BENCE JONES PROTEINS AND LIGHT CHAINS OF IMMUNOGLOBULINS

II. Immunochemical Differentiation and Classification of Kappa-Chains*, ‡

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The immunochemical demonstration that the Bence Jones protein from the urine of an individual patient with multiple myeloma was antigenically identical to a portion of the homologous serum myeloma protein (1), and the establishment of the chemical and structural similarities between Bence Jones proteins and light polypeptide chains (2, 3) which are common to immunoglobulins of all known classes (4) emphasized the importance of the Bence Jones protein as a source of homogeneous material for studies on immunoglobulin structure and antibody specificity. Two distinct types of immunoglobulin light chains (κ or λ) were evidenced by immunochemical studies on Bence Jones proteins (1, 5, 6). Although Bence Jones proteins of κ type share no peptides in common with Bence Jones proteins of λ type, comparative analyses of tryptic peptide maps of Bence Jones proteins provided the initial evidence that proteins of the same type have distinctive as well as common peptides (7). Amino acid sequence determinations on Bence Jones proteins (summarized in reference 8) revealed a remarkable primary structure characterized by variancy in the first 107 amino acid residues of the amino terminal half and by constancy in the next 107 amino acid residues of the carboxyl terminal half. Hence, proteins of the same type possess distinctive variant halves but have similar constant halves.

Differences and similarities among Bence Jones proteins and isolated light chains within each major type have been evident immunochemically (9–16). Immunochemical comparisons of κ light chains with antisera developed against κ Bence Jones proteins provided evidence for extensive antigenic heterogeneity which was not related either to the Inv (3) or Inv (2) genetic type[1] of the protein (12). Amino acid

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[1] The Inv genetic factors (l), (a), and (b) are now designated as Inv factors (1), (2), and (3), respectively, as recommended in the *Bull. World Health Organ.* (1965). **33**:721.

sequence analyses (17) on two antigenically distinct κ Bence Jones proteins resulted in the localization of the antigenic differences to the amino terminal portion of the molecules (13).

Recently, we obtained an antiserum which was unique in its ability to detect antigenic differences among *intact* type K immunoglobulins as well as among isolated κ light chains and κ Bence Jones proteins (18). This antiserum, prepared against a κ Bence Jones protein with a glutamyl amino terminal residue, not only had specificity for κ light chains with glutamyl amino terminal residues but also could detect differences among κ light chains with aspartyl amino terminal residues. The multispecificity of this antiserum permitted the recognition of three distinct classes of κ light chains; the fact that the expression of this specificity did not require the isolation of the light chains permitted the direct classification of intact type K immunoglobulin molecules. The ability to cleave selectively the Bence Jones protein polypeptide chain into its variant half and constant half (19) made possible the localization of the regions of the molecule responsible for the antigenic differences detected by this antiserum.

Materials and Methods

Protein Isolation.—Bence Jones proteins were isolated by gel filtration (19) of urine samples from patients with multiple myeloma. Myeloma proteins and M-macroglobulins (Waldenström) were isolated from sera by block electrophoresis (20), and the macroglobulin preparations were purified by P-200 polyacrylamide gel filtration. Pooled normal human γ-globulin, Cohn Fraction II (FrII), was obtained from Mann Research Laboratories, N. Y.

Preparation of Light Chains.—Light chains were isolated from reduced and alkylated (21) immunoglobulins by gel filtration through P-100 polyacrylamide columns equilibrated with 1 M propionic acid.

Antisera.—Antisera to isolated κ Bence Jones proteins were prepared in albino New Zealand rabbits. An emulsion of 1 ml of complete Freund's adjuvant and 5 mg of antigen (5 mg in 1 ml of 0.15 M NaCl) was prepared. Two rabbits were immunized with the same antigen by intramuscular injection at two sites with 1.25 mg of protein (0.5 ml). Injections were made weekly for 4 consecutive wk and then at monthly intervals. The first sample of blood was obtained 5 wk after the initial injection, and thereafter samples were obtained 1 wk after the monthly injection. Specificity of the antisera for κ light chain determinants was assured by absorption with urine specimens containing λ Bence Jones proteins and with isolated G and A myeloma proteins and M-macroglobulins of type L.

Immunochemical Techniques.—Immunodiffusion (Ouchterlony) analyses were performed in a 2% agar medium prepared in a barbital buffer, pH 8.6, ionic strength 0.05. Bence Jones proteins and isolated light chains were tested at a concentration of 0.25 mg/ml, as determined by a modification of the Folin-Ciocalteu method (22).

The type K light chain content of immunoglobulins in normal, hypergammaglobulinemic, and hypogammaglobulinemic sera was quantitated by electroimmunodiffusion (23). Representative sera were diluted in 0.15 M NaCl to yield a final γ-globulin concentration of approximately 0.25 mg/ml. In the quantitation of the total type K light chain content, known concentrations of FrII γ-globulin were used as standards; the calculation of the standard concentrations was made on the basis that 70% of the FrII γ-globulin molecules possessed type K

light chains (6). The concentration of immunoglobulins G, A, and M was determined by radial immunodiffusion analyses in Hyland Immuno-Plates.

