Bence Jones Myeloma with a Tetramer of Kappa-Type Globulin in Serum
M. L. Gallango, R. Suinaga, and M. Ramírez

We describe a case of Bence Jones multiple myeloma involving two kappa-type monoclonal proteins. One was isolated from serum, the other from both serum and urine. After biochemical and immunochemical analyses, we concluded that one of the proteins was a tetramer, present in serum but not in urine, whereas the other was a monomer, which was excreted in large amounts in the urine during the terminal stage of the disease.

Patients with multiple myeloma often have Bence Jones protein in their sera and urine, even when myeloma protein is not detectable, but Bence Jones proteinemia without proteinuria is not so common (1). Only few such cases have been described so far (2–6), all with Bence Jones lambda-type protein, and all attributed to the presence of an abnormal protein, mainly a tetramer.

To our knowledge, no case of a tetrameric kappa-type Bence Jones globulin has been reported hitherto. We describe a case of multiple myeloma with large amounts of two monoclonal proteins of kappa light chains circulating in the serum, one of them a tetramer, the other a monomer. There was no evidence of Bence Jones proteinuria until the final stage of the disease.

Case Report

A 53-year-old woman was admitted to the hospital because of generalized bone pain. On physical examination she appeared chronically ill. There was exquisite tenderness to palpation over the sternum, anterior chest wall, and the lumbar spine. Roentgenograms demonstrated generalized osteoporosis with vertebral compression and fracture of the lower right ribs, and multiple osteolitic lesions of the skull. Examination of a bone-marrow aspirate showed no lymphoid activity but infiltration of mature and immature plasma cells, many of them vacuolated. The diagnosis of multiple myeloma was established.

Hemoglobin concentration was 106 g/L, erythrocytes 400 \( \times 10^{6} \) cells/mm\(^3\), leukocytes 1200/mm\(^3\). The blood sedimentation rate was 20 mm in the first hour. Serum proteins were (g/L, and percent of total): albumin 33.4 (64%), \( \alpha_1 \)-globulin 2.1 (4%), \( \alpha_2 \)-globulin 5.8 (11%), \( \beta \)-globulin 7.4 (14%), \( \gamma \)-globulin 3.7 (7%). Total protein concentration was 53 g/L. The concentration of urea nitrogen in the serum was 47 mg/dL, of creatinine 12.6 mg/dL, and of calcium 87 mg/dL. Urinalysis disclosed a moderate proteinuria, about 95 mg/24 h. Results of the classical heat test for Bence Jones proteinuria were negative. Serum immunoglobulins as determined by radial immuno-

diffusion were (mg/L): IgG 4090, IgA 60, IgM 90, IgD 18, and IgE 0. No cryoglobulin or pyroglobulin was found.

Five months after discharge, the patient was readmitted to the hospital, at which time the laboratory results were only slightly changed, except that the proteinuria was more pronounced. Despite therapy, she continued to deteriorate and died. No autopsy was performed.

Material and Methods

Fresh serum and urine were used and total protein was estimated by the biuret method (7). Electrophoresis was at room temperature on cellulose acetate membranes, with use of a barbital buffer (0.1 mol/L, pH 8.6). The membranes were scanned with a densitometer (Auto-Scanner; Helena Labs., Beaumont, TX).

We used Scheidegger's micromethod (8) for immunoelectrophoresis, with antisera against IgA, IgG, IgM, IgD, IgE, kappa, lambda, Fab and Fc fragments, and whole human serum. Some of these antisera were obtained commercially; others were obtained in our laboratory, by immunizing adult New Zealand albino rabbits subcutaneously in multiple sites with 2 mg of the purified Bence Jones protein emulsified in complete Freunds adjuvant, followed by intravenous injections of the antigen in buffered saline weekly for four weeks. The rabbits were bled a week after the last injection, and the antiserum appropriately absorbed with Bence Jones protein and isolated myeloma proteins of type lambda. For quantitative immunodiffusion (9) of IgG, IgA, IgM, and IgD we used Immunoplates (Hyland, Division of Travenol Labs. Inc., Costa Mesa, CA); IgE was quantitated by radioimmunoassay (Phadebas IgE, PRIST; Pharmacia Diagnostics, Uppsala, Sweden).

