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Amino Acid Sequence Similarities in Two Human Anti Gamma Globulin Antibodies

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Abstract. Certain individuals with hypergammaglobulinemic purpura contain in their serum anti gamma globulins of restricted heterogeneity. These antibodies, characteristically of the γG type, were isolated from two unrelated individuals and their light polypeptide chains were subjected to amino acid sequence analysis. Although these anti gamma globulin antibodies conform to the basic VK_1 sequence, substitutions not previously encountered were found in five positions. The two antibodies were identical with each other up to residue 40 from the *N*-terminus of the light chains.

Introduction. A major goal in the field of immunology has been the elucidation of the primary structure of antibodies and the correlation of antibody structure with antibody specificity. Previous studies on the primary structure of immunoglobulins have been carried out primarily on myeloma proteins and Bence-Jones proteins devoid of recognizable antibody activity.[1–4] On the basis of these studies, antibody activity is thought to relate to the variable regions of the heavy and light chains. It seems logical to assume that an area of such variability would be associated with the many different specificities attributed to antibodies. Since restricted heterogeneity is not usually a property of the immune response to an antigen, homogeneous populations of antibodies have not been readily available for primary structural studies. However, it has been recently demonstrated that certain carbohydrate antigens often induce a homogeneous antibody response, for example the production of certain antibodies to dextran and levan[5] and of rabbit antibodies to streptococcal polysaccharides.[6] Most protein antigens, however, induce a heterogeneous population of antibodies.[7]

Previous investigations from this laboratory demonstrated that certain individuals with a benign disorder called hypergammaglobulinemic purpura contain in their serum relatively high concentrations of anti gamma globulin antibodies.[8] More recent studies have indicated that many of these antibodies have restricted heterogeneity.[9,10] These anti gamma globulin antibodies are of the γG type and have specificity for the Fc portion of the gamma globulin molecule. They were isolated and have been subjected to amino acid sequence analysis in an attempt to delineate structural similarities between antibodies with similar specificities. A preliminary report of this work has been presented elsewhere.[11]

Materials and Methods. Isolation of anti gamma globulins: The anti gamma globulins utilized for this study were isolated from 250–500 ml of plasma obtained by

plasmapheresis from patients with hypergammaglobulinemic purpura. The basic procedure for isolation has been described in detail.[10] Briefly, Fraction II gamma globulin was heat-aggregated and cross-linked with bisdiazotized benzidine. This insoluble material was then suspended in 0.1 M pH 4.0 acetate buffer and the suspension was added to an equal volume of serum. The mixture was stirred overnight in the cold and then centrifuged. The precipitate was washed and the anti gamma globulin antibody was eluted from it at 37°C with pH 4.0 acetate buffer. Further purification was accomplished by gel filtration chromatography in pH 4.0 acetate buffer. The γG fraction was removed and again subjected to the isolation procedure (beginning with the cross-linking step). The final eluate could be prepared in large quantities and had anti gamma globulin activity as detected by latex agglutination tests. Generally, 2–10 mg of purified anti gamma globulin of the γG type could be isolated per ml of plasma. Studies establishing the restricted heterogeneity of many of these antibodies have been presented elsewhere.[10]

Preparation of light chains for primary structural studies: The purified anti gamma globulin antibodies were reduced and alkylated[12] and heavy and light chains were prepared by gel filtration on Sephadex G-100 in 1 M propionic acid. After lyophilization, the light chains were subjected to complete reduction and alkylation in 8 M urea in preparation for automated amino acid sequence analysis.

Protein sequencing techniques: A Beckman model 890 Protein Sequencer was utilized for automated Edman degradations.[13] The program provided sequential additions of phenyl isothiocyanate and Quadrol buffer (Wyandotte Chemical Co.), washing with benzene and ethyl acetate, cleavage with heptofluorobutyric acid, and extraction of the thiazolinone derivative with butyl chloride. Aldehyde-free glacial acetic acid (0.1%) was added to the ethyl acetate[13] and 0.1 mM dithioerythrytol was added to the butyl chloride extraction solvent.[14] The former is useful in eliminating the Quadrol buffer from being carried into the extraction step and the latter improves recoveries of serine and threonine phenyl thiohydantoins. The repetitive yield in most instances was greater than 97%. The yield for individual amino acids varied from 87 to 30%.

