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Research Article

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Macroglobulinemia with Bence Jones Proteinuria: Comparison of Urinary Protein and L Chain of Serum Proteins *

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Edelman and Gally (1) have recently shown that an individual's Bence Jones proteins are chemically and physically similar to the low molecular weight polypeptides (L chains) obtained by reductive cleavage of his myeloma proteins. Indeed, Putnam (2) has shown that peptide maps of the tryptic digests of oxidized Bence Jones proteins correspond to a portion of the peptide map of similarly treated autologous serum myeloma protein.

Edelman and Benacerraf (3) have postulated that the manufacture of the L polypeptide chains is asynchronous in multiple myeloma, and thus a part of the cell output appears in the urine as Bence Jones protein, whereas other chains are incorporated into the circulating myeloma globulin. It was of interest, therefore, to examine the uncommon instance of Bence Jones proteinuria in a patient with Waldenström's macroglobulinemia and particularly to compare the L chains of the serum macroglobulins and γ_2-globulins with the urinary Bence Jones protein.

Evidence is presented to show that the Bence Jones protein is chemically and antigenically similar to the L chain derived from the patient's macroglobulin and antigenically deficient when compared to the L chain of the autologous 7 S γ_2-globulin.

Materials and Methods

Clinical information. The patient in the present study, a 74-year-old Caucasian male, was admitted to the Ear, Nose, and Throat Service of the University of California Medical Center, in January 1963, because of gradual, progressive loss of hearing during the preceding year. He had always been in excellent health except for several episodes of unexplained epistaxis one year before admission. In the course of the initial physical examination, hepatomegaly was detected, and initial laboratory studies revealed a 3+ proteinuria positive for Bence Jones protein and a normochromic, normocytic anemia with marked rouleaux formation. Additional laboratory studies showed a hemoglobin of 9.3 g per 100 ml, hematocrit of 29%, and leukocyte count of 5,100 per mm^3. The total serum protein was 11.4 g per 100 ml; serum electrophoretic analysis showed 20% albumin and 61% γ-globulin. A Sia test showed a 4+ reaction. Bone marrow aspiration detected lymphocytosis and plasmacytosis, and an X-ray bone survey demonstrated generalized demineralization.

Materials. The sodium salts of reagent grade phosphate and acetate were used for the preparation of the buffers; reagent grade urea was used. Additional materials were mercaptoethanol,[1] iodoacetamide,[2] carboxymethyl-cellulose (CM-cellulose, 0.8 mEq per g),[3] and diethylaminoethyl-cellulose (DEAE-cellulose, 0.9 mEq per g).[3] The exchangers were recycled through acid and base washes before use. Also used were cross-linked dextran (140 to 400 mesh) [4] and a crystalline preparation of trypsin.[5]

Protein analysis. Protein concentrations were estimated by optical absorption at 280 mμ in 1-cm cells or by the modified Folin-Ciocalteau method (4). One OD unit at 280 mμ was assumed to be the equivalent of 1 mg of protein per ml.

Ultracentrifugation. Proteins were analyzed in the Beckman Spinco Model E analytical ultracentrifuge using schlieren optics. Sedimentation coefficients were determined by the procedures described by Schachman (5). The sedimentation coefficients for the macroglobulins were derived by extrapolation of the experi-

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[1] Eastman Organic Chemicals, Rochester, N. Y.

[2] Mann Research Laboratories, New York, N. Y.

[3] CM-cellulose, Selectacel no. 76; DEAE-cellulose, Selectacel no. 70. Carl Schleicher and Schuell Co., Keene, N. H.

[4] Sephadex G-100, Pharmacia, Uppsala, Sweden.

[5] Lot no. 6112, Worthington Biochemical Corp., Freehold, N. J.

mentally obtained values at various concentrations to infinite dilution. The sedimentation coefficients of other protein samples were determined from data using single concentrations of the proteins. The partial specific volume was assumed to be 0.73 ml per g. All samples were dialyzed against 0.1 M NaCl and 0.01 M phosphate at pH 7 or pH 8 before centrifugation.

Isolation of the macroglobulins and 7 S γ-globulins. Ultracentrifugal analysis of the patient's serum had revealed 4.5 g of protein per 100 ml having a sedimentation value of 17 S or higher. This serum was dialyzed against 0.15 M NaCl, and the macroglobulin was isolated as a precipitated euglobulin by the addition of one part of the dialyzed serum to six parts of deionized water. This mixture was allowed to remain at 4° C for approximately 1 hour before centrifugation. The macroglobulin was further purified by four more euglobulin precipitations and then characterized by analytical ultracentrifugation.

The γ_2-globulin of the patient's serum was isolated from the supernatant fluid acquired during the first euglobulin precipitation. This fluid was equilibrated against 0.02 M phosphate at pH 8 and passed through a DEAE-cellulose column (4.0 × 39 cm) equilibrated with the same buffer (6). The first effluent containing diluted 7 S γ-globulin was further purified and concentrated on CM-cellulose. The final product was dialyzed against water and lyophilized.

The γ_2-globulin of a normal human serum was isolated by modification of the described chromatographic procedures (6).