Fresh samples of serum and of urine were stored at 4°C for 24 or 48 h, to test for cryoglobulins; for pyroglobulins, the serum was gradually warmed to 56°C. The Bence Jones test was done on acidified urine, according to the technique of Snapper and Ores (10).

Protein isolation: Urine specimens (24 h) were filtered through Whatman No. 3 filter paper and concentrated by positive-pressure ultrafiltration with an Amicon ultrafiltration cell (Model 52; Amicon Corp., Lexington, MA). Samples of urine containing Bence Jones protein were precipitated by 45% saturation with ammonium sulfate. Precipitates and supernates were extensively dialyzed against distilled water, lyophilized, and tested with specific antisera for the presence of Bence Jones proteins. Fractions containing the Bence Jones protein were dialyzed against phosphate buffer (10 mmol/L, pH 8.3) for 48 h and then applied to a 2 X 30 cm column of Sephadex DE52 that had been equilibrated with the same buffer and eluted with an increasing gradient up to 0.3 mol/L.

Serum: Monoclonal proteins were isolated from the whole

---

Received Jan. 7, 1980; accepted June 6, 1980.

---

Department of Experimental Medicine, Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas 101, Venezuela.

CLINICAL CHEMISTRY, Vol. 26, No. 12, 1980 1741
serum by gel filtration on a 5 x 100 cm Sephadex G-200 column equilibrated with tris(hydroxymethyl)methylaminoacetic acid (0.1 mol/L, pH 8.00) containing 0.15 mol of sodium chloride per liter, or by precipitation with 45% saturated ammonium sulfate, followed by passage through Sephadex DE52 column, as for the urine.

Proteins eluted from the columns were dialyzed and concentrated and the purity of the monoclonal proteins was verified by electrophoresis on cellulose acetate and by immunoelectrophoresis with use of several specific antisera. When necessary, the proteins were rechromatographed and again dialyzed and lyophilized.

Reduction and alkylation were carried out according to Fleischman et al. (11), with 2-mercaptoethanol (0.2 mol/L) as reducing agent, followed by alkylation by addition of iodoacetamide to a concentration of 90 mmol/L. To learn more about the subunit structure of the reduced and alkylated proteins, we did gel filtration on Sephadex G-100, with a buffer containing 1 mol/L acetic acid as dissociating agent.

Relative molecular mass of purified Bence Jones proteins from the serum and urine was estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate according to Weber and Osborne (12), with use as markers proteins of known relative molecular mass: cytochrome c (13 x 10^3), k light chains (23 x 10^3), myoglobin (17.6 x 10^3), ovoalbumin (43 x 10^3), and transferrin (88 x 10^3) (Schwarz/Mann, Division of Becton Dickinson & Co.).

Results

During the course of the disease, we performed several studies on this patient.

On the first admission to the hospital, cellulose acetate electrophoresis of the serum showed a small M component in the β region and the presence of albumin in the urine. The serum immunoelectrophoresis showed no abnormal precipitin arc with antisera to IgG, IgA, IgM, IgD, IgE, Fab, Fc, or anti-Bence Jones type lambda, but one abnormal precipitin arc was developed with antisera to kappa light chain (Figure 1). The urine showed precipitin arcs identified as transferrin, haptoglobin, and albumin by use of antisera specific for these proteins.

No Bence Jones protein could be demonstrated in the urine of the patient at this time, either by the heat test or by immunoelectrophoresis of untreated, 200-fold concentrated, or diluted urine.

