MU-Chain Disease in an African Patient

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A new case of μ-chain disease occurring in an African patient is described. The main clinical feature was a massive splenomegaly. Contrary to five previously reported cases, this patient was not affected with chronic lymphocytic leukemia, and vacuoles were not found in the cytoplasm of the bone marrow plasma cells. A considerable increase of the plasma volume was noted without hyperviscosity. The electrophoretic pattern showed an abnormal band in the α2 region. The anomalous protein was devoid of light chains and was present in the form of disulfide-linked polymers of incomplete μ-chains. The molecular weight of the monomer was approximately 58,000. The protein comprised the Fc fragment and a part of the Fd segment. Bence Jones protein was not found in the urine, and light chains were not present in the cells producing the μ-chain disease protein.

HEAVY-CHAIN diseases (HCD) are defined by the presence in the serum of a homogeneous population of molecules consisting of incomplete heavy polypeptide chains belonging to a given class or subclass of immunoglobulins (Ig) and devoid of light chains. Three types of HCD are now recognized; they correspond to the three main classes of Ig heavy chains. Gamma heavy-chain disease was initially described by Franklin in 1963. Twenty-five additional cases have been published or brought to our knowledge (see reference 2). Alpha-chain disease (αCD) was first recognized in 1968 and appears not to be an uncommon disorder, since we are aware of at least 60 cases. Mu-chain disease (μCD) was first described in 1970 and only four more patients with this disorder have been found since.

We report a new case of this rare lymphoproliferative disease that occurred in an African patient and differed by several features from the previously reported cases.
MATERIALS AND METHODS

Serum electrophoresis was done in agar gel and on cellulose acetate membranes with microzone apparatus. Immunoelectrophoresis was performed with 2\textdegree\textsuperscript{o} agar gel in barbital buffer, ionic strength 0.05, pH 8.2. Ouchterlony diffusion studies were carried out in 1.5\textdegree\textsuperscript{o} agar in 0.15 M NaCl at pH 7.2. Quantitative Ig determinations were performed by the radial diffusion technique. Analytical ultracentrifugation was performed with a Spinco model E ultracentrifuge at 59,780 rpm at 20\degree C. Sedimentation coefficients were obtained in borate buffer (pH 7.5), 0.15 M in NaCl, and calculated by standard methods. Density gradient preparative ultracentrifugation with 10\textdegree, 40\textdegree sucrose gradients was performed by standard methods. Twenty-four-hour urine samples were filtered and concentrated by negative pressure dialysis in Visking 23/32 tubing. Purification of the \( \mu \) CD protein was effected by gel filtration of the delipidated serum on Sepharose 6B (Pharmacia, Uppsala) columns equilibrated in 0.1 M Tris HCl buffer, pH 8.0, 1 M in NaCl. The ascending limb of the first peak contained essentially pure \( \mu \)CD protein. Sodium dodecylsulphate acrylamide gel electrophoresis was performed according to the procedure of Weber and Osborne.\textsuperscript{11} The molecular weight of the reduced alkylated \( \mu \)CD protein was measured using suitable markers (\( \mu \)-chains, \( \gamma \)-chains, alcohol dehydrogenase, and \( \kappa \)-chains). The papain pentameric F\(_{\mu}\) fragment was prepared according to Mihaesco and Seligmann,\textsuperscript{12} the tryptic (F\(_{\mu}\)) 5 fragment according to Plaut and Tomasi,\textsuperscript{13} and the F(\(ab\'))\(_{2\mu}\) peptic fragment according to Mihaesco and Seligmann.\textsuperscript{14} All antisera were prepared in our laboratory in rabbits and conveniently absorbed. The procedures used for the immunofluorescence detection of intracytoplasmic and membrane-bound Ig have been previously described.\textsuperscript{15}

RESULTS

Clinical and Hematological Findings

Patient B.O., a 45-yr-old black man, was born in Ivory Coast, where he had always lived in the tropical bush. He was admitted to the University Hospital of Abidjan for weakness, anorexia, recent diarrhea, and weight loss. He was further investigated in the Department of Hematology of the Hôpital Saint-Louis in Paris because of findings suggestive of \( \mu \)-chain disease.

The main clinical feature was huge splenomegaly, reaching the umbilicus on first admission and the pubis 3 mo later. The spleen was firm and irregular without palpable nodules. Moderate hepatomegaly was found. There was no significant peripheral lymph node enlargement. The fundi were normal.