Isolation and purification of the urine proteins. Random urine specimens from the patient showed 0.15 g of protein per 100 ml of urine as measured by the biuret method. Twenty-four hour collections of urine were dialyzed against multiple changes of distilled water for 3 days, with sodium ethyl mercurithiosalicylate (Merthiolate) used as a preservative for the first 2 days. Some protein precipitated and could not be redissolved in salt solutions; this precipitated protein was not investigated further. The dialyzed preparations were stored frozen or as a dry powder after lyophilization.

The dialyzed urine was equilibrated against 0.01 M acetate at pH 5 and chromatographed on CM-cellulose, using salt and pH gradients as described under Results. A fraction obtained from this column and having characteristic properties of Bence Jones proteins was further purified on DEAE-cellulose.

Thermosolubility characteristics of the proteins were examined in an acetate buffer, pH 5.0, ionic strength (μ) = 0.2 (7). The turbidity of the protein solutions was observed visually in a water bath that was gradually heated to boiling.

Reduction and alkylation of proteins. A slight modification of the bulk dialysis method of Edelman and Poulik (8) was utilized for reduction and alkylation of the macroglobulin. Two to 10 ml of a 1 to 3% solution of the protein in 0.05 M phosphate, 7 M urea (apparent pH 8), 0.1 M mercaptoethanol, and 0.001 M disodium ethylenediaminetetraäcetate were placed in a dialysis bag and surrounded by 100 ml of the above solvent containing the mercaptan. After reaction at room temperature for 16 to 20 hours, a fivefold molar excess of iodoacetamide was added to the contents of the dialysis bag and the apparent pH adjusted to 8 with tris(hydroxymethyl)aminomethane. After 20 minutes the reaction mixture was dialyzed against changes of water for several days. The resulting product was insoluble in both water and saline. A sample of the purified Bence Jones protein was also treated in this manner.

To obtain soluble reduction products and to examine the antigenic character of the protein fractions, reduction and alkylation were performed in 0.2 M phosphate at pH 8 in the absence of urea but with identical concentrations of the other reactants used in the above procedure (8). After alkylation, the protein was dialyzed against 0.1 M NaCl solutions. When very small amounts of protein were available, the dialysis against the reducing agent was eliminated during reduction.

Dissociation and isolation of light chains of the macroglobulin. The reduced and alkylated γ-globulin proteins obtained by both methods of reduction mentioned above were dissociated by dialysis against 7 M urea with 0.05 M phosphate, apparent pH 6 (8).

Separation of the L chains from the heavy polypeptide chains (H chains) (3) was achieved by gel filtration in a manner modified but similar to that used in the separation of components of γ_2-globulin by Fleischman, Pain, and Porter (9). Columns containing Sephadex (G-100) equilibrated with 7 M urea and 0.05 M phosphate (apparent pH 6) were prepared immediately before use. Samples of the reduced and alkylated macroglobulins in the urea buffer were applied to the columns. Elution with the same buffer yielded two peaks on analysis by the Folin method. The second peak was dialyzed against 0.1 M NaCl buffered at pH 8 and then against water and concentrated by lyophilization. Under similar conditions the reduction products of 7 S γ_2-globulin, reduced and alkylated in buffers containing no urea, were isolated.

Starch gel electrophoresis. Vertical starch gel electrophoresis in urea at pH 3 was performed in a manner essentially as described by Edelman and Poulik (8) with the modification that the concentration of urea in the gel was 7 M. Electrophoresis was conducted at 4° C for 16 to 20 hours at a gradient of 6 to 7 v per cm.

Peptide mapping of the proteins. One to 1½% suspensions of the lyophilized L chain of the macroglobulin or the Bence Jones protein previously reduced and alkylated in urea were reacted with trypsin in 0.02 M ammonium acetate at pH 8.6 for 18 hours at 37°. The trypsin to protein ratio was approximately 1 to 50. A slight precipitate remaining in each of the samples was removed by centrifugation. The samples were then lyophilized to total dryness.

Solutions containing approximately 1.5 to 2.0 mg of the trypsin digest were applied to Whatman 3MM paper (46 × 57 cm) at one position. The peptides were separated by electrophoresis in an acetic acid-pyridine buffer (acetic acid 10 : pyridine 1 : water 189) at pH 3.6 in the