The γ heavy chain classification of G myeloma proteins was performed by Dr. H. G. Kunkel, Dr. H. M. Grey, and Dr. W. D. Terry. The Inv typing of Bence Jones proteins was performed by Dr. A. G. Steinberg.

Analytic Procedures.—Total serum protein concentration was measured by refractometry. The concentration of myeloma proteins and M-macroglobulins in sera was determined by the densitometric tracings of the cellulose acetate electropherograms; and in the immunochemical analyses, sera were diluted in 0.15 M NaCl to yield a final concentration of 0.5–1.0 mg/ml.

The amino terminal residue of κ Bence Jones proteins and light chains was determined by the "dansyl" technique (24). The dansylated residue was compared with dansyl-aspartic and dansyl-glutamic standards (Calbiochem, Los Angeles, Calif.) by high-voltage electrophoresis in a pyridine:acetic acid:water (1:2:250, v:v:v) buffer, pH 4.38. The electrophoresis on Whatman No. 3 MM paper was performed on a flat plate apparatus (Savant Instruments, Inc., Hicksville, N. Y.) at 70 v/cm for 120 min at 15°C.

Electrophoresis in alkaline (0.05 M glycine, 0.005 M NaOH, pH 8.8) and alkaline-8 M urea (0.05 M glycine, 0.006 M NaOH, pH 9.3) starch gels was performed as previously described (25, 26).

RESULTS

Differences in the Specificity of Antisera to κ Bence Jones Proteins.—Antisera prepared against nine κ Bence Jones proteins were used in the immunochemical comparison of 37 κ Bence Jones proteins with the homologous immunizing protein. The antisera were evaluated on the basis of the results of these comparative studies. Five antisera possessed idiotypic specificity, i.e., recognition of antigenic determinants unique to the immunizing protein. All 37 heterologous Bence Jones proteins were antigenically deficient to the homologous protein with each of these five antisera, and no differences were detected among the heterologous proteins. With three antisera, (R160, R182, and R2), the homologous protein gave a reaction of identity with seven heterologous Bence Jones proteins, and furthermore, antigenic differences were detected among the other 30 heterologous proteins which were antigenically deficient to the homologous protein. Of prime importance was the finding of one antiserum (R185) which had specificity for the most antigenically deficient proteins detected by these three antisera as well as idiotypic specificity.

The precipitin reactions between six heterologous κ Bence Jones proteins and antiserum R160 were compared with the precipitin reactions between the same six proteins and antiserum R185 (Fig. 1). Proteins 4, 5, and 6 were recognized by antiserum R160 as antigenically deficient to proteins 1, 2, and 3. The immunochemical reactions with R160 were typical of those obtained with antisera R182 and R2. The specificity of R185 for Bence Jones proteins 4, 5, and 6, which R160 revealed as antigenically deficient, was obvious.

Detection and Designation of Three Classes of κ-Chains by Immunochemical and Amino Terminal End-Group Analyses on Bence Jones Proteins.—Antiserum R185, prepared against a κ Bence Jones protein with a glutamyl amino terminal

residue, was used in immunodiffusion analyses which resulted in the division of 38 κ Bence Jones proteins into three distinct classes. The determination of the amino terminal residue of Bence Jones proteins in each of the three classes revealed that each of the proteins for which R185 had major specificity possessed a glutamyl amino terminal residue. This class of proteins was designated as κ_{glu}. Each protein analyzed in the two classes of proteins, antigenically deficient to the proteins of the major class, possessed an aspartyl amino terminal

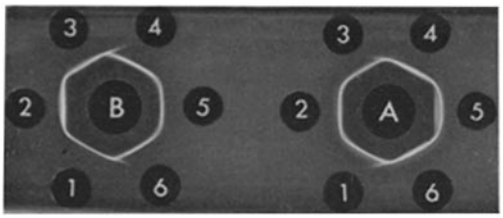

FIG. 1. Immunodiffusion analysis of six κ Bence Jones proteins. With antiserum B (R160) proteins 4, 5, and 6 were antigenically deficient to proteins 1, 2, and 3. The opposite specificity of antiserum A (R185) for the same proteins was evident from the precipitin reactions.

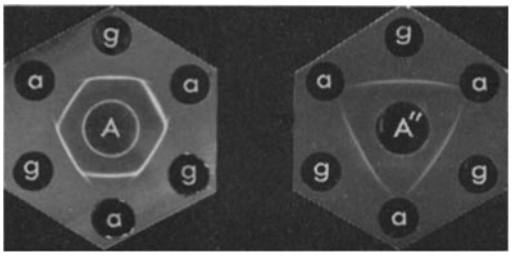

FIG. 2. Immunochemical demonstration of the specificity of antiserum R185 for κ_{glu} Bence Jones proteins. The outer antigen wells contained three κ_{glu} (g) and three κ_{asp} (a) Bence Jones proteins. A, antiserum R185; A″, R185 rendered specific for κ_{glu} proteins by absorption with a κ_{aspII} Bence Jones protein.

residue. The two classes were designated as κ_{aspI} and κ_{aspII}, in which case the proteins of class κ_{aspI} were antigenically deficient to the proteins of class κ_{aspII}. The specificity of antiserum R185 for proteins with glutamyl amino terminal residues was evident from the immunochemical comparison of three Bence Jones proteins, each having an aspartyl amino terminal residue with three Bence Jones proteins, each having a glutamyl amino terminal residue (Fig. 2).

