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Studies on IgA and IgG Monoclonal Proteins Derived from a Single Patient. Evidence for Identical Light Chains and Variable Regions of the Heavy Chain†

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ABSTRACT: Two immunoglobulins, IgA(κ) and IgG(κ), were isolated from the serum of a single patient with two monoclonal components (biclonal proteins). After chain separation, the light chains from each molecule were found to be identical by the following criteria: electrophoretic mobilities under various pH and dissociating conditions, amino acid composition, fingerprint analysis of tryptic peptides and of ^{14}C-succinylated chymotryptic peptides, and amino acid sequence of the N-terminal 40 residues. The heavy chains were indistinguishable for the N-terminal 45 amino acid residues. These data are consistent with the hypothesis that a single heavy chain variable (V_H) region may be associated with two different heavy chain constant (C_H) genes.

Multiple myeloma is a neoplasm of antibody-producing cells which may result in the appearance of a monoclonal immunoglobulin in the serum. Biclonal myeloma, on the other hand, shows an increase in two distinct immunoglobulins. This condition, which occurs in a much higher frequency than what would be expected for two independent neoplastic events, may reflect the normal differentiation process of antibody-producing cells (Sledge et al., 1975). Therefore, immunoglobulins from patients with biclonal myeloma may be used as a model to investigate the nature of genetic control during clonal differentiation of these cells.

There are three pieces of evidence which suggest a shared structural relationship between the individual members of biclonal immunoglobulin pairs. First, antigenic sites located within the H and L chain variable (V)[1] regions appear to be unique for a given immunoglobulin and are termed idiotypic determinants. The existence of shared idiotypic determinants between the individual members of a pair of biclonal proteins suggests that the V region genes of both the H and

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[1] Abbreviations used are: H chain, heavy chain; L chain, light chain; V region, variable region; C region, constant region; Tos-PheCH$_2$Cl, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone; Pth, phenylthiohydantoin.

L chains, whose products sterically interact to form the antigen binding site, are very similar for both molecules (Wang et al., 1970; Yagi and Pressman, 1973; Rudders et al., 1973; Fair et al., 1974a). Second, L chains isolated from biclonal immunoglobulins have been shown to be very similar by their electrophoretic mobilities, amino acid compositions, peptide maps, and N-terminal amino acid sequences (Wang et al., 1969; Pink et al., 1971; Seon et al., 1973a; Wolfenstein-Todel et al., 1974). These data suggest that the genes responsible for the synthesis of both L chains (the V and the C region) are very similar, if not identical, for both biclonal myeloma immunoglobulins. Finally, because no differences can be detected in the N-terminal amino acid sequences of both H chains in biclonal immunoglobulins, the heavy chain variable region (V_H) genes appear to be shared for both immunoglobulins (Wang et al., 1970; Seon et al., 1973b). However, not one H chain hypervariable region located within the antigen-combining site has been completely sequenced for any biclonal pair of proteins and thus the data are only suggestive that identical V_H genes can combine with one or more C_H genes. Moreover, because biclonal myeloma patients share idiotypic determinants and structural analysis of H and L chains indicates apparent similarities, biclonal myeloma immunoglobulins may indeed be the products of two clones of antibody-producing cells which have differentiated from a common precursor cell (Sledge et al., 1975).

Previously, we presented evidence for the sharing of common idiotypic antigenic determinants between IgA and IgG biclonal immunoglobulins for patient GR (Fair et al., 1974a). In this report, we describe our results which indicate that the κ light chains of the IgA and IgG molecules are identical and that differences in the N-terminal region of the heavy chains of these molecules could not be detected. A preliminary report of these data has been presented elsewhere (Fair et al., 1974b).

Materials and Methods

Protein Purification. Isolation of IgA and IgG monoclonal proteins from the plasma of patient GR has been described previously (Fair et al., 1974a).

Separation of Heavy and Light Chains. Each intact immunoglobulin (15 mg/ml) was mildly reduced in 0.5 *M* Tris-HCl (pH 8.2) with 0.2 *M* 2-mercaptoethanol for 2 hr at room temperature. Carboxylmethylation was achieved with iodoacetic acid in 10% molar excess at 0°C for 1 hr in the dark, and addition of NaOH maintained the pH between 8.2 and 8.5. The mixture was dialyzed against 0.15 *M* NaCl and then exhaustively against H_2O at 4°C.