Routine hematologic examinations were made difficult by the high tendency to rouleaux formation. The RBC count was 1,500,000/cu mm with 5 g/100 ml of hemoglobin. WBC count was 3000–5000/cu mm with 30\textdegree, 50\textdegree granulocytes, 40\textdegree, 55\textdegree mature lymphocytes, and 2\textdegree, 3\textdegree, plasma cells. Platelet count was 70,000/cu mm. Repeat bone marrow aspiration revealed 30\textdegree, 40\textdegree, 60\textdegree mature lymphocytes and 8\textdegree, plasma cells with a few intermediate cells. All plasma cells were mature with a normal appearance, and they never contained cytoplasmic vacuoles. No hypereosinophilia was noted. On histologic examination, the bone marrow was hypercellular with marked diffuse lymphoplasmacytic infiltration. After special stains, neither densification of the reticulin fibers nor amyloidosis were observed.

Only the nodes on the left side were opacified after abdominal lymphangiography, and they showed a moderate hyperplasia. Chest and skeletal x-ray examination showed no abnormality. Hepatosplenic scintigraphy (\( ^{99m}\text{Tc} \) sulfur colloid) demonstrated inhomogeneity of the spleen.

The ESR was 118 mm/hr. Serum proteins were 8.5 g/100 ml with, on electro-
Electrophoresis, a low albumin (2.5 g/100 ml) and a broad abnormal band in the region of the α2 globulins (Fig. 1) accounting for approximately 3 g/100 ml. Gamma globulin was 1.1 g/100 ml. Proteinuria was 0.2–0.3 g/24 hr.

The RBC volume measured by 51Cr was normal (1600 ml), whereas extremely high figures (close to 8 liters) were found for the plasma volume. The serum viscosity was very slightly increased (2 min 30 sec compared to a control of 1.30 min).

Intradermal reactions to PPD, Candida, and streptokinase streptodornase were negative. DNCB sensitization was not performed. The direct antiglobulin (Coomb's test) and the tests for rheumatoid factors were negative. Antinuclear, smooth muscle, gastric, and mitochondrial antibodies were not demonstrated.

The feces were found to contain 0.5 g of fat per 24 hr. The glucose tolerance test and serum folate concentration were normal. x-Ray films of the GI tract, including the small bowel, were within normal limits. Parasitologic investigations were essentially negative, except for the presence of Ancylostoma eggs in the stools and immunofluorescence positivity for Plasmodium berghei.

Bromosulfonephthalein elimination, serum alkaline phosphatase, glutamic transaminase, and bilirubin levels were normal. The study of the clotting factors revealed a decrease in the level of factors II (40%) and VII + X (38%), which was not modified after administration of vitamin K1 and of fibrinogen (0.15 g/100 ml). There was no fibrinolysis nor detectable degradation products. Laparoscopic examination showed a slightly congested liver without portal hypertension. A needle biopsy specimen of the liver showed only a moderate infiltration of mononuclear cells in portal spaces. A marked decrease in the level of total cholesterol (60 mg/100 ml) and total lipids (0.17 g/100 ml) was observed. Normal results were found for serum calcium and iron, and blood urea nitrogen and ECG. Rectal biopsy was not performed because of the clotting defects.

Treatment by plasmapheresis, chlorambucil, and spleen irradiation was instituted. A decrease in the size of the spleen was observed.
Immunoglobulin Studies

The abnormal broad band found in the α2 region on the agar gel and cellulose acetate electrophoretic pattern of the serum was easily identified by immunoelectrophoretic analysis. Immunoelectrophoresis with polyvalent antisera to normal human serum revealed an abnormal precipitin line (Fig. 2) in the α2-β1 region, crossing at its extremities the cathodic end of the serum albumin line and the anodic end of the IgG line. This abnormal protein reacted with all specific antisera to IgM. The fast mobility and the curvature of this precipitin line were different from those seen in cases of macroglobulinemia. This anomalous component with μ-chain determinants was not seen on immunoelectrophoretic patterns developed with several antisera specific for κ- or λ-light chains (Fig. 2).

