous study (Florin-Christensen, Roux, and Arana, 1974), 18 showed an abnormal electrophoretic pattern suggestive of paraproteinaemia. Eight of these patients had acute viral hepatitis, five of them with positive HB Ag. Seven had clinical, biochemical, and histological evidence of active chronic hepatitis and three of primary biliary cirrhosis. HB Ag was not detected.
in either of these groups. Blood was obtained by
venepuncture and allowed to clot at 37°C. LE cell
tests were performed according to Hargraves (1954).
All sera were submitted to paper electrophoreses and
then stored at −20°C until tested, except for a small
aliquot which was kept at 4°C for the detection of
cryoglobulins. Electrophoresis in gels containing 1-5g
agar (Noble Difco) per 100 ml 0·1 M veronal buffer (pH 8·2) was performed on an LKB apparatus
employing micromethods. Immunelectrophoresis
was done according to Scheidegger (1955).
Antisera to whole human serum and plasma and to
IgG and IgA were prepared in the laboratory by
immunizing rabbits. For this purpose IgG was
obtained from pooled normal sera by ion-exchange
chromatography on DEAE-cellulose, and IgA from
myeloma sera purified by gel filtration on Sephadex
G200 columns and/or ion-exchange chromatography.
Rabbit antihuman IgM, IgD, and IgE and anti-
kappa and -lambda chains were obtained commer-
cially (Hyland). Antisera to gamma, alpha, mu, delta,
and epsilon chains were rendered monospecific
by absorption with purified immunoglobulins of another
class. Antisera to kappa and lambda chains were absorbed with myeloma proteins known to belong
to the opposite type.

Isolation of homogeneous proteins was per-
formed by several techniques. IgM was isolated from
euglobulin fractions by gel chromatography on
Sephadex G200 columns (100 × 2·5 cm) with 0·1 M
Tris-HCl/1 M NaCl buffer (pH 8·0). IgG was iso-
lated and purified by chromatography on DEAE-
cellulose using a sodium phosphate buffer (pH 8·0).
For IgA, pseudoglobulin fractions were employed and
chromatographed on DEAE-cellulose columns.
The initial buffer was 5 mM sodium phosphate and,
after IgG elution, a linear gradient was applied up to
0·1 M. Fractions of 3 ml were collected and
concentrated by dialysis against polyvinylpyrrolidone
in 0·14 M NaCl. Protein concentrations were
estimated by the biuret (Weichselbaum, 1946) or
micro-Kjeldahl (Kabat and Mayer, 1964) methods.

Antibodies to human gamma globulin were
detected by the latex fixation test according to Singer
and Plotz (1956). Antiglobulins to heterologous
gamma globulin were looked for by means of the
sensitized sheep cell agglutination test as described
in the WHO Manual of Autoimmune Serology
(Roitt and Doniach, 1969).

Antibodies to nuclei, liver cytoplasm, gastric
parietal cells, smooth muscle, renal tubules, and
glomeruli were detected by immunofluorescence
(Hamard, Cannat, and Seligmann, 1964; Roitt
and Doniach, 1969). Cryostat sections (4 μm) of
mouse liver, kidney, and stomach were used as a
substrate. In some tests human thyroid and stomach
were also employed. Antiserum to human gamma
globulin (IgG, IgM, and IgA) was prepared in a
goat and labelled with 12·5 μg of fluorescein iso-
thiocyanate per mg protein (Nairn, 1968). The
protein: fluorescein ratio was 1:5 and the labelled
antiserum was used at a 1:8 dilution according to
standardization. FITC-conjugated antihuman C3
was obtained commercially (Miles Laboratories).

Antibodies to native DNA and single stranded,
heat-denatured DNA and to a saline-soluble calf
thymus extract (Tan and Kunkel, 1966) were
detected by complement-fixation and precipitin tests
(Cannat, Arana, and Seligmann, 1967). The presence
of HB Ag and HB Ab was established by double
diffusion in agar and by immunelectrophoresis.
Reference sera for HB Ag and HB Ab were at first
provided by Austragen Laboratories and later by
Academia Nacional de Medicina, Buenos Aires,
from polytransfused haemophilic patients. When
looking for HB Ab, HB Ag-positive sera were
pooled in order to test patients' sera against a wider
number of HB subtypes.