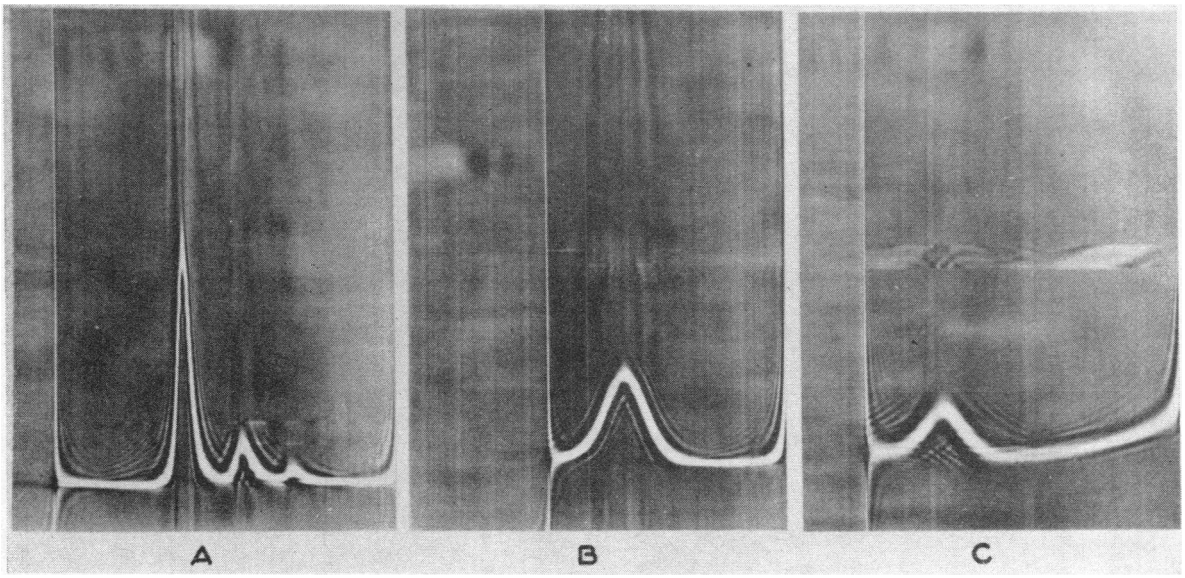

FIG. 1. ULTRACENTRIFUGAL ANALYSIS OF PURIFIED PREPARATIONS. All pictures were taken at the indicated time after attaining full speed. Temperature: 20° C. Solvent: 0.1 M NaCl and 0.01 M phosphate at the indicated pH.
A. Purified macroglobulin. pH 7. Speed: 52,640 rpm. Time: 20 minutes. Angle: 60°. Concentration: 0.69%.
B. Purified Bence Jones protein. pH 8. Speed: 59,780 rpm. Time: 48 minutes. Angle: 60°. Concentration: 0.90%.
C. Light chain of macroglobulin. pH 7. Speed: 59,780 rpm. Time: 48 minutes. Angle: 45°. Concentration: 0.22%.

apparatus described by Katz, Dreyer, and Anfinsen (10). Descending chromatography in the second dimension was performed using an *n*-butanol: acetic acid: water (120: 30: 50) solvent.

Peptides were detected by dipping the paper in 0.25% triketohydrindene hydrate (Ninhydrin) in acetone, heating the paper to 60 to 70° C for 15 minutes, and observing the development of the spots over a period of 48 hours. Photographs were taken after 48 hours of development.

Immunological techniques. Double-diffusion analysis was performed in agar buffered with a barbital buffer, $\mu = 0.075$, pH 8.6. Immunoelectrophoresis was performed by standard techniques at this ionic strength and pH. A horse antihuman serum, a specific horse antihuman γ_1-A (β_{2A}),[6] and a rabbit antiserum to human γ-globulin were used in this study.

Results

Characterization of the isolated macroglobulin. Ultracentrifugal examination of a 3.5% solution of the purified macroglobulin indicated no components with a sedimentation coefficient less than 17 S. The macroglobulin showed three components (Figure 1A). The sedimentation coefficients $S_{20,w}$ for the two slowest components were 17.6 S and 25.8 S. The relative concentrations were 77 and 17%, respectively. The remaining 6% of the protein had a sedimentation coefficient between 30 and 32 S.

Isolation of urinary Bence Jones protein. The usual methods for isolation of Bence Jones protein by ammonium sulfate or euglobulin precipitation proved unsuitable in this case because of the relatively low concentration of protein (approximately 0.15%) in the urine. Isolation of the nonalbumin urinary protein was accomplished by rapid adsorption of the dialyzed urine onto a CM-cellulose column at pH 5 with 0.01 M acetate (Figure 2a). About 40% of the protein (representing albumin) readily passed through the column and emerged in the first fraction. A gradient was applied to the column at point A in the figure. This gradient was formed by passing 0.025 M phosphate, pH 7.4, into a constant volume-mixing chamber containing 400 ml of 0.01 M acetate, pH 5.0. A second gradient, formed by passing 0.3 M NaCl and 0.025 M phosphate, pH 7.4, into the contents of this mixing chamber, was applied at B. A final stepwise elution was performed with 0.5 M NaCl at point C. Just before application of the

[6] Hyland Laboratories, Los Angeles, Calif.

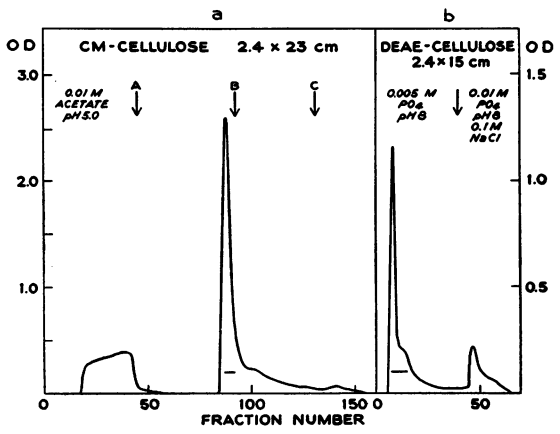

FIG. 2. CHROMATOGRAPHIC ISOLATION OF THE BENCE JONES PROTEIN. The ordinate is OD at 280 mμ. a) Chromatography on CM-cellulose. 347 ml of urine in 0.01 M acetate, pH 5, was passed through a CM-cellulose column. After removal of the first fraction, a gradient was applied at A and a second gradient at B. Stepwise elution with 0.5 M NaCl was started at C. Fraction size: 11 ml. Protein recovery: 86%. b) Chromatography on DEAE-cellulose. The fraction underlined in the second peak of "a" in 0.005 M phosphate, pH 8.0, was passed through DEAE-cellulose equilibrated with the same buffer. The underlined initial fraction emerging from this column was used for the described work. Further material was eluted at higher ionic strength. Fraction size: 4 ml. Protein recovery: 75%.