Separation of H and L chains was achieved by adding propionic acid to the protein solution to a final concentration of 1 *M*, incubating for 2 hr (Björk and Tanford, 1971), and passing the solution over a Sephadex G-100 column (3 × 100 cm) equilibrated in 1 *M* propionic acid, according to the method described by Fleischman et al. (1962). Following elution, the separated proteins were dialyzed against 0.01 *M* sodium acetate buffer (pH 5.5) and then against 0.01 *M* sodium acetate (pH 5.5), containing 0.15 *M* NaCl. This procedure produced three peaks on elution from Sephadex G-100. The first peak contained aggregated H chains with some L chain contamination. Heavy chains were present in the second eluting peak, and light chains were contained in the third peak. The isolated polypeptide solutions were concentrated by ultrafiltration by employing Amicon PM-10 membranes. Occasionally, aggregated material eluting in the first peak from the Sephadex G-100 column was concentrated to approximately 25 mg/ml, brought to 6 *M* guanidinium chloride, and the H and L chains further separated over a 6% agarose column (1.5 × 85 cm) equilibrated in 6 *M* guanidinium chloride (Mann et al., 1973). After elution, the separated proteins were treated as described above, or dialyzed exhaustively against H_2O, lyophilized, and stored at −20°C.

Electrophoresis. The electrophoretic mobilities of the isolated L chains were analyzed in a variety of polyacrylamide gel systems. First, analyses of electrophoretic mobilities in sodium dodecyl sulfate–10% polyacrylamide gels using nonreduced and reduced treatments with 2-mercaptoethanol have been previously described (Weber and Osborn, 1969; Mann et al., 1971). Second, the urea–acetic acid system described by Panyim and Chalkley (1969) for 10% polyacrylamide gels served as another denaturing solvent but at an acid pH. Third, disc gel electrophoresis in 10% polyacrylamide was carried out in a dilute aqueous solvent at alkaline pH as described by Davis (1964). Protein bands were resolved in the above gels by staining with Coomassie blue as described by Fairbanks et al. (1971).

Isoelectric focusing of the κ chains was carried out by two separate methods. In the first, the L chains were focused in 7% acrylamide gels containing 2% ampholine (pH range 3–10) between solutions of 2% phosphoric acid and 0.05 *M* NaOH at 250 V for 3 hr. The proteins were fixed in the gels with 12% trichloroacetic acid (Cl_3CCOOH) and the ampholines removed by several washes in 2% Cl_3CCOOH. The resulting protein pattern was developed by staining with 0.1% amido black in 7% acetic acid–0.1% Cl_3CCOOH solution and destained electrophoretically in 7% acetic acid–0.1% Cl_3CCOOH. In the second procedure, the polypeptides were focused in 6% acrylamide gels (1 × 11 cm), containing 0.6% ampholine (pH range 3–10) and 6.7 *M* urea between solutions of 5% phosphoric acid and 5% ethylenediamine at constant voltage (1 W/gel) for 12 hr. After removing the ampholines by soaking the gels in a 20% IPA–25% sulfosalicylic acid solution, the proteins were resolved by staining in 0.0025% Coomassie blue in 10% acetic acid and destaining in 10% acetic acid.

Amino Acid Analysis. The amino acid composition of the light chains was determined on a Beckman Model 119 automated amino acid analyzer utilizing 3-hr, single column methodology. Duplicate protein samples were hydrolyzed in evacuated tubes with 6 *M* HCl for 25 hr at 110°C. The samples were applied to a column matrix of Beckman's spherical resin (AA-15), and the amino acid residues were eluted at 53.5°C under pressure by stepwise changes of three buffers: (a) 0.2 *N* sodium citrate (pH 3.49); (b) 0.2 *N* sodium citrate (pH 4.13); (c) 0.2 *N* sodium citrate (pH 5.72).