Identification of PTH amino acids: A modification of the Pisano and Bronzert technique utilizing a Beckman GC-45 gas chromatograph was used to identify the amino acid phenyl thiohydantoins.[15] For certain residues the PTH derivatives were acid-hydrolyzed[16] and the amino acids were identified on a Beckman model 121 amino acid analyzer. Generally, 6% of the thiohydantoin derivative was subjected to gas chromatography and the remaining 94% was hydrolyzed and subjected to amino acid analysis on the amino acid analyzer. Most of the results presented here were obtained in a single programmed run on the Sequencer. However, both antibody light chains have been analyzed three times and there were no discrepancies between any of these experiments. Human kappa chain Hac, previously sequenced by Dr. Leroy Hood (personal communication) was sequenced 10 residues from the *N*-terminus by the above technique and no discrepancies were noted. In addition, on two occasions, the sequence of the first 24 residues from the *N*-terminus of sperm whale myoglobin was determined; the same sequence was obtained as is derived by other methods.

Results. Three major classes of kappa light chains have been designated, on the basis of *N*-terminal sequences, VK_1, VK_2, and VK_3.[17,18] The available sequence data on VK_1, the most common type, up to residue 23 are shown in Figure 1. As can be seen, there are only a few exceptions to the basic VK_1 sequence up to the first cystine at position 23. Five additional VK_1 proteins derived from either myeloma light chains (Tei) or from Bence-Jones proteins (Die, Car, Pot, Joh) have been sequenced in the present study. As can be seen in Figure 1, these data are quite consistent with those in previous studies. The bottom two sequences were obtained on the light chains of two purified anti gamma globulin antibodies from two different patients and an unambiguous sequence was obtained for each. The recovery of aspartic acid in step 1 was