second gradient at B, a large protein fraction emerged from the column. This fraction was concentrated and then equilibrated against 0.005 M phosphate at pH 8. This material was placed upon a DEAE-cellulose column previously equilibrated with the same phosphate buffer (Figure 2b).

Stepwise elution with 0.005 M phosphate, pH 8, 0.1 M NaCl, and 0.01 M phosphate, pH 8, yielded two fractions. The first fraction, which was used for all subsequent work, demonstrated the typical thermosolubility characteristics of Bence Jones protein.

This purified fraction consisted of a major and several very minor components as judged by starch gel electrophoresis in urea (Figure 4–5). Immunoelectrophoresis indicated a major and two minor components in the γ-globulin region, and with some antisera a trace of albumin could be detected.

Analytical ultracentrifugation of this purified Bence Jones protein revealed a single component with an $S_{20,w}$ value of 3.5 S (Figure 1B).

Isolation of the L chains of the macroglobulin and γ_2-globulin. Reduction of the macroglobulin in urea solutions with mercaptoethanol and subsequent alkylation with iodoacetamide led to a product insoluble in dilute saline or water. Reduction and alkylation of the macroglobulin in buffer free of urea yielded a product soluble in salt solutions. This soluble product appeared as a single peak in the ultracentrifuge with an $S_{20,w}$ value of 6.0 S (10.7 mg per ml; 0.10 M NaCl and 0.01 M phosphate, pH 7).

Gel filtration chromatography of each of these preparations on Sephadex G-100 in 7 M urea yielded a fraction that was excluded from the gel and one that entered the gel, the latter peaks being the L chains (Figure 3). As judged by the protein distribution, about 21% of the protein was in the second peak.

Separation of the two peaks in the case of the macroglobulin reduced in the absence of urea was virtually complete (Figure 3). However, separation of the components of the macroglobulin reduced in urea yielded some overlap of the two peaks. In these cases, rechromatography of the second peak under the same conditions was at times considered necessary.

The L chain of the macroglobulin reduced in the presence of urea was insoluble in saline and

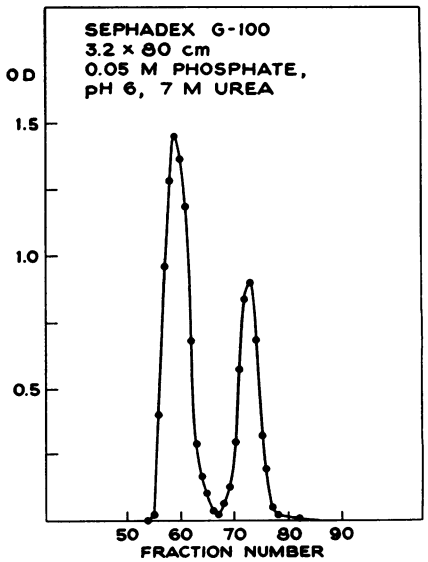

FIG. 3. GEL FILTRATION OF THE MACROGLOBULIN PREVIOUSLY REDUCED AND ALKYLATED IN A BUFFER CONTAINING NO UREA. Applied sample: 4 ml. Fraction size: 4 ml. Ordinate: OD of Folin color at 750 mμ. Recovery: 87%.

water. The L chain of the macroglobulin reduced in the absence of urea was soluble in saline. This latter L chain had an $S_{20,w}$ value of 3.6 S (Figure 1C).

The γ_2-globulins isolated from serum of the patient and from a normal serum failed to reveal the presence of γ_{1A} or γ_{1M} globulins by immunoelectrophoresis. These proteins were subjected to reduction and alkylation in the absence of urea. Chromatography of these reduced proteins on Sephadex G-100 with the urea buffer yielded patterns similar to those obtained with the macroglobulins. The L chains of these γ_2-globulins were soluble in saline.

Electrophoresis in urea-starch gel. In Figure 4 is seen the pattern of the various protein preparations after electrophoresis in urea-starch gel at pH 3. Under these conditions the purified macroglobulin (Figure 4–1) showed a total inability to penetrate the starch gel and no indication of breaking into smaller and more mobile subunits.