Alpha 1 fetoprotein (α1 FP) and anti-α1 FP were
looked for by double diffusion in agar and agarose
gels. Immunelectrophoresis was also used,
employing gels containing 0·85 g agarose per 100
ml 50 mM veronal buffer (pH 8·2) as described
previously (Florin-Christensen and Arana, 1973).
Single radial immunodiffusion was used to look for
small amounts of α1 FP in the cryoprecipitates
(Mancini, Carbonara, and Heremans, 1965). Refer-
ence α1 FP serum and antiserum were obtained
commercially (Hoechst Laboratories), and later
prepared in the laboratory by immunizing rabbits
with the antigen obtained from foetuses of 14 to 16
weeks' gestation.

Antigenic analyses were done according to
Ouchterlony (1953) using gels with 1 g agar per 100
ml phosphate-buffered saline (pH 7·2).

Detection of Bence Jones proteins was done
at pH 6. Immunelectrophoretic analysis was per-
formed on 100-fold concentrates of urine.

Results

The paraproteins found in the 18 patients are shown
in table I. Of the eight patients with acute viral
hepatitis three had an M-component of the IgM
class, four had an IgG paraprotein, and one had
an IgMK, free kappa chains, and a cathodic bifurca-
tion of the IgG precipitin arc, consistent with gamma
chains devoid of light chains (figure). The spikes
were all small and could not be accurately quanti-
tated. They were first detected in the second week of
illness and disappeared on recovery, between two
and three months after onset.
were generally small but they were constantly detected over a follow-up period of up to three years, during which they did not increase significantly.

All three patients with primary biliary cirrhosis had IgMK paraproteins. One of them showed, in addition, a second spike (IgGK) and circulating free gamma and kappa chains, with an immunoelectrophoretic appearance similar to that in the figure. In two cases of primary biliary cirrhosis, the monoclonal spikes were small but in the third patient, who also had free heavy and light chains in the serum, the paraproteinemia was more pronounced and there was a kappa Bence Jones proteinuria. In this patient, as also in the other cases of primary biliary cirrhosis, the condition did not progress to overt myelomatosis and the paraproteins remained unchanged for follow-up periods of up to four years. Marrow aspirations carried out in some of the active chronic hepatitis and all three patients with primary biliary cirrhosis, showed no significant increase in plasma cells or abnormal forms.

All the sera were tested for autoantibodies and, whenever possible the M-components were isolated by chromatography and tested in the same way. The results are shown in table II. It can be seen that IgMK from two patients with acute viral hepatitis reacted with human gamma globulin down to concentrations of 1.5 and 6.2 μg/ml, both these patients having a mixed cryoglobulinaemia. The third case of acute viral hepatitis IgMK proved to be an antibody to α1 FP forming cold precipitable complexes. Only one of the seven paraproteins from patients with active chronic hepatitis showed antibody activity; this was an IgGK reactive with smooth muscle by immunofluorescence down to a concentration of 50 μg/ml. This paraprotein was also the sole component identified in the cryoprecipitate.

IgMK from two patients with primary biliary cirrhosis were responsible for the antimitochondrial activity of the sera from which they were derived. Fluorescent staining of distal renal tubules and gastric parietal cells with the paraprotein was positive down to concentrations of 20 and 100 μg/ml respectively. Both monoclonal antibodies fixed complement with the same staining pattern. With whole serum the reaction was positive in each case using anti-IgM and antikappa conjugates and negative with anti-IgG-IgA and γ-lambda. After chromatography of the sera only the IgM gave mitochondrial fluorescence and this could be abolished after pretreatment of the fractions with anti-IgM and antikappa, but not with anti-IgG, -1gA, or -lambda. The antibody specificity of the paraproteins was further confirmed by absorption of the mitochondrial