Peptide Maps. Tryptic digestion of the light chains was performed by a modification of the method of Stears et al. (1965). Lyophilized samples were dissolved in 6 *M* guanidinium chloride (10 mg/ml) and completely reduced with 2-mercaptoethanol (1:10, v/v). The pH was adjusted to 8.6 with 1 *M* NaOH, and reduction proceeded at room temperature for 8 hr. Samples were then dialyzed exhaustively against 0.001 *M* HCl–0.01 *M* 2-mercaptoethanol, and heat denatured at 100°C for 15 min. This solution was brought to 0.2 *M* NH_4HCO_3 while still hot, and the protein coagulated upon cooling. The denatured protein solution was made 0.01 *M* in 2-mercaptoethanol and incubated with dry L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (Tos-

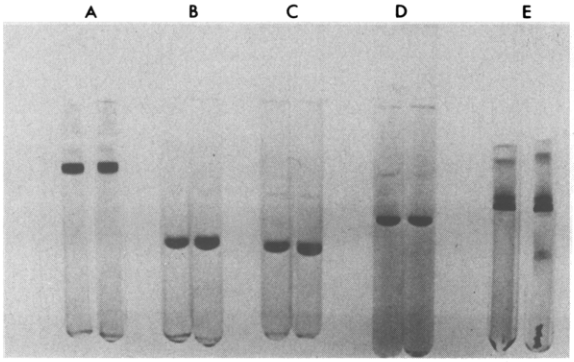

FIGURE 1: Polyacrylamide gel electrophoretic analysis of GR κ light chains. In each pair of gels, the light chain from IgA is to the left and the light chain from IgG is to the right. Migrations of each polypeptide are depicted in a discontinuous system at pH 8.9 (A), in an unreduced sodium dodecyl sulfate system (B), in a sodium dodecyl sulfate gel reduced with 2-mercaptoethanol (C), in a urea–acetic acid system at pH 3.2 (D), and isoelectric focusing in 7% acrylamide gels containing 2% ampholine (E).

PheCH$_2$Cl)–trypsin (1:100, w/w) at 37°C for 24 hr. Following hydrolysis, the samples were lyophilized, and the dry hydrolysate was taken up in 0.01 *M* acetic acid. One to two milligrams of hydrolysate was spotted on a sheet of Whatman No. 3MM paper (57 × 45 cm), and the peptides were initially separated by descending chromatography in a solvent of butanol–acetic acid–water (4:1:5, v/v) for 16 hr. The peptides were further separated by high-voltage electrophoresis in a pyridine–acetic acid–water (1:10:289, v/v) buffer (pH 3.7) for 1 hr at 3000 V at a right angle to chromatographic separation. The chromatogram was developed by a ninhydrin solution, and the spots and colors were noted. Duplicate peptide maps were done for each hydrolysate.

Following the modification of the L chains by ^{14}C-succinylation the polypeptides were digested with chymotrypsin and analyzed by radioautography, according to the procedure of Waterson and Konigsberg (1974). Modification of 1.5 mg of L chain was carried out with [^{14}C]succinic anhydride in a 0.5-ml solution of 0.5 *M* Tris-HCl–6 *M* guanidinium chloride (pH 9.0) and the mixture was permitted to react for 20 min at 25°C. Unlabeled succinic anhydride (1.5 mg) was then added, and the reaction was allowed to proceed for an additional 30 min while maintaining the pH at 9.0 by the addition of 6 *N* NaOH. After dialysis against 0.1 *M* ammonium bicarbonate, the protein concentration was adjusted to 1 mg/ml (specific activity 0.5 μCi/mg of protein) and subjected to chymotryptic hydrolysis (6%, w/w) for 24 hr at 25°C. Separation of the peptides was first accomplished by spotting 15 μl of the digest on a cellulose thin-layer plate (20 × 20 cm) and then electrophoresing at pH 5.5, using a pyridine–acetic acid–water (25:1:225, v/v) solvent system on a DESAGA Brinkman cold plate electrophoresis unit. Next, ascending chromatography was carried out at a right angle to the electrophoretic separation using a solvent system of butanol–acetic acid–water (3:1:1, v/v). The ^{14}C-succinylated peptides were detected by radioautography after 64-hr exposure on Kodak No-Screen x-ray film.