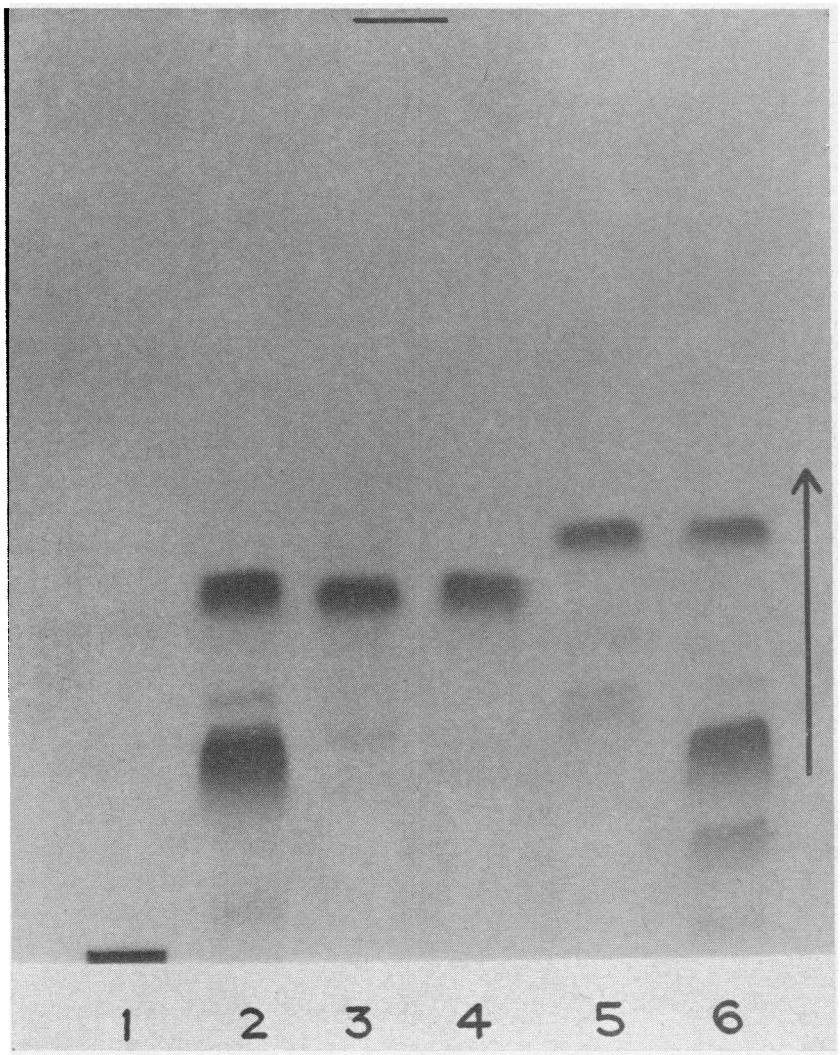

FIG. 4. ELECTROPHORESIS OF VARIOUS FRACTIONS IN UREA-STARCH GEL AT pH 3. The origin is at the bottom of the photograph, and the direction of migration is toward the cathode. 1) Purified macroglobulin, 2) macroglobulin reduced and alkylated in urea, 3) Bence Jones protein reduced and alkylated in urea, 4) L chain obtained from the macrogloblin reduced and alkylated in urea, 5) unmodified Bence Jones protein, and 6) macroglobulin reduced and alkylated in the absence of urea. The protein concentrations were 0.5 to 1.0%.