Ultracentrifugal analysis of whole serum in sucrose density gradient indicated that the molecules bearing μ-determinants and lacking light-chain determinants were mainly distributed in a peak situated between the 7S and 19S areas. Molecules able to precipitate with the antiserum specific to IgM were also found in the 7S and 19S peaks. The schlieren pattern of the serum in the analytical ultracentrifuge showed an abnormal peak whose apparent sedimentation coefficient at a protein concentration of 10 mg/ml was 11S and which accounted for 36% of the proteins (Fig. 3). The values for the 4S, 7S, and 19S components were 37%, 19%, and 8%, respectively.

Immunochemical quantitation of the pathologic IgM protein by radial diffusion could not be performed because of the lack of valid standard for a calibration curve. The values for IgG and IgA were 90 and 29 IU/ml, respectively.

The amount of protein excreted in the urine was low and ranged between 0.05 and 0.4 mg/ml. The thermosolubility test for Bence Jones proteins was negative. The immunoelectrophoretic analysis of the concentrated urine proteins showed that the main constituent was the fast-moving component that

Fig. 2. Immunoelectrophoretic analysis of the serum of the patient (BO) compared to normal human serum (N) with (1) antiserum to whole normal serum, (2) antiserum specific for IgM, (3) antiserum to κ-light chains, (4) antiserum to λ-light chains. The anode is to the left. The precipitin line given by the μCD protein is shown by an arrow.
Fig. 3. Ultracentrifugal schlieren pattern of serum BO at a protein concentration of 10 mg/ml. The photograph was taken at 37 min after the speed of the rotor had reached 59,780 rpm.

reacted with the antiserum to IgM and not with the antisera to light chains. Only trace amounts of free light chains of both antigenic types were found.

Intracytoplasmic immunofluorescence studies performed on fixed smears of bone marrow cells showed that 10% of the nucleated cells were stained with the conjugated antiserum to the \( \mu \)-chains, whereas they did not react with the antisera to \( \kappa \)- or \( \lambda \)-light chains. The majority of these \( \mu \)-positive cells were plasma cells. The percentage of cells reacting with antisera to \( \gamma \), \( \kappa \), and \( \lambda \) chains were 1.5\%, 0.5\%, and 1\%, respectively. Only occasional cells were stained by the antiserum specific for IgA. Surface staining of the living bone marrow cells with double labeling experiments showed that the \( \mu \)-containing plasma cells bore membrane-bound \( \mu \)-determinants in the absence of light-chain determinants. The study of membrane-bound Ig of the peripheral blood lymphocytes showed a moderate decrease of IgM-bearing cells. The figures were the following: 6% for \( \mu \), 5% for \( \gamma \), less than 0.1% for \( \alpha \), 8% for \( \kappa \), and 3% for \( \lambda \). Eighty-four percent of the blood lymphocytes were able to form spontaneous rosettes with sheep erythrocytes.

The isolated \( \mu CD \) protein was found to be essentially pure when tested in gel double diffusion at a concentration of 10 mg/ml. The purified protein did not precipitate with a number of antisera to \( \kappa \)- or \( \lambda \)-light chains and did not combine with anti-\( \kappa \) or anti-\( \lambda \) antibodies. No light chains were seen following reduction and alkylation on urea starch-gel or SDS polyacrylamide electrophoresis. The antigenic analysis of the protein was performed in Ouchterlony plates with selected and conveniently absorbed antisera to IgM, \( \mu \)-chains, and IgM proteolytic fragments. The protein contained all antigenic determinants found in the papain and trypsin pentameric Fc\( \mu \) fragments. Both these fragments were found to be deficient when compared with the \( \mu CD \) protein, the spur over the papain fragment being stronger than that over the trypsin frag-
ment. Some μ-chain determinants of the peptic F(ab)''2μ fragment unrelated to conformational specificity were found in the pathological protein. Moreover, this protein was able to precipitate with an antiserum to Fabμ fragment previously absorbed with light chains. The presence in the μCD protein of some Fd determinants, presumably located in the Cl region, could thus be assumed. Antigenic deficiency of the μCD protein when compared to μ-chains from Waldenström macroglobulins could not be demonstrated using several antisera. The molecular weight of the reduced and alkylated monomer of the μCD protein, estimated in SDS polyacrylamide gel electrophoresis, was 55,000–60,000. The ultracentrifugal pattern of the unreduced purified protein showed two components with corrected sedimentation coefficients of S_{20w}^{0} = 11.7 s and S_{20w}^{0} = 18.7 s.

