figurations when their heads are above water. Although it is theoretically possible for them to see stars from beneath the surface, this would only occur in perfectly calm water. The normal ocean waves would disrupt the star images and, because of the critical angle of refraction, would impose a new and shifting horizon. Other types of celestial navigation involving the position of the sun or moon cannot be ruled out; but any theories must be consistent with knowledge of the green turtle's inability to see clearly outside its marine environment.

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Evolution of Immunoglobulins: Structural Homology of Kappa and Lambda Bence Jones Proteins

Abstract. *The amino acid sequence of a human lamba chain has been determined. There are many identities in sequence with human kappa chains, but this intraspecies homology is less than the interspecies homology of kappa light chains of man and mouse. Structural relationships suggest a common evolutionary origin and early differentiation of light- and heavy-chain genes.*

Immunoglobulin molecules consist of a pair of heavy chains that determine that γ-globulin class (γG, γA, or γM) and a pair of light chains (kappa or lambda) that determine the antigenic type (K or L, respectively) (*1*). Patients with multiple myeloma excrete Bence Jones proteins identical in structure and antigenic type with the light chains of the myeloma globulin in their serum and similar to the light chains of normal γ-globulin (*2*). Comparison of amino acid sequences of type K Bence Jones proteins of man (*3, 4*) and the mouse (*5*) has established that the polypeptide chains consist of a variable portion and an invariant portion. In man the invariant portion comprises the 107 amino acids in the COOH-terminal end, except that residue 191 is leucine in molecules of the genetic type Inv(a+) and valine in the Inv(b+) type (*6*). The NH_2-terminal portion (also about 107 residues) is subject to variation; up to 23 positions have been found to be substituted when limited sequence areas of some half dozen human Bence Jones proteins of type K have been compared (*7*) and 42 positions in two mouse Bence Jones proteins of type K (*5*). Thus, the hypothesis has been proposed that all light chains (and probably also heavy chains) contain a variable and an invariant region, the amino acid sequence of which is related to antibody specificity (*3, 4*).

Although the two antigenic types of human Bence Jones proteins and of the light chains of immunoglobulins (types K and L) share no antigenic determinants and have no tryptic peptides in common (*8*), it has been predicted that they should exhibit considerable homology in primary structure (*7, 8*). This prediction has now been verified by analysis of amino acid sequence of a human Bence Jones protein of type L. Between one protein of type K (designated Ag) for which the tentative complete sequence is known (*3, 7*) and one protein of type L (designated Sh) for which the probable sequence is given in Fig. 1, there are many positions of identity or probable identity and many others where the amino acid pairs are chemically homologous or are related to the genetic code through single nucleotide changes within single codons.

The type L Bence Jones protein designated Sh was purified on a Sephadex A-50 column. The protein was reduced with mercaptoethanol in 7*M* guanidine hydrochloride; the reduced protein was then aminoethylated by reaction with ethylenimine for subsequent tryptic digestion (*9*), or it was alkylated with monoiodoacetic acid for subsequent chymotryptic digestion. The peptides of the tryptic digest were first fractionated on a Dowex-1–X2 column by use of a gradient system with volatile buffers (*4*). The peptide fractions thus obtained were purified on Dowex-50–X2 columns by use of pyridine–acetic acid buffers. Altogether, 21 major tryptic peptides were characterized. Two of them contained two basic amino acids: one contained one lysine and one arginine, the other contained two arginines. Amino acid analysis on the intact protein indicated that all the basic amino acids (11 lysine, 7 arginine, and 5 aminoethylcysteine residues) were recovered in the tryptic peptides.

In a separate experiment, the chymotryptic digest of the *S*-carboxymethylated protein was placed on a Dowex-1–X2 column, and the peptides were eluted with pyridine–acetate buffers graded with respect to *p*H and ionic strength. Dowex-50–X2 columns were used for further purification of the peptide fractions, as well as for the tryptic peptides. A sufficient number of peptides was isolated to permit us to propose a unique arrangement of the tryptic peptides in protein Sh. In some instances, the overlapping chymotryptic peptides were digested with trypsin in order to confirm the assignment of linkages between tryptic peptides. The amino acid sequence of the tryptic and chymotryptic peptides was determined by stepwise degradation by the modified Edman method (*10*) and by use of leucine aminopeptidase and carboxypeptidase A.