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**Table I**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>HB Ag</th>
<th>Paraprotein(s)¹</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute viral hepatitis</td>
<td></td>
<td>IgMK</td>
</tr>
<tr>
<td>2</td>
<td>Acute viral hepatitis</td>
<td></td>
<td>IgML</td>
</tr>
<tr>
<td>3</td>
<td>Acute viral hepatitis</td>
<td></td>
<td>IgGL</td>
</tr>
<tr>
<td>4</td>
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<td>+</td>
<td>IgGK</td>
</tr>
<tr>
<td>5</td>
<td>Acute viral hepatitis</td>
<td></td>
<td>IgGL</td>
</tr>
<tr>
<td>6</td>
<td>Acute viral hepatitis</td>
<td>+</td>
<td>IgMK, IgGK, free gamma and kappa</td>
</tr>
<tr>
<td>7</td>
<td>Acute viral hepatitis</td>
<td>+</td>
<td>IgMK</td>
</tr>
<tr>
<td>8</td>
<td>Acute viral hepatitis</td>
<td>+</td>
<td>IgGK</td>
</tr>
<tr>
<td>9</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgAK</td>
</tr>
<tr>
<td>10</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgGK</td>
</tr>
<tr>
<td>11</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgGK, IgM²</td>
</tr>
<tr>
<td>12</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgGL</td>
</tr>
<tr>
<td>13</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgM²</td>
</tr>
<tr>
<td>14</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgAL</td>
</tr>
<tr>
<td>15</td>
<td>Active chronic hepatitis</td>
<td></td>
<td>IgMK</td>
</tr>
<tr>
<td>16</td>
<td>Primary biliary cirrhosis</td>
<td></td>
<td>IgMK</td>
</tr>
<tr>
<td>17</td>
<td>Primary biliary cirrhosis</td>
<td></td>
<td>IgMK</td>
</tr>
<tr>
<td>18</td>
<td>Primary biliary cirrhosis</td>
<td></td>
<td>IgMK, IgGK, free gamma and kappa</td>
</tr>
</tbody>
</table>

¹K = type of immunoglobulin bearing kappa light chains, L = type of immunoglobulin bearing lambda light chains

²Not typed.

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**Fig**

Diagram of immunoelectrophoretic analysis of serum from patient no. 6 (acute viral hepatitis) demonstrating the presence of IgGK paraprotein and free gamma and kappa chains. Troughs contained: A, antihuman whole serum; B, antihuman IgG; C, antihuman lambda chains; D, antikappa chains.

Two of the seven patients with active chronic hepatitis showed IgM paraproteinemia. Two had monoclonal IgA and one had an IgG M-component. The remaining case presented two paraproteins, identified as IgG and IgM. Here again the spikes
fluorescence with mitochondria-rich tissue fractions (Roitt and Doniach, 1969). It is of interest that the cryoprecipitates in these two cases both contained IgM and IgG and both had antiglobulin activity, but only one showed antimitochondrial specificity. Alpha1 FP was found in the cryoprecipitates of three patients with acute viral hepatitis and in one case of active chronic hepatitis. Antibodies to alpha1 FP were found in two patients with acute viral hepatitis.

The Bence Jones protein in case 18 was present in small quantities and concentrates from 3 litres of urine were necessary to establish that it was of kappa type.

Discussion

The patients described in the present study were highly selected since a cryoglobulinaemia of more than 100 mg/100 ml occurred in less than 1% of sera tested. However it was surprising to find paraproteins in as many as two-thirds of these patients. The autoantibody activities of the sera were typical of those usually found in these diseases (Doniach et al, 1966), i.e., smooth muscle antigen and antiglobulins were present in the cases of acute viral hepatitis; the patients with active chronic hepatitis had ANA, antiglobulins, smooth muscle antigens, antibodies to heat-denatured DNA in two cases, and microsomal antibodies directed primarily against liver and kidney (Rizzetto, Swana, and Doniach, 1973) in two others. The patients with primary biliary cirrhosis had high titre mitochondrial antibodies and two also had antiglobulins, as reported more fully in the cryoglobulin study (Florin-Christensen et al, 1974).

Some of the paraproteins possessed antibody activities which were found in whole sera. Follow-up studies showed that all three phenomena, i.e., antibodies, paraproteinaemia, and cryoprecipitation, were of a transient nature in acute viral hepatitis while they appeared to be constant in active chronic hepatitis and primary biliary cirrhosis.