Bence Jones proteins representative of each of the three κ-chain classes were differentiated immunochemically with antiserum R185. The deficiency of κ_{aspI} and κ_{aspII} proteins to κ_{glu} proteins, as well as the deficiency of a κ_{aspI} protein to a κ_{aspII} protein, was evident (Fig. 3). After antiserum R185 was absorbed with

a $\kappa_{asp I}$ protein, precipitin reactions resulted only with $\kappa_{asp II}$ and κ_{glu} proteins. The antiserum was rendered specific for κ_{glu} proteins by absorption with a $\kappa_{asp II}$ protein, and consequently, precipitin reactions were obtained only with κ_{glu} proteins.

Electrophoretic Heterogeneity among Bence Jones Proteins within Each κ-Chain Class.—The Bence Jones proteins in each of the three κ-chain classes were compared by starch gel electrophoresis in alkaline, alkaline-urea, and alkaline-urea-mercaptoethanol buffer systems. There was no correlation between the κ-chain class and the electrophoretic mobility of the Bence Jones proteins. The

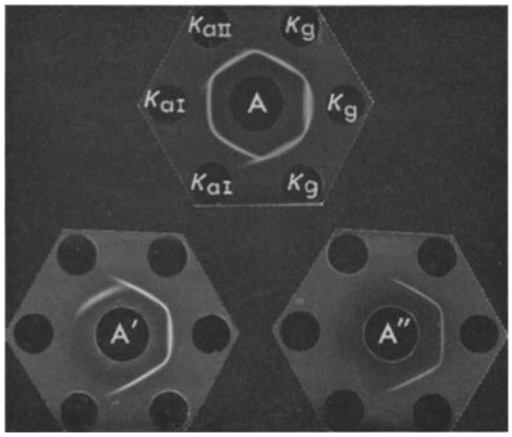

FIG. 3. Immunochemical differentiation of three classes of κ Bence Jones proteins. The outer antigen wells in each Ouchterlony pattern contained either a $\kappa_{asp I}$ protein ($\kappa_{a I}$), a $\kappa_{asp II}$ protein ($\kappa_{a II}$), or a κ_{glu} protein (κ_g) as labeled in the upper pattern. A, antiserum R185; A′, antiserum R185 absorbed with a $\kappa_{asp I}$ protein; A″, antiserum R185 absorbed with a $\kappa_{asp II}$ protein.

electropherogram of representative Bence Jones proteins of each of the three κ-chain classes and light chains isolated from FrII γ-globulin is shown in Fig. 4. Wide differences in electrophoretic mobility were apparent among proteins of the same class. Immunodiffusion analyses of the protein bands cut from the unstained half of the gel revealed that light chains representative of all three κ-chain classes were distributed throughout the electrophoretic range of the FrII light chains.

Distribution of Inv Genetic Factors among Bence Jones Proteins within Each κ-Chain Class.—34 κ Bence Jones proteins were tested for Inv (1) activity, and four were Inv (1+). The distribution of Inv (1+) proteins among the three classes, $\kappa_{asp I}$, $\kappa_{asp II}$, and κ_{glu}, was 2,1,1, respectively. 15 of the Inv (1−) proteins were tested for the Inv (3) factor, and all were Inv (3+). The distribution of the Inv (3+) proteins was as follows: six $\kappa_{asp I}$, six $\kappa_{asp II}$, and three

κ_{glu}. Of the 15 remaining Inv (1−) proteins, 6 belonged to the κ_{aspI} class, 5 to the κ_{aspII} class, and 3 to the κ_{glu} class.

Division of Intact Type K Immunoglobulins into κ-Chain Classes.—The antigenic differences which we detected among κ Bence Jones proteins were evident