Because of previous difficulty in determining the NH_2-terminal amino acid of type L Bence Jones proteins (*8, 11*), special attention was given to the assignment of the NH_2- and COOH-terminal residues. Serine was obtained as the NH_2-terminal residue when the dinitrophenyl method was applied to the intact protein, but in low yield. Also, serine is the NH_2-terminal residue of the chymotryptic peptide which has the same starting sequence as the tryptic peptide given as NH_2-terminal in Fig. 1 (the initial octadecapeptide ending in arginine). The sequence of the octapeptide shown as COOH-terminal in Fig. 1 confirms that given by Milstein (*12*) for γG light chains of type L and accords with the composition of the COOH-terminal peptide A_1 reported by Putnam and Easley in all type L Bence Jones proteins they studied (*8*).

Because protein Ag contains 214 amino acids according to sequence analysis, the previous numbering system based on the assumed 212 residues in the Roy protein has been changed (*13*). Although the Sh protein appears to have 213 residues, a numbering system is not proposed yet since the proposal for the sequence is still tentative. However, when the sequences

of the two proteins are aligned as shown in Fig. 1, the maximum number of positions having identical amino acids (identities) is obtained. This alignment pairs 209 amino acids in each of the proteins but requires the introduction of five gaps relative to protein Ag (positions 1, 9, 169, 201, and 202) and four insertions (two residues between positions 97 and 98 of Ag, one between 106 and 107, and one after 214). This procedure is analogous to that adopted to show the maximum homology between α- and β-chains of hemoglobin.

Table 1 lists the positions of definite identity and the maximum number of identities including positions of probable identity. The positions of definite identity are those where the amino acid is known in both proteins and is the same. The probable identities are of two kinds: (i) where the amino acid is definitely established in only one of the proteins but by sequence analogy appears to be the same in both, and (ii) where it is not yet certain whether aspartic acid and glutamic acid (or either) are present as the amides or not, in which case the residues are designated Asx or Glx. Tentative assignments (not shown), which can be made in many of the latter cases, support the assumption that most of these pairings represent positions of identity.

Of the 209 paired positions, the amino acids are identical in 59 and probably identical in another 35, yielding a maximum number of 94 positions of identity. This represents 44 percent of the amino acids in each of the two types of molecules; thus, the degree of homology of κ- and λ-chains is almost identical with that of the α- and β-chains of human hemoglobin (45 percent). If, in addition to identical pairs, chemically homologous pairs such as valine and leucine are considered, the structural similarity of κ- and λ-chains becomes even more striking. Aside from the dicarboxylic acids and their amides, there are 35 such chemically homologous pairs indicated by underscoring in Fig. 1. Thus, 129 of the 209 pairs are probably identical or similar in structure, or a total of about 60 percent of the molecule.

Many of the chemically homologous pairs of amino acids, as well as others, differ in their codons by only a single nucleotide base. If all the 115 pairs of amino acids that are probably not identical in structure are compared, the differences could have arisen by one-step mutations in the corresponding codons in 72 cases but would have required at least two steps in 43 cases. This suggests that the κ- and λ-chain genes differentiated early in evolutionary development through an accumulation of successive mutations.

The distribution of positions of chemical identity, chemical homology, and sites of possible one-step mutations is seemingly random throughout the two chains and is almost equally divided between the variable and invariant portions. Hence, during evolutionary divergence the two segments of the κ- and λ-genes corresponding to the variable and invariable portions were subject to almost equal change. This makes it appear less likely that the NH_2-terminal and COOH-terminal halves of light chains are coded by separate genes. The random distribution of identities also accounts for the lack of cross-reaction between antigenic types K and L; the presence of serine in position 191 explains the absence of the Inv antigenic character in type L.

Although little sequence data has so far been published for other λ-chains, we assume, on the following basis, that the residues paired as 1 to 107 represent the variable portion and those paired