DISCUSSION

Mu CD appears to be the rarest of the three known forms of HCD. This report presents the sixth known patient with this disorder. Three previous cases have been reported in detail and two others in abstract form or as an unpublished observation. All five subjects had long-lasting chronic lymphocytic leukemia. No additional cases were found when more than 150 patients with chronic lymphocytic leukemia were screened for this biochemical abnormality in the Research Institute on Blood Diseases in Paris. The patient reported by Josephson et al. presented quite a different syndrome. He was suffering from an indurated ulcerating lesion over a parotid gland and massive splenomegaly. He was found to have two distinct serum Ig abnormalities: a monoclonal IgA and a protein fragment immunologically related to IgM and devoid of light-chain activity.

Our patient differs in several respects clinically and in his laboratory abnormalities from the five first patients with μCD. He was not diagnosed as having chronic lymphocytic leukemia. The number of peripheral blood lymphocytes was not increased, and no B cell monoclonal proliferation similar to that found in chronic lymphocytic leukemia was found by the study of membrane-bound Ig. The main clinical finding was massive splenomegaly. The lymphoid cells and plasma cells showed no cytoplasmic vacuoles, which were found in all four previous patients. Neither bony lesions nor amyloidosis were observed in our patient. The considerable increase in the plasma volume leading to a "false anemia" is worth noting.

The amount of μCD protein in serum was much greater than in all previously published cases in which the routine electrophoretic pattern was normal except for marked hypogammaglobulinemia. In the present patient, the decrease in IgG was moderate, and a clearly abnormal band was easily detected in the α2 region of the electrophoretic pattern. As seen in other varieties of HCD, this band was broader than the usual spike of monoclonal Ig. As in all other patients with μCD, the abnormal component migrated more rapidly than normal or monoclonal IgM. An important difference between the presently reported case and four of the five previous patients is the absence of Bence Jones protein in the urine and the absence of light chains in (and on) the bone marrow cells producing the μCD protein. Zucker-Franklin and Franklin had
established in their patient that μ-chain fragments and κ-chains were present in the same cell and had postulated that the μ-chain defect interfered with proper assembly of the IgM molecule. In this regard, the situation in our patient is thus similar to that found in all cases of γHCD and αHCD. The μCD protein was excreted in small amounts in the urine. One may recall that a similar finding was made in most patients with α-chain disease and in a number of cases of γHCD.

The immunological characterization of the μCD protein BO and the estimation of the molecular weight of its monomer indicate that the portion of the μ-chain which is missing is of a relatively small size. The molecular weight of the monomer of this anomalous protein appears to be higher than that of the single μCD protein which has been previously isolated. Our immunological and physicochemical data suggest that the entire Fc portion and part of the Fd segment are present in protein BO, and, in view of its sedimentation coefficient, this protein is possibly made of ten disulphide-linked incomplete μ-chains. Studies are currently in progress to elucidate the structure of these μ-chains and the location in the Fd segment of the presumed deletion.

In view of the geographic distribution of patients with α-chain disease and of the postulated role of intestinal microorganisms in the etiology and pathogenesis of this disease, it is of interest that our patient originated from and had been living in subtropical Africa, where parasitic infestation constitutes an important stimulus for the proliferation of IgM-producing plasma cells. Note added in proof. Since this manuscript was submitted, we have been aware of four other cases of μ-chain disease. In one case reported by Biserte et al. as a letter in Nouv. Presse Méd. 2: 1997, 1973, the level of the pathologic protein in the serum is high enough to give an abnormal band on the electrophoretic pattern. There is no Bence Jones proteinuria; there are no vacuolated plasma cells in the bone marrow; and the patient does apparently not suffer from chronic lymphocytic leukemia. In another patient studied by Dammacco and Franklin (personal communication), the main features were chronic lymphocytic leukemia with vacuolated marrow plasma cells, no abnormal spike at serum electrophoresis, and presence of a κ Bence Jones protein. The third patient was studied by Radl et al. (unpublished observation). This 70-yr-old woman was affected with a reticulum cell sarcoma in the lymph nodes without peripheral blood or marrow abnormalities. There was no spike on the serum electrophoretic pattern and a transient Bence Jones proteinuria of the λ type was noted. Lastly, we have found a new case of μ-chain disease in another patient from Ivory Coast with enlarged spleen and without chronic lymphocytic leukemia.

REFERENCES
of abdominal lymphoma and a new type of IgA abnormality ("alpha chain disease"). Digestion 1:321, 1968


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