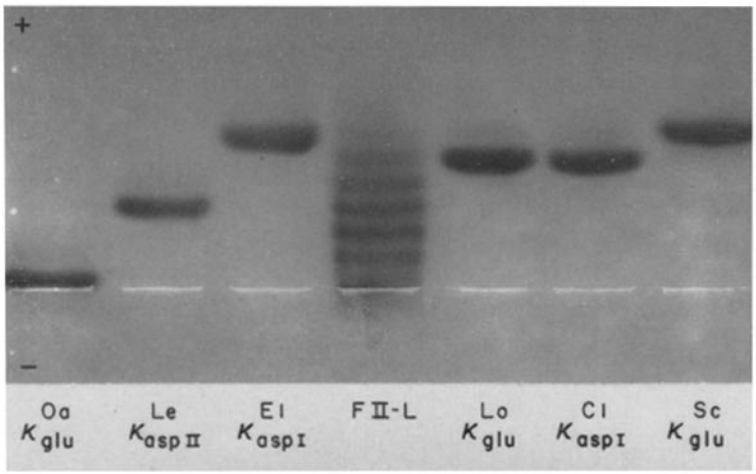

FIG. 4. Starch gel electropherogram of Bence Jones proteins representative of each κ class and of FrII light chains. The electrophoresis was performed in an alkaline 8 M urea, 0.1 M 2-mercaptoethanol, pH 9.3 starch gel. FII-L: light chains isolated from FrII γ-globulin.

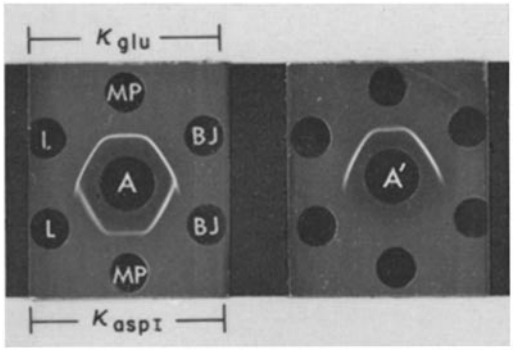

FIG. 5. Immunochemical comparison of the precipitin reactions of an intact myeloma protein, and the isolated light chains and the homologous Bence Jones protein. The antigens placed in the outer wells of both Ouchterlony plates are indicated on the left pattern. The identity among the precipitin reactions of the intact myeloma protein (MP), the isolated light chains (L), and the homologous Bence Jones protein (BJ) is evident. The specificity of antiserum R185 (A) for κ_{glu} proteins was apparent by the precipitin reactions between the κ_{glu} and κ_{aspI} proteins, as well as by the reactivity of the κ_{glu} proteins with antiserum R185 absorbed with a κ_{aspI} protein (A′).

among κ light chains isolated from the homologous myeloma proteins; however, prior to the development of antiserum R185, the immunochemical differences detected among κ Bence Jones proteins or isolated κ light chains were not apparent among the intact homologous type K myeloma proteins. Unique to antiserum R185 was its ability to detect on the intact immunoglobulin the antigenic differences evident among the isolated homologous κ light chains. The

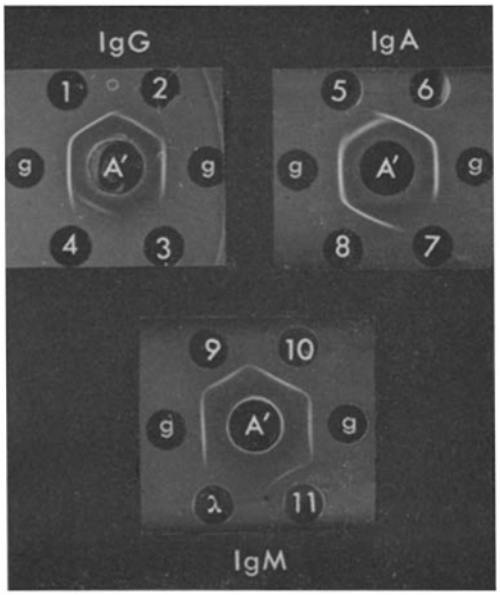

FIG. 6. Immunochemical classification of intact type K myeloma proteins and M-macroglobulins. The antigen wells contained sera diluted in 0.15 M NaCl to yield a final concentration of myeloma protein or M-macroglobulin of 0.5–1.0 mg/ml.; g, reference protein of κ_{glu} class (G myeloma protein); λ, M-macroglobulin of type L used in the absorption; A′, antiserum R185 rendered specific for κ_{glu}- and κ_{aspII}-light chains. The IgG protein 3 and IgA protein 7 were classified as κ_{aspI}; IgG proteins 2, 4, and IgA protein 5 were classified as κ_{aspII}; IgG protein 1, IgA proteins 6, 8, and IgM proteins 9, 10, and 11 were classified as κ_{glu}.

precipitin reactions with R185 of the homologous Bence Jones protein, the intact myeloma protein, and the light chains isolated from the myeloma protein were equivalent (Fig. 5). The specificity of R185 for κ_{glu} determinants of the whole myeloma protein, as well as the isolated light chains or the homologous Bence Jones protein, was evident. Likewise, the specificity of R185 for κ_{aspII} antigenic determinants could be demonstrated by immunodiffusion analyses.