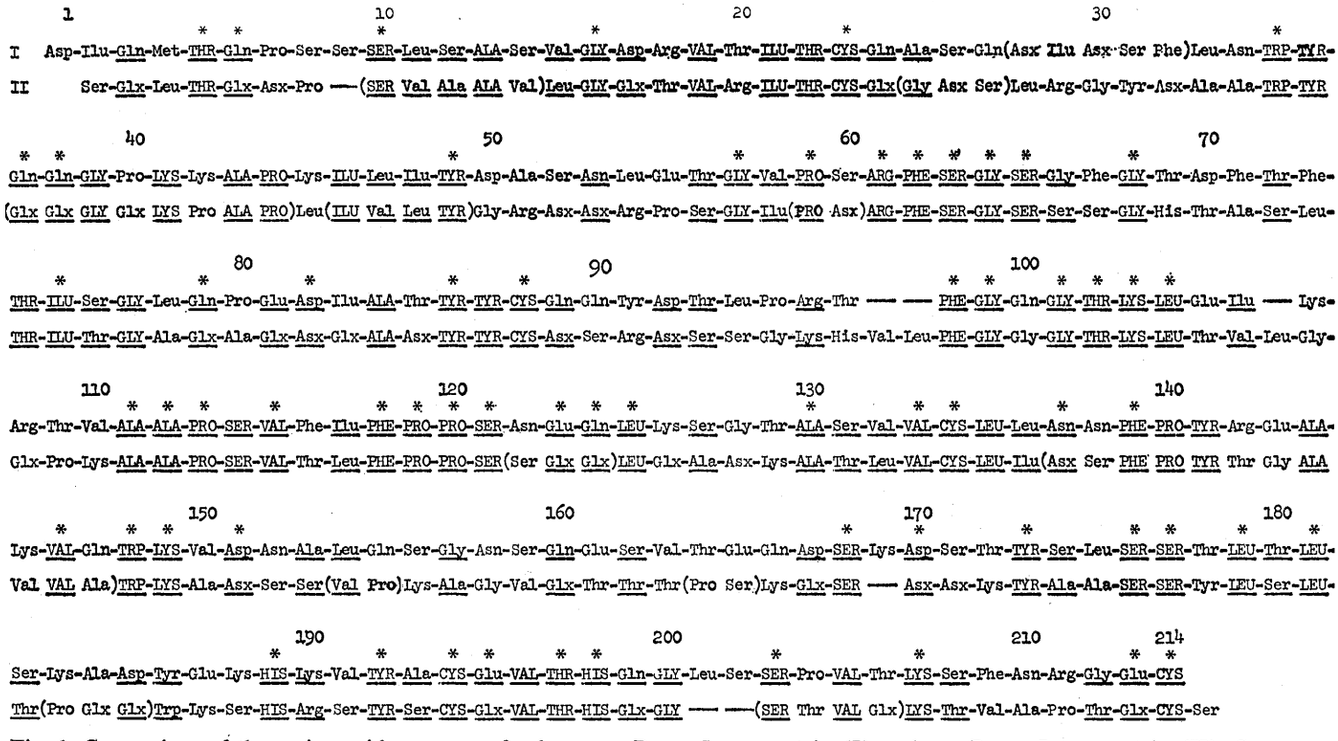

Fig. 1. Comparison of the amino acid sequence of a human κ Bence Jones protein (I) and a λ Bence Jones protein (II). Sequences joined by hyphens were determined by analysis. Areas of undetermined sequence are enclosed in parentheses, but the amino acid residues are assigned positions on the basis of maximum homology. A revised numbering system from 1 to 214 is employed for the κ protein Ag (*13*). The sequence of the λ protein Sh is aligned to achieve maximum homology. Thus, residues are inserted at four positions and gaps left at five in the λ-chain. Residues identical at the same position in the two chains are designated by underscored capital letters. Other residues that are probably identical or are chemically homologous are underscored. Asterisks designate residues that are also identical or probably identical with those in corresponding positions of two mouse κ-chains (*5*).

as 108 to 214, the invariant portion: (i) the close sequence analogy to κ-chains, (ii) the variable amino end groups and the frequent absence of detectable amino end groups in type L Bence Jones proteins (*8, 11*), (iii) the common COOH-terminal octapeptide referred to above, (iv) the apparent identity of two of our tryptic peptides in the carboxyl half of the λ-chain with two reported by Milstein (*12*), and (v) correlation with peptide maps of type L Bence Jones proteins. Of ten tryptic peptides from protein Sh, for which the composition was given by Putnam and Easley (*8*), five reported as constant are in the COOH-terminal half and the five reported as variable are in the NH_2-terminal half of the sequence shown in Fig. 1.

The κ- and λ-chains must be similar in tertiary structure as well as in primary sequence because both types of chains have two long intrachain loops each of which contains about 60 residues. We (*7*) and Milstein (*12*) have shown that the two half-cystines in the NH_2-terminal half of κ-chains (positions 23 and 88) are linked through a disulfide bridge and likewise the two in the COOH-terminal half (positions 134 and 194). Work in our laboratory (*14*) has established that the two half-cystines in the NH_2-terminal half of the λ-chain Sh are joined in a disulfide bridge and likewise the half-cystines of this λ-chain that correspond to positions 134 and 194 of the κ-chain.

In immunoglobulin molecules, light chains are normally joined to heavy chains through the COOH-terminal half-cysteine, which is position 214 in κ-chains and is the penultimate residue in λ-chains. In the κ Bence Jones protein Ag, which exists predominantly as a monomer, the COOH-terminal cystine is stabilized by combination with the free amino acid cysteine, whereas in the λ Bence Jones protein Sh, a dimer is formed through an interchain linkage of the COOH-terminal half-cystines (*14*).