Amino Acid Sequence Analysis. Each chain was subjected to 40–45 steps of degradation by the automated Edman procedure using a Beckman 890A protein sequenator employing the standard DMBA program. Samples were dried under N_2 and converted to the phenylthiohydantoin

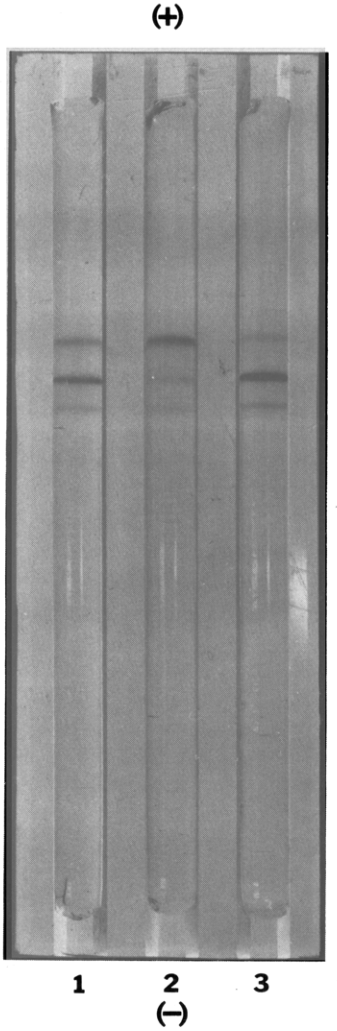

FIGURE 2: Isoelectric focusing gels of GR κ light chains in 6% acrylamide gels containing 0.6% ampholine and 6.7 *M* urea: (1) IgA; (2) IgG; (3) IgA + IgG.

(Pth) amino acids by treatment with 1 *N* HCl–0.001 *N* ethanethiol for 10 min at 80°C. Following extraction in ethyl acetate, 5% of each sample was run on a Hewlett-Packard gas chromatograph equipped with dual glass columns packed with 7.5% DC-560. The remainder of each sample was then regenerated by hydrolysis in hydriodic acid for 18 hr at 120°C (Smithies et al., 1971), and analyzed on a Durrum D-500 amino acid analyzer. Throughout these experiments, Pth-norleucine was added to the sequencer tubes prior to conversion to normalize for any losses due to handling. The methodologies used in these studies have been described in a previous publication (Hood et al., 1973). Yields of approximately 50% of the theoretical values were noted at step 1 and the repetitive yields averaged 90% for all chains.

Results

Light Chain Studies. Light chains isolated from mildly reduced immunoglobulin and separated on Sephadex G-100 equilibrated in 1 *M* propionic acid always revealed less than 2% contamination by heavy chain. However, after passing the isolate through a 6% agarose column equilibrated in 6 *M* guanidinium chloride contaminants could not be detected.

Light chains isolated from IgA and IgG of patient GR were compared for similarities in their electrophoretic be-

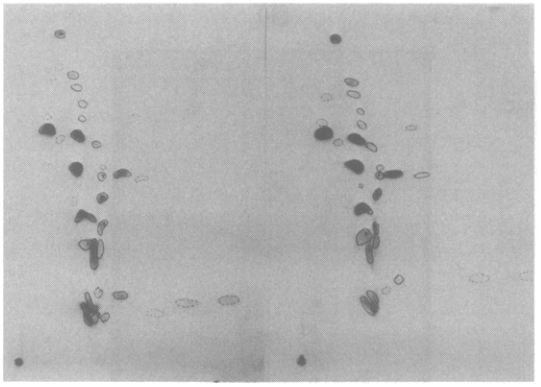

FIGURE 3: Tryptic peptide maps of GR κ chains from IgA (left) and IgG (right). Descending chromatography is from left to right, and electrophoresis is from bottom to top in each map. Solid lines indicate pronounced color while dashed lines outline faint spots. The Y and G represent spots colored yellow and gray, respectively, by the ninydrin reagent.

Table I: Amino Acid Compositions of GR κ Chains.[a]

Amino Acid	κ_G	κ_{Am}	κ_{Ap}
Asp	14.66	14.71	14.64
Thr	14.54	14.60	14.69
Ser	28.93	28.83	28.91
Glu	28.37	28.90	28.37
Pro	10.28	9.07	10.23
Cys	2.64	2.73	2.91
Gly	12.16	12.30	12.60
Ala	16.07	16.58	16.34
Val	13.63	13.64	13.66
Met	1.66	1.74	1.62
Ile	6.51	6.64	6.56
Leu	15.21	15.29	15.08
Tyr	8.73	8.57	7.98
Phe	8.35	8.12	8.43
Lys	10.38	10.31	10.57
His	2.76	2.83	2.99
Arg	9.73	9.84	9.83

[a] Twenty-five hour hydrolysis in 6 *M* HCl at 110°C.