The direct classification of type K myeloma proteins and M-macroglobulins in sera was made by immunodiffusion analyses with antiserum R185 which was rendered specific for type K_{glu} and type K_{aspII} proteins by absorption with a

type K_{aspI} protein. The precipitin reactions of the test samples of diluted sera (see Materials and Methods) were compared with that of a reference G myeloma protein with type K_{glu} light chains. The failure of the test sample to react with the absorbed antiserum indicated a type K_{aspI} protein. A reaction of partial identity between the reference type K_{glu} protein and the test sample indicated a type K_{aspII} protein; and a reaction of complete identity with the known type K_{glu} protein connoted a type K_{glu} protein (Fig. 6). Data on the immunochemical classification of Bence Jones proteins, type K myeloma proteins, and M-macroglobulins (Waldenström) are presented in Table I.

TABLE I

Classification of κ Bence Jones Proteins and Type K Immunoglobulins

Proteins	No.	κ-chain class		
		κ_{aspI}	κ_{aspII}	κ_{glu}
Bence Jones proteins	38	14	16	8
G myeloma proteins	60	16	29	15
subclass: IgG1	19	8	5	6
IgG2	4	0	4	0
IgG3	5	1	4	0
IgG4	3	1	2	0
A myeloma proteins	16	5	7	4
D myeloma protein	1	1	0	0
M-macroglobulins	10	0	1	9

Quantitation of Total Type K and Type K_{glu} Immunoglobulins in Normal, Hypergammaglobulinemic, and Hypogammaglobulinemic Sera.—Antiserum R185, specific for type K light chain determinants, was utilized in the quantitation of the total content of type K immunoglobulins in whole sera (see Materials and Methods).[2] The type K_{glu} immunoglobulin content was determined with the antiserum rendered specific for κ_{glu} antigenic determinants, and known concentrations of a G myeloma protein with type K_{glu} light chains were used as standards. The percentage of immunoglobulins with type K_{glu} light chains in each serum was calculated; the total type K and type K_{glu} immunoglobulin concentrations of each serum are compared in Fig. 7. The concentration of type K_{glu} chains in six normal sera represented 24–31% of the total type K chains. Although the total type K light chain concentration in the four poly-

[2] Absorption of antiserum R185 with a heterologous κ_{glu} Bence Jones protein removed the capacity of the antiserum to react with all κ Bence Jones proteins except the homologous protein; furthermore, the fact that this absorbed antiserum did not react with FrII γ-globulin, tested in concentrations as high as 20 mg/ml, negated the possibility of spurious quantitative results which could have occurred if there had existed in the FrII γ-globulin standards a large pool of molecules antigenically identical to the homologous protein.

clonal hypergammaglobulinemic sera was two to three times that in normal sera, the percentages of type K_{glu} chains (21–32%) were similar to that of normal sera. The sera from four patients with non–sex-linked hypogammaglobulinemia contained one-tenth to one-fourth of the total type K light chain

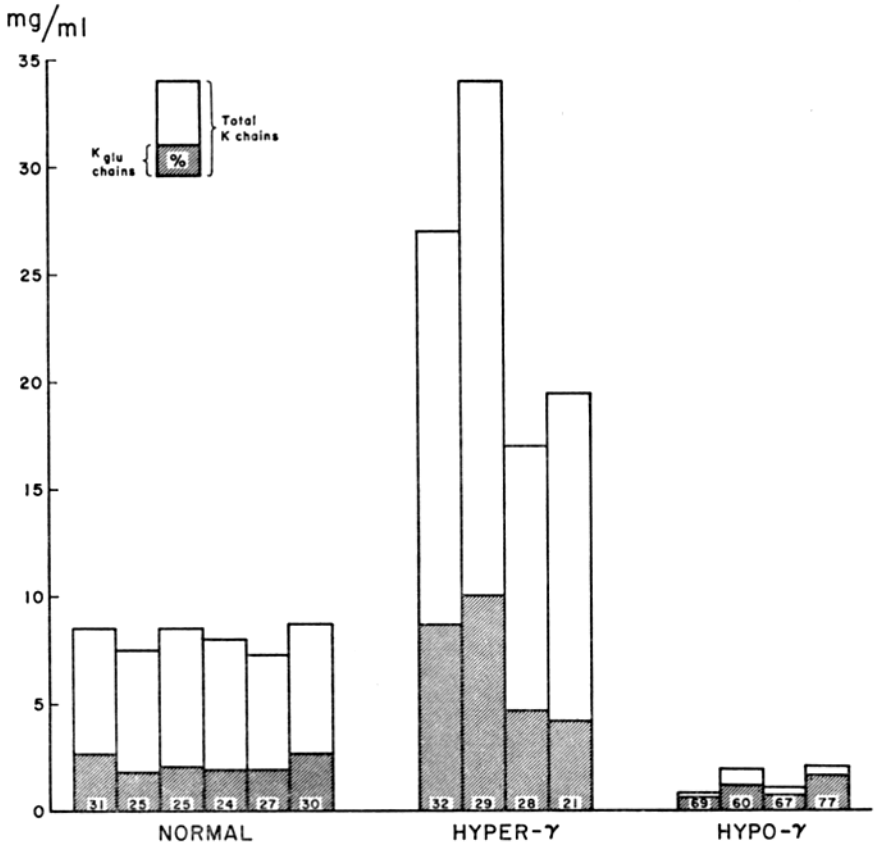

FIG. 7. Concentrations of K_{glu} chains and total K immunoglobulin chains in normal, hypergammaglobulinemic, and hypogammaglobulinemic sera. The per cent of K_{glu} chains in the total K immunoglobulin chains of each serum is indicated by the number at the bottom of each bar.

content of normal sera; the type K_{glu} chain concentration represented 60–77% of the total type K light chains.