The presence of the two intrachain bridges in each of the two kinds of light chains suggests an internal symmetry in three-dimensional conformation as well as in primary sequence. Our present hypothesis is that the variable portion of the light chains is related to the specificity of the antigen-combining site and that the invariant portion enhances affinity for the heavy chain. However, there is still no positive evidence that the invariant portion does not confer antibody specificity. Indeed, the importance of the whole molecule for antibody specificity is emphasized by our findings of the overall homology in the primary structure of κ- and λ-light chains and of the genetic conservation of their tertiary structure.

Table 1. Homology of types K and L Bence Jones proteins: Distribution of probable identities in position and of possible nucleotide base changes in codons.

Identities in position		Minimum base changes	
Definite	Maximum	One	Two
Variable part (residues 1–107)			
28	47	34	24
Invariable part (residues 108–214)			
31	47	38	19
Whole protein			
59	94	72	43

A further demonstration of this is the close analogy in primary structure in the vicinity of the half-cystine residues of κ- and λ-chains: for example, the Ilu·Thr·Cys·Glx (*15*) and the Tyr·Tyr·Cys sequences in the variable regions and the Val·Cys·Leu and the Cys·Glx·Val·Thr·His·Glx·Gly sequences in the invariant regions of the two chains (see Fig. 1).

A common evolutionary origin of the light chains of man and mouse, and presumably other species, is indicated by the fact that of the 94 positions that are probably identical in human κ- and λ-chains 65 are the same as in two mouse κ-chains (the positions marked with an asterisk in Fig. 1). These, too, are distributed in a similar proportion between the NH_2- and COOH-terminal halves. This indicates a genetic stability in part of the molecule; in contrast, of 23 positions known to be variable in human κ-chains, only one is identical in the four cases compared in Fig. 1.

Although the data available are limited to the tentative complete sequence of one human type K Bence Jones protein (and partial sequences of others), to one human type L Bence Jones protein, and to two mouse type K Bence Jones proteins, it is probable that interspecies differences among κ-chains are less pronounced than intraspecies differences between κ- and λ-chains. Whereas human κ-light chains such as the Bence Jones proteins Ag and Roy may have as few as 13 differences in sequence and thus an identity in primary structure of 94 percent (*7*), two mouse κ-chains (Bence Jones proteins 41 and 70) have 44 probable differences or only 80 percent identity in sequence (*5*). Indeed, in the NH_2-terminal half, the mouse protein 41 is more like the human protein Ag (64 percent identity) than it is like mouse protein 71 (60 percent identity). On the other hand, the human κ-chain Ag is more like the mouse κ-chains than like the human λ-chain Sh (only 44 percent identity). This suggests an early evolutionary divergence of the genes for the κ- and λ-chains. Since lower species do not make immunoglobulins, one might predict a close structural relationship of these proteins in higher forms because of their more recent origin.

Others (*16, 17*) have noted that homologous relationships may exist between heavy and light chains. Although the modified theory of Burnet (*18*) predicts that the F_D fragment and the light chain are identical or have considerable segments in common, Hill *et al.* (*17*) have pointed out the remarkable homology between the F_C fragment of the heavy chain of rabbit γG-globulin and a human κ-light chain. To obtain maximum homology they introduced seven small gaps into the sequence of the 161 amino acids in the rabbit F_C fragment and nine single-residue gaps into the sequence of the human type K Bence Jones protein (Ag) that we studied. With this alignment there are 42 positions of identity or probable identity; this is equivalent to 25 percent of the total number of positions compared. Using the same alignment for the Sh protein, we find 37 residues in identical positions or 22 percent. Furthermore, there are still 23 amino acids in identical positions, or 14 percent, if the human κ- and λ-chains Ag and Sh and the mouse κ-chains 41 and 70 are all compared in the same alignment with rabbit F_C fragment. The agreement is even more striking if only the invariant or COOH-terminal halves of the light chains are compared with the 108 amino acids in the COOH-terminal part of the rabbit heavy chain; then, 20 positions or almost one-fifth have identical amino acids. This homology in structure is even greater than that exhibited by sperm-whale myoglobin and the α- and β-chains of human hemoglobin which share 21 positions of identity in sequences ranging from 141 to 151 amino acids (*19*). With this exception, such a degree of homology is almost unprecedented for such large segments of polypeptide chains of proteins that differ in species origin, antigenic type,

and size. It indicates that there is both a genetic and functional relation between light and heavy chains, which is probably related to their role in antibody function. Furthermore, despite the variation in sequence to which the NH_2-terminal portions of light and heavy chains are subject, portions of the tertiary structure may be rather stable since certain sequence regions as well as the disulfide bridges are similar from species to species and within antigenic types and classes.