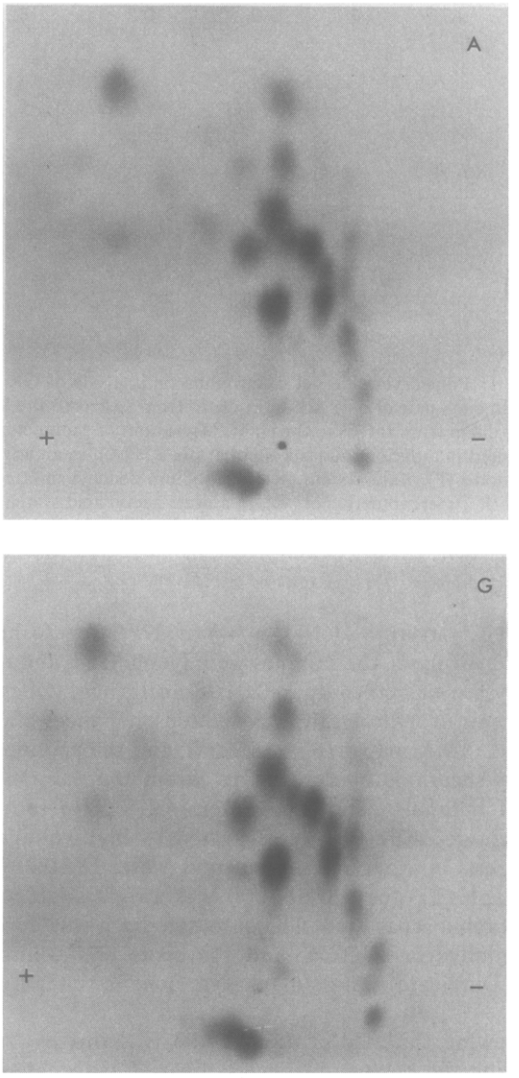

FIGURE 4: Autoradiograms of the chymotryptic peptide maps of ^{14}C-succinylated GR κ chains from IgA (top) and IgG (bottom).

havior under a variety of denaturing and pH conditions. Figure 1 depicts the results for both L chains. Both polypeptides had the same mobilities in disc electrophoresis with a running gel of pH 8.9, using nondenaturing conditions (Figure 1A). Protein mobilities are also the same when the molecules were analyzed under denaturing conditions with sodium dodecyl sulfate, whether or not a reducing agent was used (Figure 1B,C). No difference in migration was detected when electrophoresis was carried out using 10% polyacrylamide gels in urea–acetic acid (Figure 1D). Finally, when the isoelectric focusing patterns of both light chains were compared, they were very similar (Figures 1E and 2). Because it was not possible to detect differences in electrophoretic mobilities between the L chain from IgA or IgG when analyzed under various denaturing and pH conditions, the data suggested that the two light chains are very similar, if not identical.

A further comparison of the light chains was carried out by determinations of their amino acid composition. Included in Table I are the average molar quantities of the amino acid residues of duplicate samples present in the κ chains isolated from IgG, IgA monomer, and IgA polymer. The results indicate that the three polypeptides are very similar, if not identical. Only the number of proline residues in the light chain from IgA monomer is different from the other two molecules, and this difference of one residue is within experimental error.

Mapping the polypeptides produced after enzyme cleavage was employed to show similarities in primary sequence structure between both the IgA κ chain and the IgG κ chain. Figure 3 depicts representative maps of the fingerprint pattern of both polypeptides after Tos-PheCH_2Cl–trypsin digestion. Because the map patterns were very similar, this suggested that the primary amino acid sequence of both L chains was also very similar. In addition, analogous results were observed when the polypeptides were labeled with [^{14}C]succinate and hydrolyzed with chymotrypsin (Figure 4). Again, the autoradiograms indicated a very similar distribution of labeled peptides, when the two L chains were compared. It, therefore, appeared that both chains were very similar, if not identical, in their primary structure.