The finding of alpha1 FP in certain cryoprecipitates led to the search for antibodies to this protein (Florin-Christensen and Arana, 1973). These were found in two patients positive for HB Ag. In one case the anti alpha1 FP specificity was confined to the IgMK paraprotein, while in the other the response was polyclonal.

Dielonar spikes are known to occur in lymphoproliferative disorders but have not been found so far in acute viral hepatitis or autoimmune liver diseases. When present, they usually share a common type of light chain (Natvig and Kunkel, 1973) and this is probably true of our three cases with dielonal spikes.

The presence of free heavy and light chains is not uncommon in plasma cell malignancies and in primary amyloidosis (Osserman, Takatsuki, and Talal, 1964) but has not been previously reported in the present context. These incomplete immunoglobulins could result from a disturbance of synthesis in the affected clone(s) as postulated for myeloma, but the possibility cannot be excluded that normal immunoglobulin molecules undergo enzymatic proteolysis in the serum.

The fact that all these patients were selected for their cryoglobulinaemia probably explains why the six cases with monoclonal antibodies had antiglobulins, since it is now known that antigamma globulin factors are produced in response to circulating immune complexes. Antiglobulins are also the commonest autoantibody activity found in monoclonal proteins whether benign or malignant. It must be supposed that the factor which triggers the uncontrolled proliferation of a B cell to produce monoclonal proteins is more likely to meet with cells from an already expanded or expanding clone. In acute viral hepatitis, the transient nature of the

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Serum Ab Titres</th>
<th>Isolated Paraprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Human Gamma Globulin (Crohn's II)</td>
<td>alpha1 FP</td>
</tr>
<tr>
<td>1</td>
<td>Acute viral hepatitis</td>
<td>2560</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>Acute viral hepatitis</td>
<td>160</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Acute viral hepatitis</td>
<td>10240</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Active chronic hepatitis</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>Primary biliary cirrhosis</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>Primary biliary cirrhosis</td>
<td>+</td>
<td>—</td>
</tr>
</tbody>
</table>

Table II Autoantibody activities in serum and isolated paraproteins of six patients with liver disease and cryoglobulinaemia

*Not titred*
autoantibodies and of the uncontrolled clonal proliferation suggest that both are directly related to the virus. In chronic autoimmune liver disorders such as active chronic hepatitis or primary biliary cirrhosis the initiating cause is unknown, but the persistent stimulus to autoantibody formation probably causes a similar selection of an already expanded cloneseckering smooth muscle antibodies or ANA in the case of active chronic hepatitis and mitochondrial antibodies in primary biliary cirrhosis as likely candidates for paraprotein synthesis.

Antibody activity to self antigens has been found in myeloma proteins Wager, Rasanen, Haltia, and Wasastjerna (1971) studied two myeloma proteins, one IgA and one IgM with smooth muscle antibody activity and a further IgG myeloma with anti-thyroglobulin activity. However the most commonly detected antibody activities in myeloma proteins are those directed against streptococcal and staphylococcal antigens, which again suggests that the trigger for malignant proliferation affects clones that are likely to be expanded since these organisms constantly stimulate immune responses. The unique physicochemical properties of cryoprecipitates make it possible to isolate immune complexes. In the case of cryoglobulins with autoantibody activity, particularly if the antibody is monoclonal, there is a possibility of isolating all the constituents. In mixed cryoglobulins with antiglobulin activity this has been achieved. In addition to IgG and anti-IgG antiglobulin, some systemic lupus erythematosus sera show DNA in the cryoprecipitate (Bluestone, Golberg, Cracchiolo, and Barnett, 1970; Arana, Roux, Garcia Morteo, and Florin-Christensen, 1971), and although the source of this DNA could be foreign, it is clear that it constitutes the primary antigen in the soluble complexes which then evoke the antiglobulin response.

In the case of mitochondrial and smooth muscle reaction, the antigens have not so far been demonstrated in the cryoprecipitates, and a detailed analysis of such cryoprecipitates will make it possible to find out whether the antigens are normal body constituents or if they are in some way modified by a hypothetical infective agent.

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