Most theories of hypermutation are not compatible with these findings. Indeed, these structural relationships are difficult to explain by any mechanism other than an accumulation of mutations through many separate genes for κ- and λ-light chains, and probably also for γ-, α-, and μ-heavy chains. As in the myoglobin-hemoglobin example, this structural homology can be accounted for by the hypothesis of common ancestry of light and heavy chains from a single primitive gene that gave rise to light and heavy genes through a process of duplication and independent mutation as proposed by Hill *et al.* (*17*). Our results suggest that κ- and λ-chain specialization preceded interspecies differentiation and probably occurred on an evolutionary time scale at about the same period as the separation of the genes for α- and β-hemoglobin chains.

Note added in proof. Hood *et al.* (*20*) have demonstrated variation in the sequence of the NH_2-terminal octadecapeptide of human λ-chains analogous to that known for κ-chains. On this basis they, too, have suggested that the genes coding for κ-chains and λ-chains have evolved from a common ancestral gene.

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15. Abbreviations for amino acid residues: Lys, lysine; His, histidine, Arg, Arginine; Asp, aspartic acid; Asn, asparagine; Asx, aspartic acid or asparagine, identity not established; Thr, threonine; Ser, serine; Glu, glutamic acid; Gln, glutamine; Glx, glutamic acid or glutamine, identity not established; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ilu, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan; CyS, half-cystine.
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Leukocyte Oxidase: Defective Activity in Chronic Granulomatous Disease

Abstract. *The intact leukocytes of two children with chronic granulomatous disease fail to reduce nitroblue tetrazolium during phagocytosis. This is due to defective operation of an oxidase of reduced nicotinamide adenine dinucleotide that is insensitive to cyanide and that indirectly stimulates the oxidation of glucose-6-phosphate in leukocytes. Such leukocytes undergo no increase in oxygen consumption or in activity of the hexose monophosphate shunt during phagocytosis, although lactate production is normal. The addition of nitroblue tetrazolium to a leukocyte suspension appears to provide a sensitive diagnostic screening test for this disease.*

Chronic granulomatous disease observed in childhood is a fatal disorder characterized by increased susceptibility to infection, which begins during the first year of life (*1*). Although many types of organisms have been isolated from the affected patients during the later phases of their illness, the initial series of infections is usually caused by staphylococci. The patients frequently develop granulomas in nodes, lungs, liver, and spleen.

Studies by Holmes and her co-workers (*2*) suggest that the circulating granulocytes of these patients ingest but do not destroy bacteria. These workers have also stated that the respiration of such granulocytes does not increase during phagocytosis (*3*).

We have recently observed two unrelated male children with chronic granulomatous disease, one of whom has succumbed to this disorder. We studied the metabolic response of their granulocytes to the phagocytosis of polystyrene spheres in an attempt to define a defect of direct oxidation of glucose-6-phosphate in these leukocytes. Some of the metabolic responses of guinea pig granulocytes to the phagocytosis of inert particles include, in addition to increased lactate production, stimulation of the activity of a reduced nicotinamide adenine dinucleotide (NADH) oxidase, increased oxygen consumption, and marked increase in the activity of the hexose monophosphate shunt (*4*).

Leukocytes were isolated from the peripheral blood of these patients and from infected controls with similar distributions of granulocytes by means of fibrinogen sedimentation and differential centrifugation. The isolated cells were thrice washed in tubes coated with silicon with Krebs Hensleit buffer (*p*H 7.4) to which glucose (11 m*M*) was added. Erythrocyte contamination of the leukocyte suspension was less than 10 percent. Phagocytosis was initiated by the addition of 0.1 ml of a dilution (one part in ten) of dialyzed polystyrene spheres (*5*) to the leukocyte concentrate which numbered 2 to 4×10^7 leukocytes per milliliter. We assessed ingestion of spheres by observing cells with Nomarski optics. The percentage of leukocytes containing more than two spheres was enumerated. Activity of the hexose monophosphate shunt in leukocytes was determined in the resting state and following ½ hour of phagocytosis in stoppered 15-ml serum bottles. The $C^{14}O_2$ was trapped on filter paper, soaked with KOH, which was suspended from the serum cap, and the C^{14} activity of the dried paper was determined in a liquid scintillation counter. Lactate production was determined by measurement of the lactate concentration (*6*) of a 3 percent perchloric acid filtrate of the leukocyte suspension. The oxidase activity manifested during phagocytosis was de-