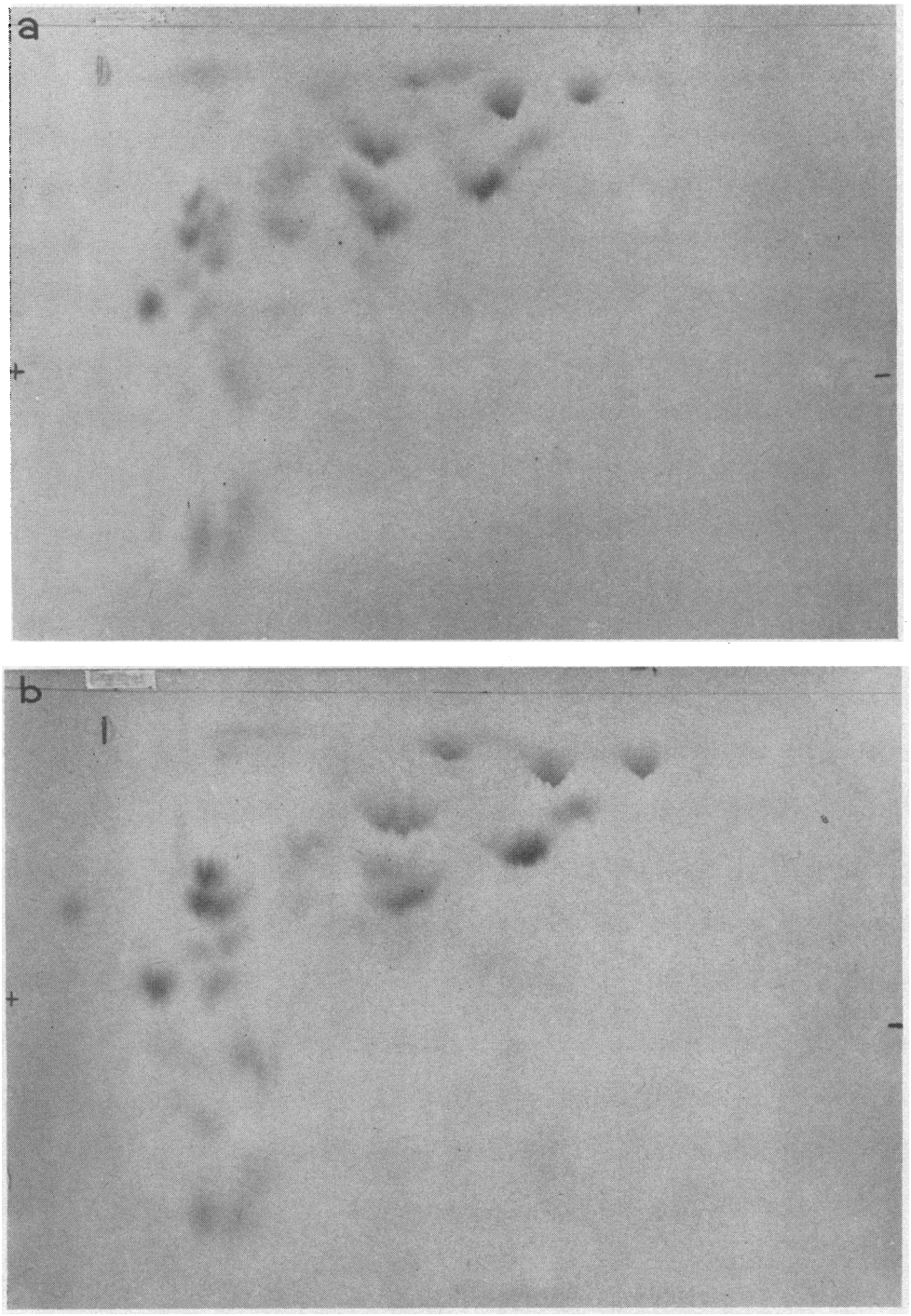

FIG. 5. PEPTIDE MAPS DERIVED FROM TRYPTIC HYDROLYSIS OF BENCE JONES PROTEIN AND THE ISOLATED L CHAIN OF THE MACROGLOBULIN. Electrophoresis was at 1,000 v (20 v per cm) for 2½ hours. Descending chromatography was with an *n*-butanol, acetic acid, water solvent. Stain: Ninhydrin. Approximately 2 mg of the digests was applied to the papers in the upper left-hand corner. a) Bence Jones protein; b) L chain of the macroglobulin.

The macroglobulin reduced and alkylated in urea gave two well-stained bands (Figure 4–2), the more rapid band (L chain) penetrating the gel about twice as far as the other band (H chain). There were several less well-stained bands apparent.

The L chain isolated by gel filtration from the reduced macroglobulin (Figure 4–4) had the identical mobility of the more rapid component of this macroglobulin (Figure 4–2). This L chain preparation appeared to be free of the other components present in the reduced parent macroglobulin.

The Bence Jones protein reduced and alkylated in urea (Figure 4–3) showed the same mobility as the fast component of the macroglobulin or the isolated L chain of this macroglobulin reduced and alkylated under similar conditions (Figure 4–2, 4).

The macroglobulin reduced and alkylated in the absence of urea (Figure 4–6) also showed two strongly stained bands in addition to three faint bands. The more mobile component (L chain) of this preparation penetrated further into the gel than did the corresponding L chain of the macroglobulin reduced in the presence of urea. The unreduced and unmodified Bence Jones protein (Figure 4–5) showed a rapid mobility in the gel and appeared to have the identical mobility of the L chain of the macroglobulin reduced and alkylated in the absence of urea.

Peptide mapping. Comparison of the Bence Jones protein with the L chain of the macroglobulin was performed by peptide mapping. Electrophoresis and chromatography of the trypsin hydrolysate of the Bence Jones protein and the L chain of the macroglobulin yielded apparently identical "fingerprints" (Figure 5a, b). The comparison was judged on the basis of relative position and relative color density of the Ninhydrin spots. There were few minor variations in color intensity between the two maps. No other stain was used.

Thermosolubility characteristics. Heating of the Bence Jones protein (4.7 mg per ml) and the soluble macroglobulin L chain (5 mg per ml) in an acetate buffer, $\mu = 0.2$, pH 5, produced distinct turbidity beginning at 56° C. For the Bence Jones protein, decreased turbidity was noted at 90° with greatly increased clearing at 100°. At this latter temperature a slight opalescence in the solution was noted. For the L chain, clearing was noted near 80° with a distinct clearing at 90°. A slight turbidity remained at 100°.

Immunodiffusion. Figure 6 shows the results of the diffusion of the Bence Jones protein and various soluble light chain preparations against a rabbit antiserum to human Fraction II. A line of complete identity appeared between the Bence Jones protein and the soluble light chain obtained from the macroglobulin, which is of antigenic Type I.[7] The light chain of the patient's γ_2-globulin produced strong spur formation across the macroglobulin L chain and the Bence Jones protein. The precipitin line of the latter two proteins fused with the precipitin line of the γ_2-globulin L chain and showed no spur formation beyond the γ_2-globulin precipitin line. The L chain of the patient's γ_2-globulin formed a line of complete identity with the L chain of a γ_2-globulin obtained from a normal person. The faint precipitin band near the wells containing the L chain of the patient's γ_2-globulin is unexplained.