Localization of the Antigenic Specificity of Antiserum R185.—To localize on the light polypeptide chain regions for which antiserum R185 had specificity, we chose a representative Bence Jones protein from each κ-chain class for special

study. The Bence Jones protein was cleaved by pepsin into its respective variant half (V_L) and constant half (C_L) (19). The conditions of cleavage were controlled so that some intact protein remained. The homologous antiserum to each representative Bence Jones protein recognized the V_L and the C_L antigenic determinants, and by appropriately absorbing the antiserum, we could identify both halves (19). Each sample containing the intact Bence Jones protein and its V_L and C_L was analyzed immunoelectrophoretically with the following antisera: antiserum R185, antiserum R185 absorbed with a κ_{aspI} protein, antiserum R185 absorbed with a κ_{aspII} protein, and the homologous antiserum. The comparison of the reactivity of the V_L, C_L, and intact protein with these antisera made possible the localization of the reactive regions on the light chain molecule

TABLE II

Localization of the Antigenic Specificity of Antiserum R185

Antiserum	Reactivity of the variant half (V_L) and constant half (C_L) of Bence Jones proteins (BJP) in each κ-chain class								
	κ_{aspI} class			κ_{aspII} class			κ_{glu} class		
	V_L	BJP	C_L	V_L	BJP	C_L	V_L	BJP	C_L
R185-A*	0	+	+	0	+	+	+	+	+
A′‡	0	0	0	0	+	0	+	+	0
A″§	0	0	0	0	0	0	+	+	0

* A, antiserum R185 specific for κ light chain determinants.

‡ A′, antiserum R185 A absorbed with a κ_{aspI} protein.

§ A″, antiserum R185 A absorbed with a κ_{aspII} protein.

for which antiserum R185 had specificity. Antiserum R185 reacted with the C_L of the κ_{aspI} Bence Jones protein, but no precipitin reaction was obtained with the V_L. Absorption of R185 with a heterologous κ_{aspI} protein or with a κ_{aspII} protein removed all reactivity to the C_L and the intact protein. Similarly, antiserum R185 reacted with the C_L and not with the V_L of a κ_{aspII} Bence Jones protein. While absorption of R185 with a κ_{aspI} protein removed the reactivity of the antiserum with the C_L, the reactivity of this antiserum with the intact κ_{aspII} protein remained. The V_L, as well as the C_L of a κ_{glu} Bence Jones protein, was recognized by R185. Absorption of the antiserum with a κ_{aspI} or a κ_{aspII} protein removed the reactivity with the C_L; both absorbed antisera reacted with the V_L and with the intact protein. Data from these immunoelectrophoretic analyses are summarized in Table II.

DISCUSSION

An antiserum developed in our laboratory recognized antigenic differences among κ-chains, and permitted the division of κ Bence Jones proteins and either isolated κ light chains or intact type K immunoglobulins into three distinct

classes. The antiserum (R185), prepared against a κ Bence Jones protein with a glutamyl amino terminal residue, had specificity for κ-chains with glutamyl amino terminal residues, and further differentiated proteins with aspartyl amino terminal residues into two classes. The three classes have been designated as κ_{glu}, κ_{aspII}, and κ_{aspI}.

The unique ability of this antiserum to detect with equal sensitivity antigenic differences on either the intact immunoglobulin, the isolated light chain, or the homologous Bence Jones protein made possible the direct classification of type K myeloma proteins and M-macroglobulins (Waldenström) in sera. Immunochemical classification of G and A myeloma proteins revealed a comparable distribution of immunoglobulins G and immunoglobulins A among the three κ-chain classes; the percentage of proteins with K_{glu} light chains among the G or the A myeloma proteins was 25. A comparison of the κ light chain class and the γ heavy chain subclass of G myeloma proteins showed that the known IgG2, IgG3, and IgG4 proteins belonged either to the κ_{aspI} or to the κ_{aspII} class; however, 32% of the proteins of the IgG1 subclass belonged to the κ_{glu} class (see Table I). The preference of μ heavy chains of M-macroglobulins (Waldenström) and IgM cold agglutinins for type K light chains has been established (27, 28). The predilection of μ heavy chains for κ_{glu} light chains was evident from our finding that 9 of 10 M-macroglobulins possessed K_{glu} light chains, and from the finding by Cohen and Cooper (29) that three of four cold agglutinins possessed type K light chains with glutamyl amino terminal residues.

