A New Case of Mu Heavy Chain Disease: Clinical and Immunochemical Studies

By Franco Dammacco, Lorenzo Bonomo, and Edward C. Franklin

A new case of mu heavy chain disease is described. The patient, a 48-yr-old Italian housewife, suffered from chronic lymphocytic leukemia of 3 yr duration with anemia and hepatosplenomegaly, but no peripheral lymph node enlargement. Serum protein studies disclosed a small M component with gamma-1 mobility ascribable to free kappa chains, hypogammaglobulinemia, and mu chain fragments with abnormally anodic mobility. Appreciable amounts of kappa chains were also detected in the urine. The mu chain disease protein had a molecular weight of approximately 54,000 and a heterogeneous NH$_2$ terminus, alanine being the main identifiable residue. The NH$_2$ terminal of the kappa chain was glutamic acid. No J chain was detected ascribable to free kappa chains, hypergammaglobulinemia, and mu chain disease protein.

Following the discovery by Franklin and associates of heavy chain disease of the IgG type in 1963,\(^1\) alpha chain and mu chain diseases have been identified.\(^2\) In all cases, an abnormal protein immunologically related to the heavy chain of IgG, IgA, or IgM and devoid of light chains has been found in serum, and sometimes also in urine.

In at least four gamma chain disease proteins, the basic abnormality has been found to be an internal deletion affecting the Fd fragment to a variable extent, while several others appear to be the result of proteolytic digestion of a larger precursor.\(^6\)-\(^10\) Although it is conceivable that a structural deletion of the heavy chain is also present in alpha and mu chain disease proteins, the demonstration of such an abnormality is still lacking.

Five cases of heavy chain disease of the IgM type have been published so far.\(^1\) It is the purpose of the present report to describe the clinical and immunologic studies performed in a new (sixth) case of mu chain disease.

CASE REPORT

I.A., a 48-yr-old housewife from Altamura, Italy, was first admitted to the local hospital in March 1971 complaining of fatigue, weakness, anorexia, and a heavy feeling in the left hypochondrium of 6 mo duration. Past history was unremarkable except for the fact that the patient had undergone a hysterectomy in 1965 because of a large fibroma. On physical examination...
diffuse pallor, intermittent fever with temperature elevations to 37.5–38°C (99.5–100.4°F), and moderate hepatosplenomegaly were noted. Laboratory studies revealed a hemoglobin of 9.5 g/100 ml, white blood cell count 19,600/cu mm, with 43% polymorphonuclear leukocytes, 50% mature lymphocytes, 3% monocytes, and 4% eosinophils. Platelets were 80,000/cu mm. Urinalysis showed 1+ proteinuria; blood urea nitrogen was 27 mg/100 ml. A bone marrow aspirate was reported as showing an over-all increase of small lymphocytes. No comment was made concerning the presence of vacuolated cells.

A diagnosis of chronic lymphocytic leukemia was made, and the patient was discharged on treatment with prednisone (20 mg daily). In the ensuing 18 mo she was relatively well except for two bouts of acute pneumonia which were treated at home and responded promptly to antibiotics. In September 1972, during a recurrence of pneumonia, she was readmitted to the same hospital. Physical examination disclosed a temperature of 39°C (102°F), rhonchi and rales over both lung bases, dyspnea, and no major changes in the hepatic and splenic enlargement. Hemoglobin was 9 g/100 ml, white blood cells 22,000/cu mm with 50% lymphocytes. Ampicillin administration (2 g daily intramuscularly) brought about a remarkable improvement of the patient’s general condition, and on October 3, 1972, she was referred to us for further evaluation of her hematologic picture.

Weakness, weight loss, nocturnal sweats, and a mild pain in the left hypochondrium were her main complaints on admission. Physical examination revealed a thin, pale woman with a slightly distended abdomen showing a vertical umbilico-pubic scar referable to previous hysterectomy. The liver was enlarged two fingerbreadths below the right costal margin, and the spleen tip was felt about 5 cm below the left costal margin. Peripheral lymph nodes could not be palpated.

During 2 mo hospitalization a number of laboratory tests were performed, but for the sake of brevity only the significant results will be reported. Hemoglobin fluctuated from 8.5 to 10 g/100 ml, hematocrit 30–33%, erythrocytes 2,700,000–3,300,000/cu mm, white blood cell count on admission was 18,500/cu mm with 47% neutrophils and 43% lymphocytes, but fell gradually to 5000/cu mm (32% lymphocytes) when chlorambucil was added to the therapeutic regimen. Platelets ranged between 60,000 and 95,000/cu mm. The ESR was 92 mm in 1 hr, urinalysis showed 3+ proteinuria, and blood urea nitrogen was 48 mg/100 ml. The patient refused to undergo bone marrow biopsy. A rectal biopsy did not reveal amyloid deposits. Skeletal roentgenograms showed a moderate degree of osteoporosis, but no punched-out skeletal lesions.

Because of the finding of hypogammaglobulinemia and a small M component on serum protein electrophoresis, as well as a positive urinary Bence Jones test, further immunologic studies were undertaken, which will be described in detail below.