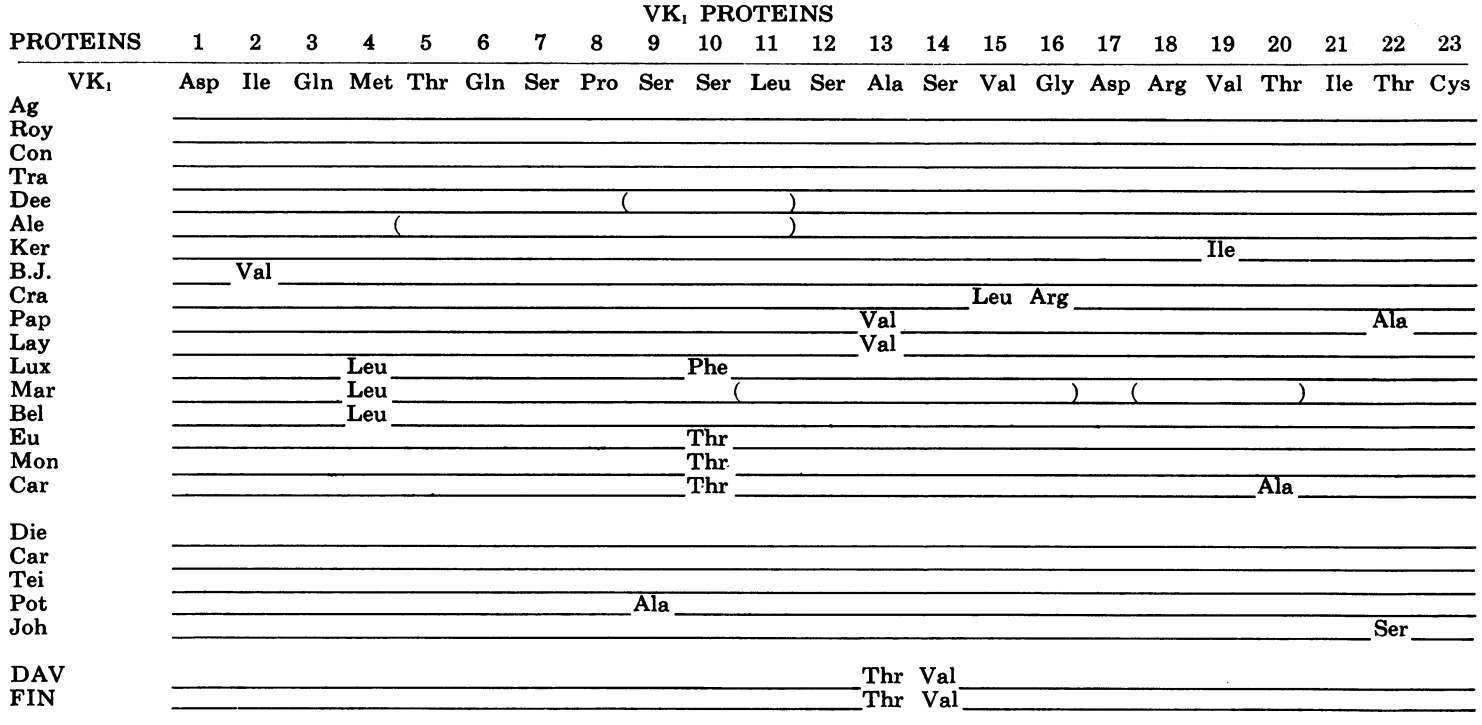

FIG. 1.—Amino acid sequences of human VK_1 proteins. The prototype sequence is given first and only those residues which differ from the prototype are shown. Most of the data on proteins Ag–Car have been summarized by Hood and Talmage.[30] Proteins Die–Joh represent five new light-chain sequences, from myeloma (Tei) or Bence-Jones proteins (remaining four). The last two proteins, Dav and Fin, are the anti gamma globulin light chains.

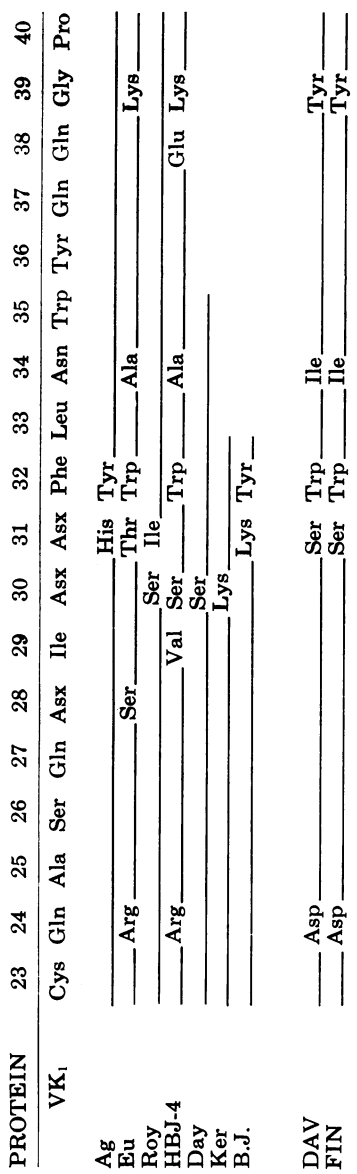

FIG. 2.—Amino acid sequences of the first hypervariable region of the kappa chain. The HBJ-4 sequence was provided by H. Sox and L. Hood (personal communication). The last two proteins, Dav and Fin, are the anti gamma globulin light chains.

very similar to that for myeloma and Bence-Jones proteins. Over 98% of the PTH derivative identified was aspartic acid, with less than 2% background. For a mixture of Fraction II light chains we, as well as others, have noted 65–70% yield of aspartic acid in step 1. This confirms the relative homogeneity of these antibodies. Their amino acid sequences are seen to be quite homologous with the basic VK_1 sequence established previously. The available amino acid sequence data of VK_1 proteins sequenced between positions 23 and 40 are shown in Figure 2, above the sequences for the two anti gamma globulin antibodies that are the subject of this report.

Discussion. These data show that two anti gamma globulin antibodies from two genetically unrelated individuals have identical amino acid sequences up to residue 40 of their respective light chains. In addition, six unusual amino acid substitutions have occurred.

As shown in Figure 1, up to position 23 several light chains (Ag, Roy, Con, Tra, Dee, Ale, Die, Car, Tei) as well as the two anti gamma globulin antibody light chains have the same sequence. Beyond residue 23, however, there have not been any two light chains with identical sequences. The region between cystine 23 and tryptophan 35 is quite variable compared to the first 23 amino acids (Fig. 2). Particularly, positions 30, 31, and 32 display a high degree of variability. As can be seen, the two antibodies continue to have identical sequences through this region. After position 35, except for position 39, there is little variability in kappa light chains until residue 52. Milstein,[19] Kabat,[20] and Franêk[21] have suggested that the antibody-combining site on the light chain involves positions 23–35 and positions 89–97 primarily because of the extreme degree of variability that has been noted in these positions. Thus, the identity of the two anti gamma globulin antibody light chains (at least to residue 40) has additional significance, since it involves the first hypervariable portion of the light chain and presumably a portion of the antibody-combining site.