The frequency of occurrence of proteins belonging to a particular antigenic class within a group of myeloma proteins parallels the frequency of occurrence of proteins in that class within normal immunoglobulins (30). The correlation between the distribution of type K_{glu} light chains in normal and hypergammaglobulinemic sera and the distribution of proteins of the κ_{glu} class among myeloma proteins was evident from the comparison of quantitative data on the K_{glu} chain content of these sera with the frequency of occurrence data on the myeloma proteins (cf. Fig. 7 and Table I). The type K_{glu} light chains of the normal sera represented 24–31% of the total type K light chains; and although the total type K light chain content in the hypergammaglobulinemic sera was double to triple that in normal sera, the type K_{glu} light chains represented a comparable percentage of total type K light chains. Among the Bence Jones proteins, G myeloma proteins, and A myeloma proteins tested, 21%, 25%, and 25%, respectively, were in the κ_{glu} class.

Quantitation of the type K_{glu} light chain content in the sera from four patients with non–sex-linked hypogammaglobulinemia revealed that type K_{glu} light chains represented 60–77% of the total type K light chain content. The total type K light chain content in these sera was one-fourth to one-tenth the content in normal sera. The IgG, IgA, and IgM concentrations in these four sera were determined; the quantitative data revealed a relatively equiva-

lent reduction in the level of all three classes of immunoglobulins in each serum, and therefore indicated that the high K_{glu} light chain content of these hypogammaglobulinemic sera was not the result of a selective suppression of immunoglobulins G or A. These data suggest that this hypogammaglobulinemic state may be associated with a selective suppression of the genetic mechanism controlling synthesis of κ_{aspI}- and κ_{aspII}-light chains. Yount et al. (31) reported a preponderance of the IgG3 subgroup associated with the absence or marked diminution of the IgG1, IgG2, and IgG4 subgroups of immunoglobulin G in the sera of patients with non-sex-linked hypogammaglobulinemia, and they were able to demonstrate by studies on one family an association between structural gene abnormalities and observed immunoglobulin G subgroup deficiencies.

While many type-specific (κ or λ) antisera have had the capacity to detect differences among Bence Jones proteins (9–16), heretofore, no single antiserum has had the capacity to differentiate κ Bence Jones proteins, isolated κ light chains, and intact type K immunoglobulins into classes which correlate with the basic sequences defined by structural studies on the isolated light polypeptide chains.[3] By comparing the sequence data on the first 22 amino acid residues of the amino terminal portion of κ Bence Jones proteins and light chains isolated from type K immunoglobulins, Niall and Edman (32) were able to categorize κ-chains into two structurally distinct classes. The prototype of the class designated κ_{Tra} had an aspartyl amino terminal residue, whereas the prototype of the class κ_{Smi} had a glutamyl amino terminal residue. Representative samples from their two classes were supplied to us, and our immunochemical analyses were in concordance with their structural analyses. Antiserum R185 had specificity for proteins in their κ_{Smi} class, and we were able to differentiate samples in their κ_{Tra} class. On the basis of homologies evident from comparisons of the available sequence data on the primary structure of the variant halves of κ Bence Jones proteins, Milstein (33) defined three basic sequences of κ-chains. Proteins in his basic sequences I and II had aspartyl amino terminal residues, and proteins in his basic sequence III possessed glutamyl amino terminal residues. Samples representative of his three basic sequences were made available to us for immunochemical classification.[4] Each of the proteins Ker (34), Roy (17, 35), and Ag (36), prototypes of basic sequence I, gave a precipitin reaction of identity with a known κ_{aspI} protein;

[3] At least three subclasses of κ Bence Jones proteins were detected by immunochemical comparisons among κ Bence Jones proteins of different genetic (Inv) types; however, there was no dependence between a subclass and an Inv factor (12). This independency was of special significance: the immunochemical detection of subclasses among proteins with the same genetic type reflected similarities as well as differences in the variant half of the light polypeptide chain.

[4] Bence Jones proteins Ker, Fr4, and Bell were supplied by Dr. C. Milstein; proteins Ag and Tew by Dr. F. W. Putnam; and protein Cum by Dr. H. G. Kunkel.

neither protein Ker, Roy, nor Ag reacted with R185 absorbed with a κ_{aspI} protein. Proteins Cum (17, 35) and Tew (37), prototypes of basic sequence II, reacted with R185 absorbed with a κ_{aspI} protein, but were deficient to a known κ_{glu} protein. Protein Fr4 (38), a prototype of basic sequence III, gave a precipitin reaction of identity with a known κ_{glu} protein. Thus, proteins of basic sequence I, II, and III were classified immunochemically as κ_{aspI}, κ_{aspII}, and κ_{glu} proteins, respectively.