Since November 1972, the patient has been kept on prednisone (10 mg daily) and chlorambucil (2 mg each day orally), with good symptomatic improvement. She has been seen twice (in February and June 1973) in the outpatient department. Anemia (hemoglobin 9 g/100 ml) is still present, and both serum and urine protein abnormalities appear unchanged, but she is otherwise doing well.

**MATERIALS AND METHODS**

**Immunological Studies**

Routine electrophoresis on cellulose acetate membranes, immunoelectrophoresis, and Ouchterlony double diffusion were performed as described elsewhere. A number of antisera were used throughout these studies which were either prepared in our laboratory or purchased from Hoechst-Behringwerke, Marburg-Lahn, Germany, and from the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. Quantitative determination of serum IgG, IgA, IgM, and IgD levels was accomplished by single radial immunodiffusion using commercially available diffusion plates (Tri-Partigen and LC-Partigen from Hoechst-Behringwerke).

For the isolation of mu chain from the patient’s serum, a combination of starch block electrophoresis and immunosorbent had to be employed. Gel filtration chromatography was performed on Sephadex G-200 columns equilibrated with 0.2 M phosphate buffered saline, pH 8.0. Analytical ultracentrifugation was carried out in a Beckman Spinco Model E ultracentrifuge at 59,780 rpm and 20°C.
Chemical Investigations

Amino terminal analysis was performed by the dansyl-chloride method. Quantitative amino acid analysis was carried out with a Beckman automatic analyzer (Model 121); the protein was first performic acid-oxidized and then hydrolyzed under vacuum at 110°C for 20 hr in constant boiling HCl containing 0.1% phenol. Partial reduction of the mu chain protein was performed with 5 mM dithiothreitol followed by alkylation with 2 mM iodoacetic acid. Further details of the chemical procedures are described elsewhere. Disk electrophoresis of the reduced and alkylated material was performed in polyacrylamide gels in the presence of urea according to the method of Reisfeld and Small. Dodecyl sulfate polyacrylamide electrophoresis was done as described by Maizel.

RESULTS

Serum total protein was 6.2 g/100 ml. The cellulose acetate electrophoretic pattern of the patient's serum revealed a small M component with a mobility intermediate between beta and gamma globulin (Fig. 1), as well as an increase of the alpha-2 fraction and marked hypogammaglobulinemia. Per cent distribution of the electrophoretic fractions was as follows: albumin, 66.2%; alpha-1 globulin, 4.4%; alpha-2 globulin, 17.0%; beta globulin, 5.8%; gamma globulin, 6.6%.

Immunoelectrophoresis of the patient's serum with a horse antihuman serum showed a reduction of the IgG precipitin line and absence of the IgA and IgM lines (Fig. 2A). When a panel of monospecific antisera (antisera to IgG, IgA, IgM, IgD, IgE, K, L) was employed, the electrophoretic M component was found to be ascribable to the accumulation in the serum of free K chains. In addition, with the anti-IgM serum, a strong precipitin line was obtained, which exhibited an abnormally fast mobility as compared with normal IgM and Waldenström-type macroglobulins (Fig. 2B). Such an abnormal IgM component showed no reactivity with either anti-kappa or anti-lambda sera. Quantitation of serum immunoglobulins by single radial immunodiffusion gave the following results: IgG, 400 mg/100 ml (46 IU/ml); IgA, 18 mg/100 ml (10.7 IU/ml); IgD, 0. IgM could not be quantitated since a large and faded precipitin ring was obtained for which no suitable standard was available.

Electrophoresis and immunoelectrophoresis of the concentrated urine disclosed traces of albumin and alpha-2 globulin and a spike consisting of free
Fig. 2. (A) Immunoelectrophoresis of serum I.A. (upper well) and of a normal human serum (lower well), developed with a horse anti-human serum. A reduction of the IgG line and the apparent absence of the IgA and IgM lines may be seen in the upper pattern. (B) Upper well, serum I.A.; lower well, serum of a patient with Waldenström's macroglobulinemia; trough, anti-human IgM serum. Note the IgM line with abnormally fast mobility given by serum I.A.

Isolation of clean mu chain protein in workable amounts proved difficult to achieve. By starch block electrophoresis, abnormal mu chains were localized in the alpha-2 region, but they were always contaminated by some other serum proteins, and purification could only be obtained by passing the protein through CNBr-activated Sepharose columns conjugated with anti-IgM serum. The yield was, however, rather low, approaching 4%, 5%, of the starting material. When tested by both immunoelectrophoresis and Ouchterlony double diffusion, the isolated component failed to react against five different anti-kappa and anti-lambda sera, even after reduction and alkylation.