Discussion

The results of the present study are an extension of existing information on the structural relationships of Bence Jones proteins and serum myeloma proteins. Studies relating Bence Jones protein to the serum myeloma protein have been in terms of the S or slow fragment obtained by papain treatment (11–15) and the L polypeptide chain obtained by reduction and alkylation of the purified serum protein (1, 16, 17). Antigenic differences between Bence Jones protein and the S fragment of autologous myeloma protein have been demonstrated (18). By double diffusion methods, the L chain of myeloma protein does show a reaction of partial identity with the S fragment of the same protein; however, the L chain more closely resembles the autologous Bence Jones protein than does the S fragment (19). Indeed, Edelman and Gally (1) have demonstrated that the L chain of a myeloma protein and the autologous Bence Jones protein after reduction and alkylation in the presence of urea had identical mobilities in urea-starch gel at pH 3

[7] The antigenic type of the macroglobulin has been established by George M. Bernier, Department of Biochemistry, University of Florida.

and showed similar amino acid compositions. The L chain prepared from this myeloma protein that had been reduced in the absence of urea was compared with the Bence Jones protein and showed similar thermosolubility characteristics. Similarly prepared L chains of normal γ_2-globulin also show thermosolubility characteristics of Bence Jones proteins and thus demonstrate a fundamental similarity between an intrinsic structural component of γ_2-globulins and the urinary Bence Jones proteins (1).

Myeloma proteins and the autologous Bence Jones proteins usually have the same antigenic configuration in that they are either Type I or II (20) and InV(+) or InV(−) (21, 22), suggesting a similarity of cellular or clonal origin.

Normal γ_2-globulin appears to be a mixture of the antigenic Types I and II (23), and recent evidence indicates that both these types also appear in macroglobulins obtained from normal serum (24), reflecting presumably the normal heterogeneity of cellular origin of both classes of γ-globulins.

Macroglobulinemia with Bence Jones proteinuria is distinctly less common than multiple myeloma and this proteinuria (25). The macroglobulins found in macroglobulinemia appear to carry only the antigenic determinants of Type I or II and to be InV(+) or InV(−) (20–22). We had anticipated that in a manner analogous to myeloma proteins and their autologous Bence Jones proteins, investigation of the present case would demonstrate a strong similarity, if not identity, of the L chain of the macroglobulin to the Bence Jones protein. We have isolated from the macroglobulin a polypeptide with a sedimentation value of 3.6 S and having thermosolubility characteristics of Bence Jones protein.

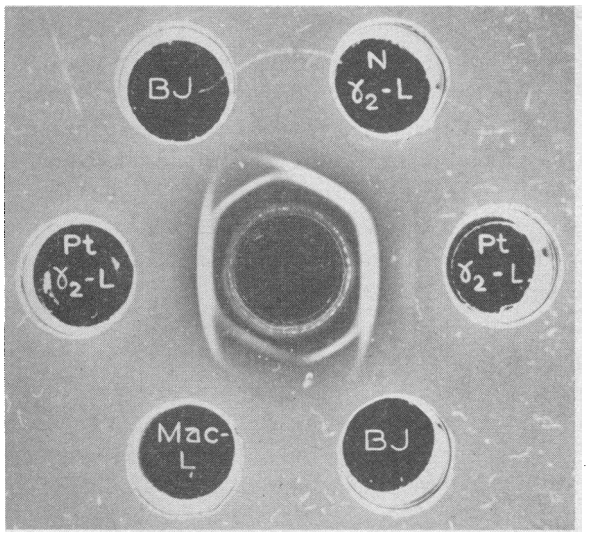

FIG. 6. IMMUNODIFFUSION OF THE VARIOUS SOLUBLE L CHAIN PREPARATIONS FROM MACROGLOBULIN (MAC-L), PATIENT'S γ_2-GLOBULIN (PT-γ_2-L), A NORMAL INDIVIDUAL'S γ_2-GLOBULIN (N-γ_2-L), AND THE UNMODIFIED BENCE JONES PROTEIN (BJ). A rabbit antiserum to human γ_2-globulin is in the center well. Diffusion was for 18 hours.

This peptide isolated from the macroglobulin, which had been reduced and alkylated in buffers containing no urea, and the unreduced and unmodified Bence Jones protein were compared by electrophoresis in urea-starch gels at pH 3 and demonstrated identical mobilities. This result, taken with the fact that the peptide map of the Bence Jones protein and the isolated L chains are essentially identical, suggests that the L chain of the macroglobulin obtained in the above manner is very similar, if not identical, in size, configuration, and charge to this Bence Jones protein. Schwartz and Edelman (26) have reported a comparable similarity in a comparison of the L polypeptide chains of myeloma globulin and autologous Bence Jones protein. Apparently the isolated L chain obtained from this macroglobulin after reduction and alkylation in buffers free of denaturing agents closely resembles native Bence Jones protein as it is excreted.