Six unique amino acid substitutions occur in the light chains of these two anti gamma globulin antibodies. Both have threonine at position 13 and valine at position 14. In 67 previously sequenced light chains from both human and mouse, threonine has not been detected at position 13 nor has valine been detected in position 14.[22] At position 24, while the number of sequences is limited, aspartic acid has not been found by others in VK_I proteins; in position 31 serine and in position 34 isoleucine have not been previously found. Finally, tyrosine at position 39 has not been detected in 17 other light-chain sequences. While positions 24, 31, and 34 are in the first hypervariable region of the kappa chain, positions 13 and 14 have previously been thought to be relatively stable.

There are at least two explanations for the finding of these unique residues. On the one hand, the amino acid differences observed at positions 13, 14, 24, 31, 34, and 39 may be related to the antibody activity of these proteins. On the other hand, the stimulus for the production of these anti gamma globulin antibodies may result in the selection of a subgroup related to the VK_I subgroup. Such a subgroup, however, has not been encountered previously in the myeloma population that has been subjected to amino acid sequence analysis. We have recently sequenced a third anti gamma globulin antibody; it belonged to the VK_{II} subgroup. In addition, another patient had a monoclonal antibody with lambda light chains. The exact incidence of the VK_I type is unclear but it is evident from these other two patients that the VK_I type is not always present.

Some previous evidence has suggested that antibodies with similar specificities shared certain structural elements. Williams *et al.*[23] studied antigenic specificities related to the cold agglutinin activity of certain γM globulins. They found that antisera prepared against isolated human γM cold agglutinins could be rendered specific for γM proteins with cold agglutinin activity but would not recognize γM proteins not having such activity. Similar findings have been reported for rabbit antibodies to streptococcal polysaccharide.[24] Antisera made against certain of these homogeneous antibodies will only recognize other rabbit antibodies with similar specificity. Recently Hood *et al.*[25] have reported limited *N*-terminal amino acid sequence studies on these antibodies and structural similarities have been documented.

In studies on the characterization of antibody to the C-carbohydrate produced by a mouse plasmacytoma, Cohn, Notani, and Rice[26] made related observations. An antiserum reacted with only 2 of 160 sera from mice bearing different plasmacytomas. One was the protein used for immunization and the other had identical antibody specificity for C-carbohydrate. Potter *et al.*[27] recently presented a preliminary report on the *N*-terminal amino acid sequence identity of the light chains of two mouse myeloma proteins with identical individual specificity and presumably identical antibody activity against phosphoryl choline. The significance of this degree of structural identity is uncertain, as the hypervariable regions of the mouse light chain have not been delineated. However, since the two light chains had identical peptide maps it is likely that they are identical. The myelomas were produced in a single inbred strain of mice.

The present findings are consistent with the early observations of Koshland[28] which suggested that the amino acid differences associated with antibody specificity are not limited to a small consecutive sequence, but appear to be single

changes distributed over a large part of the variable region of the light chain. Recently Koshland *et al.*[29] have performed amino acid compositional analysis on two heterogeneous rabbit antibodies with similar specificities. The amino acid contents of the respective heavy chains differed by a single tyrosine residue, while the light chains appeared identical.

Thus, several lines of evidence have suggested that antibodies with similar specificities share structural determinants. The data presented here show that two antibodies with similar specificities from unrelated humans have identical light-chain sequences with certain unusual features and provide additional evidence relating antibody specificity to amino acid substitutions in specific regions of the antibody molecule.

Note added in proof: Peptide maps utilizing thermolysin and chymotrypsin revealed no discernible difference between light chain Dav and Fin (done by Dr. Leroy Hood, NIH). In addition, amino acid analysis revealed the two light chains to be of identical composition within the limits of experimental error.

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Abbreviation: PTH, phenyl thiohydantoin.

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