These results suggested the presence of Bence Jones proteinemia without Bence Jones proteinuria. The monoclonal protein was then isolated. Several months later, at the time
of the second admission to the hospital, serum immunoelectrophoresis showed a double-bowed precipitin arc developed only with antisera to kappa light chain (Figure 2). The proteinuria was heavier (8 to 10 g per 24 h) and a large M component was visible in the $\beta$ region of the urine electrophrogram (Figure 3). This urinary kappa Bence Jones protein corresponded to the fast monoclonal arc observed in immunoelectrophoresis of the serum. By precipitating the serum with ammonium sulfate at 45% saturation, we separated the monoclonal protein into two homogeneous components, corresponding to the double-bowed arc of precipitation observed in immunoelectrophoresis. One of them, the slower, was found in the precipitate and the other in the supernatant fluid (Figure 4).

The slow monoclonal arc was highly contaminated with transferrin, which was notably increased in this patient. The kappa light chains corresponding to this slow-migrating arc eluted together with transferrin from Sephadex G-200, just before albumin, indicating that the molecular mass of the kappa chains was greater than that of albumin (Figure 5). We removed the contaminating proteins with further such chromatographic passage. The relative molecular mass of the purified fraction was calculated to be $87,000 \pm 1000$. After reduction and alkylation this fraction showed the presence of light chains only.

The kappa light chains of the faster migrating arc were monomers and dimers, as demonstrated by gel filtration through Sephadex G-100 that resulted in the separation of two peaks (Figure 6). The first small absorption peak was due to dimers, the second to monomers, and had a molecular mass similar to that of the control $\kappa$ light chain (Figure 7). The fast monoclonal component of the serum and the separated Bence Jones protein of the urine presented the same elution pattern from the Sephadex G-100 and DE52 columns and the same solubility in ammonium sulfate as well as the same elec-
Discussion

Berggård and Petersen (13), in a study of the physicochemical and immunochemical properties of the normal light chains of the human immunoglobulins as well as of patients with tubular proteinuria, concluded that the light chains from both sources consist of monomers, non-covalently linked dimers, covalently linked dimers, and tetramers, and that there is a high content of dimers and tetramers among the free chains from such patients, according to results obtained with starch gel electrophoresis in non-dissociating and in dissociating media.

Polymers of immunoglobulins have been considered a clinically important characteristic of monoclonal immunoglobulinopathies (14) and tetrameric Bence Jones proteinemia without proteinuria has been reported only in four instances, all of type lambda.

In addition, the coexistence of more than one monoclonal component in a single serum is relatively infrequent, being present in about 1% (15, 16) of patients with demonstrable paraproteinemia; a double paraproteinemia with two light chains of the same type is still more uncommon. Dalal and Winsten (17) recently reported as unique a case of double light-chain disease with monoclonal light chains of both the lambda and kappa type in the urine.

Our patient had several interesting and uncommon characteristics: the presence of two paraproteins of Bence Jones type kappa—one of anodic electrophoretic migration that corresponds to monomers of kappa chain, another of slower migration with higher molecular mass that corresponds to tetramers of light chain.

These light chains differ in solubility on ammonium sulfate fractionation, in electrophoretic migration, due probably to different amino acid substitution and molecular mass, and in antigenic characteristics as demonstrated by use of specific antibodies against each M component.

Another interesting feature of this patient was the sequential development of the two monoclonal proteins during the progressive deterioration of her clinical condition. Very early in the history of this patient she had no detectable Bence Jones proteins in the urine, but between the first and second study developed the ability to synthesize kappa chains of different electrophoretic mobility, which were eliminated in the urine as a protein of lower molecular weight, probably due to evolving renal insufficiency.

The presence of the tetramer in the serum but not in the urine of a patient has always been attributed to the high relative molecular mass of the protein, but the excretion of plasma proteins does not depend only on this factor. Some others such as molecular configuration, surface charges, and tubular reabsorption may substantially contribute to the phenomenon. The present case seems to be the first reported with a tetramer of Bence Jones type \( \kappa \) in the blood, and our findings emphasize the need of using immunochemical rather than simply classic methods for the identification of Bence Jones myeloma.

References