The complete amino acid sequence of the variable half of κ Bence Jones protein Ti, of basic sequence III, not only confirmed the existence of three basic sequences, but also made possible a complete structural comparison among proteins representative of each of the three basic sequences (39). Additional structural heterogeneity among proteins of basic sequence I has been reported by Milstein et al. (40), and indeed immunochemical analyses of Bence Jones proteins Ker and Bell, both of basic sequence I[5], revealed Ker to be antigenically deficient to Bell. Furthermore, the reactivity of protein Bell with antiserum R185 was not removed by absorption of the antiserum with a κ_{aspI} protein (Roy); however, Bell was deficient to κ_{glu} and κ_{aspII} proteins. Additional immunochemical heterogeneity has become apparent among proteins within the κ_{aspII} and κ_{glu} classes. This heterogeneity has been evidenced by selective absorptions of antiserum R185 with different proteins from each class.

While the extent of variability or constancy in the primary structure of the light polypeptide chain of immunoglobulins has been determined chiefly by structural analyses (17, 33–39), it is evident that differences detected by structural analyses can be detected by immunochemical analyses. This concordance asserts the importance of immunochemical techniques in providing not only a rapid method for the detection of differences among proteins, but also a sensitive means for the localization of these differences (41). The ability to cleave specifically the Bence Jones protein polypeptide chain into its respective variant half (V_L) and constant half (C_L) (19) made possible the localization of the regions of the molecule for which antiserum R185 had specificity. Immunochemical analyses of the V_L, C_L, and intact Bence Jones protein representative of each class (see Table II) revealed that the specificity of antiserum R185 for κ_{glu} proteins was confined to the V_L antigenic determinants; the expression of specificity for κ_{aspII} proteins required the intact molecule and would appear to be directed toward the "switch" region between the V_L and C_L; the specificity for κ_{aspI} proteins was limited to the C_L antigenic determinants.

While the human Inv allotypic specificity has been attributed to a single amino acid substitutuion (valyl/leucyl) at position 191 (17, 42, 43) in the constant region of the κ light chain, a complete or partial dependency upon the association of the light chain with the heavy chain for expression of the Inv

[5] Milstein, C. Personal communication.

activity has been demonstrated (44, 45). The essentiality of the complete light polypeptide chain for the expression of the Inv factor was suggested by the fact that Inv activity was not expressed after an Inv positive Bence Jones protein was cleaved into its V_L and C_L (19). Determination of the Inv allotype of proteins within each of the three κ-chain classes revealed no correlation between the Inv allotype and the κ-chain class, and substantiated the finding by Milstein (33) of no correlation between the Inv allotype and the basic sequence. The seemingly independent nature of the Inv genetic determinant has been evidenced in previous immunochemical, electrophoretic, and structural studies (12). Our immunochemical detection of proteins representative of the three κ-chain classes in each of six normal sera was in accordance with the chemical detection in normal sera of light chains of each basic κ-chain sequence which excluded the possibility of an allotypic relationship among the three basic sequences (40). While current data suggest the presence of three or more structural genes coding for the variant half of the κ light polypeptide chain, the nature of the genetic mechanism responsible for this diversity in the variant half awaits establishment. Nevertheless, complementary immunochemical and structural studies on homogeneous prototypes of normal immunoglobulins, i.e., Bence Jones proteins and myeloma proteins, should provide the additional knowledge necessary for the elucidation of the genetic mechanism controlling immunoglobulin synthesis, and thereby provide a molecular basis for antibody specificity.

Note Added in Proof: Recent immunochemical studies reveal that a second antiserum (R 198), which was also prepared against a κ Bence Jones protein with a glutamyl amino terminal residue, has major specificity for the proteins differentiated by antiserum R185 to be in the κ_{glu} class.

SUMMARY

Three distinct classes of κ light polypeptide chains have been detected immunochemically by an antiserum (R185) prepared against a κ Bence Jones protein with a glutamyl amino terminal residue. This antiserum had specificity for κ light chains with glutamyl amino terminal residues and differentiated κ-chains with aspartyl amino terminal residues into two classes: the three κ-chain classes have been designated as κ_{glu}, κ_{aspII}, and κ_{aspI}.

The ability of antiserum R185 to detect these antigenic differences on the intact immunoglobulin molecule, as well as on the isolated light chain or Bence Jones protein, made feasible the direct classification of type K myeloma proteins and M-macroglobulins (Waldenström). The multispecificity of the antiserum permitted the quantitation of type K_{glu} light chains in normal, hypergammaglobulinemic, and hypogammaglobulinemic sera. Whereas the distribution of myeloma proteins and Bence Jones proteins in the κ_{glu} class correlated with the distribution of K_{glu} chains in normal and hypergammaglobulinemic

sera, the M-macroglobulins in the κ_{glu} class represented 90% of the total M-macroglobulins tested and revealed a marked divergence from the range of 24–31% of K_{glu} immunoglobulins in normal sera. A preponderance of K_{glu} chains was detected in the sera from patients with non–sex-linked hypogammaglobulinemia and represented 60–77% of the total type K light chain content.

The controlled cleavage of a Bence Jones protein representative of each κ-chain class into its variant half and constant half made possible the localization on the light polypeptide chain, the reactive sites for which antiserum R185 had specificity. The correlations between immunochemical and structural classification of κ light chains are discussed.

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