Analytical ultracentrifugation (Fig. 3) of the patient's serum revealed no increase of the 19S peak, thus confirming that the strong immunoelectrophoretic IgM line was due to abnormally small molecules. The sedimentation coefficient of the isolated component was found to be 5.1S, but polymers with higher sedimentation rates were seen with some preparations. The tendency of the protein to form polymers was also apparent in Sephadex gel filtration experiments at neutral or alkaline pHs; indeed, IgM antigenic determinants could be detected in a broad chromatographic area ranging from the void volume to the descending half of the 7S peak. The molecular weight of the mu chain protein, calculated by both calibrated Sephadex columns and SDS-polyacrylamide electrophoresis, was found to be approximately 54,000.

Amino terminal analysis of the isolated mu chain protein disclosed a certain kappa chains with the same mobility as the serum M component. An average daily output of 3 g Bence Jones protein was calculated.
heterogeneity, in that alanine was the main residue but small amounts of valine, isoleucine, and glycine were also detected. The amino terminus of the kappa chains isolated from the patient’s urine was glutamic acid. The amino acid composition of the mu chain protein is given in Table 1.

After partial reduction and alkylation, the purified mu chain protein was tested by polyacrylamide gel electrophoresis in the presence of urea: J chains could not be detected.

Table 1. Amino Acid Composition (Average of Three Analyses) of the Mu Chain Disease Protein I.A.*

<table>
<thead>
<tr>
<th>Amino Acid†</th>
<th>Moles of Residues per Mole of Protein</th>
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<tbody>
<tr>
<td>Lysine</td>
<td>27.0</td>
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<tr>
<td>Histidine</td>
<td>8.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>17.3</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>12.5</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>41.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>49.4</td>
</tr>
<tr>
<td>Serine</td>
<td>46.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>51.1</td>
</tr>
<tr>
<td>Proline</td>
<td>35.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>36.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>31.2</td>
</tr>
<tr>
<td>Valine</td>
<td>41.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>14.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>35.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>8.3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Total 484.6

*Oxidized samples, 20 hr hydrolysis.
†Tryptophan was not calculated.
DISCUSSION

The clinical picture of our patient is quite similar to the previously reported instances of mu chain disease: indeed, fatigue, anemia, and hepatosplenomegaly without peripheral lymph node enlargement have been found so far in all the patients. In addition, the hematologic findings and the slow progression of the disease are consistent with the clinical diagnosis of chronic lymphocytic leukemia. The diagnosis of mu chain disease is based on the results of serum and urine protein studies. In serum, hypogammaglobulinemia is associated with an abnormal negatively charged IgM component which lacks light chains and usually produces a rather diffuse increase of the alpha-2 globulin fraction in the absence of a clear-cut spike. The absence of a recognizable monoclonal component may preclude recognition of the disorder, unless immunoelectrophoretic analysis with monospecific antisera is performed. In the urine, four of the five patients with mu chain disease so far described had a kappa Bence Jones protein. Our patient did, indeed, have a small M component on serum protein electrophoresis; this was, however, immunologically identified as due to free kappa chains. Appreciable amounts of kappa-type Bence Jones protein were also detected in her urine.

At variance seems to be a recent case of mu chain disease (Seligmann, personal communication): this African patient was not affected with chronic lymphocytic leukemia, exhibited a noticeable spike in the alpha-2 region on routine serum electrophoresis, and had no Bence Jones protein in the urine. Separate mention should also be made of the case reported by Josephson et al. This patient suffered from an indurated ulcerating lesion over the left parotid gland and left ear, and had no apparent chronic lymphocytic leukemia. In addition, along with IgM fragments, his serum contained an IgA monoclonal component. Since the IgM fragment was not isolated, some doubts remain as to the true nature of his protein abnormality. It seems, therefore, likely that, as new cases of mu chain disease are described, further heterogeneity of clinical and immunological features will be detected.

A peculiar characteristic of mu chain disease is the presence in the bone marrow of sheets of plasma cells, many of which appear strikingly vacuolated. Unfortunately, bone marrow biopsy could not be performed in our patient, and bone marrow smears prepared in another hospital in 1971 were no longer available for reexamination.

Biosynthetic tissue culture studies have established that the intracellular synthetic products consist of the mu chain fragments, their polymers, and free light chains, thus excluding that extracellular degradation occurs to any appreciable extent. It has therefore been postulated that vacuolization of the plasma cells depends upon the accumulation of the abnormal polypeptide chains and their polymers near the Golgi zone, the structural defect possibly interfering with full assembly of the molecule. It is tempting to speculate in this connection that failure to demonstrate the presence of J chain in our protein, as well as in protein G1 (Franklin, unpublished observation), represents still another aspect of the disordered molecular synthesis and assembly of the mu chain fragments. How the subunits may undergo polymerization in the absence of J chain and
the precise extent of the internal heavy chain deletion remain so far undetermined.

Note added in proof: After this paper had been submitted for publication, another case of mu chain disease has been published (Biserte et al.: Nouv. Presse Med. 2:1997, 1973). This 76-yr-old male patient complained with pain of the thoracic and lumbar spine; he had a small tumor of the right lower eyelid but no chronic lymphocytic leukemia and no urinary Bence Jones protein. The mu chain disease protein migrated as an M-component in the alpha-1 region simulating bisalbuminemia.

REFERENCES
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