The L chain of the macroglobulin and the Bence Jones protein, when both had been reduced and alkylated in urea solutions, demonstrated in urea-starch gel a slower but identical mobility than their counterparts obtained without the use of urea. The slower mobilities of the proteins reduced in urea would be expected if intramolecular disulfide bonds were broken by the more drastic conditions of reduction leading to a more extensively unfolded molecule. Indeed, others have shown that ribonuclease, ovalbumin, human albumin, and the L and H chains of human γ-globulin extensively reduced and alkylated in 8 M urea lead to molecules with slower mobility than the corresponding less reduced or unreduced proteins (8, 27).

In contrast to our results, Edelman and Gally (1) have given examples of unmodified Bence

Jones protein migrating more slowly than the corresponding Bence Jones protein or L chain of the myeloma proteins reduced in the presence of urea.

The differences in mobility and solubility noted between the L chains of the macroglobulin prepared by reduction and alkylation in the absence or presence of urea undoubtedly reflect a difference in the extent of reduction of disulfide bonds in these polypeptides. Reduction of proteins by mercaptans in the presence of urea is more extensive than in the absence of urea (28). When pathologic macroglobulin was reduced with mercaptoethanol, many more disulfide bonds were broken with the macroglobulin reduced in the presence of urea than in the absence of urea (8). In all probability, the L chain obtained from the macroglobulin reduced in the presence of urea represents a far more extensively reduced product than the L chain produced by reduction of the macroglobulin that was not in the presence of urea.

In our own work, comparison of the L chains of the macroglobulin with the Bence Jones protein by double diffusion established the antigenic identity of the two proteins. In addition, the spur formation shown by the patient's own γ_2-globulin L chains with the Bence Jones protein and the L chain of the macroglobulin indicated the presence of determinants of both Types I and II in the patient's γ_2-globulin. The L chain of the γ_2-globulin did not have antigenic deficiencies when compared to the L chain of the γ_2-globulin obtained from a normal serum and by this criterion, therefore, appeared to be normal. Investigation of the normal population of macroglobulin molecules in this patient was not considered feasible.

The evidence presented here indicates that the patient's lymphoid cells are producing large amounts of unique macroglobulins belonging to antigenic Type I. In addition, this same cell line probably is producing the Bence Jones protein that is chemically and antigenically indistinguishable from the L chain of the macroglobulin.

Other cells of the lymphoid series are producing γ_2-globulin of Types I and II that are presumably normal. The findings are consistent with the concept that a certain population of lymphoid cells, probably arising from a single clone of cells, has extensively proliferated in this patient and is committed to the manufacture of a limited number of unique macroglobulin proteins belonging to one antigenic group.

This case represents a situation parallel to that in multiple myeloma, where much evidence has strongly suggested that both myeloma proteins and Bence Jones proteins are produced from a clone of cells that have extensively proliferated (29). Indeed, in this case of the transplantable mouse plasma-cell tumors' producing myeloma protein, the production of these proteins is directly related to the weight of the tumor (29). In addition, some of these mouse myeloma tumors also produce Bence Jones protein. Askonas and Fahey (30) have shown that the Bence Jones protein was neither a precursor nor a breakdown product of the myeloma protein in one of these mouse tumors. Putnam and Hardy (31) have found that Bence Jones proteins were not breakdown products of the myeloma protein. The data are consistent with the view that Bence Jones proteins are manufactured at a rate independent of the myeloma proteins. Edelman and Benacerraf (3) have postulated that the uncontrolled asynchronous synthesis of the L chain of myeloma protein is responsible for the appearance of Bence Jones protein in the urine.

An over-all similarity between macroglobulinemia with Bence Jones proteinuria and myeloma and its corresponding proteinuria (26) appears to exist. There has been no definitive evidence to date that the distinctive 7 S or 19 S proteins of these diseases are abnormal. Rather it is distinctly possible that these several serum proteins and Bence Jones proteins are expressions of some regulatory dysfunction of an otherwise normal population of lymphoid cells. The Bence Jones proteinuria of macroglobulinemia is far rarer than the proteinuria of myeloma (25). Whether this fact represents a difference in the mechanism leading to increased synthesis of L chains or decreased synthesis of H chains is not known. With the establishment of additional qualitative similarities between normal serum and urinary proteins and the proteins associated with these two diseases, future studies should be directed toward the basic mechanisms regulating the rate of synthesis of γ-globulins and Bence Jones proteins.

Summary

A comparison was made between the serum and urine proteins isolated from a patient with Waldenström's macroglobulinemia and Bence Jones proteinuria. After reduction and alkylation in a solvent containing no denaturing agent, the macroglobulin was separated into H and L chains by gel filtration on urea-Sephadex G-100. The L chain of the macroglobulin and the Bence Jones protein had similar thermosolubility characteristics and sedimentation values on analytic ultracentrifugation.

On electrophoresis in urea-starch gel, the unmodified Bence Jones protein had the identical mobility of the L chain of the macroglobulin. L chains and Bence Jones protein prepared by reduction in urea buffers had identical but slower mobilities than the proteins reduced in buffers without urea. Peptide maps of these proteins appeared to be identical.

The L polypeptide chain of the serum macroglobulin and the urinary Bence Jones protein were both of Type I, whereas the L chain of the autologous serum γ_2-globulin manifested both Types I and II antigenic specificity.

This study strongly suggests the chemical and immunologic identity of a Bence Jones protein and the L chain of the autologous serum macroglobulin.

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