Because the above data suggested that the L chains were very similar in their amino acid sequence, we determined the N-terminal amino acid sequence of each polypeptide chain. The results of the first 40 amino acid residues are depicted in Figure 5. These data indicated that (1) both L chain sequences were consistent with that of the $V_\kappa I$ subgroup, (2) both were identical over that portion of the V

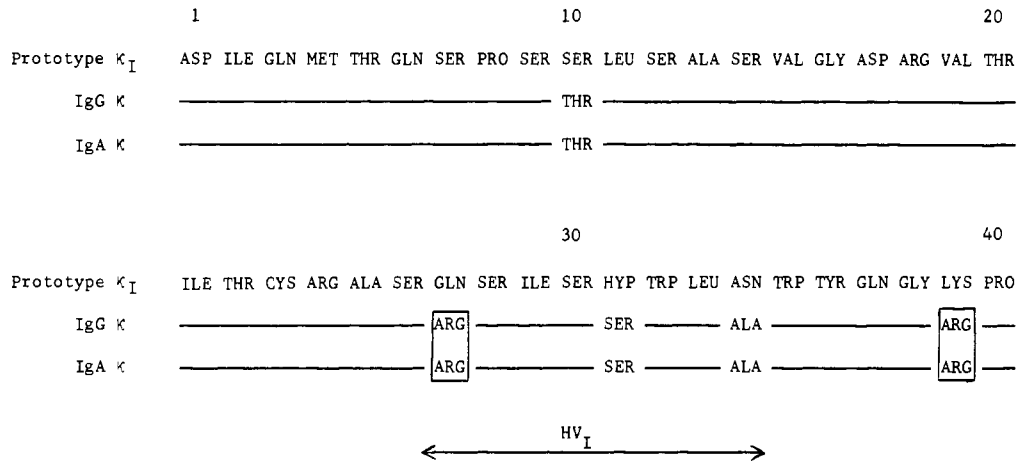

FIGURE 5: The N-terminal sequences of human κ chains from biclonal immunoglobulins from patient GR compared with a human prototype $V_\kappa I$ sequence. The boxed residues have not been found in any other human V_κ regions. HYP indicates a position with many substitutions (hypervariable). HV indicates the extent of the first hypervariable region. Parentheses designate an unidentified residue. The amides have not been identified.

region examined (~35%), (3) both contained unique residues not seen in any other human V region, e.g., the arginyl residues at positions 27 and 39, and (4) both κ chains were identical throughout the first hypervariable region.

Heavy Chain Studies. To determine if the same V_H region was shared between the two H chains, we sequenced the N-terminus of each polypeptide. Figure 6 shows our results from this analysis for the first 45 amino acid residues. The data indicated that (1) the gene encoding this sequence corresponded to a V_HIII subgroup gene, (2) the sequences of both α and γ chains were indistinguishable over that portion of the V region examined (~40%), (3) both contained unique residues not seen in any other human V_H region, i.e., the tyrosyl, prolyl, and arginyl residues at positions 33, 38, and 43, respectively, (4) the amino acid residues within the first hypervariable region were identical for both chains, and (5) although the residues at position 35 were not identifiable, their chromatographic profiles were identical.

Discussion

Immunoglobulins from patients with biclonal myeloma may be used as a model to investigate the nature of genetic control during clonal differentiation of antibody-producing cells. Two observations suggest that biclonal myeloma may reflect the normal differentiation process in which two or more daughter clones of immunoglobulin-producing cells are derived from a common precursor cell. First, the spontaneous incidence of multiple myeloma is about 1 in 20000 (Moore, 1967), whereas biclonal myeloma occurs in 1% of these individuals afflicted with this disorder (Bihrer et al., 1974). This frequency of biclonal myeloma is much higher than what would be expected for two independent spontaneous neoplastic events, and suggests that a precursor cell may undergo neoplastic conversion and subsequently differentiate to produce two or more daughter cells each synthesizing a different immunoglobulin. Second, immunochemical and biochemical studies indicate that each pair of biclonal myeloma proteins share very similar structural features. The most common feature is the sharing of similar, if not identical, light chains and V_H regions between both immunoglobulins. Accordingly, a single V_H gene may be associated with two or more different C_H genes. Moreover, the similarities in the individual molecular components from the serum of patients with this condition provide us with an opportunity to examine the pathway(s) of differentiation of antibody-producing cells.

Our study indicated that the κ chains from the monoclonal IgA and monoclonal IgG from the serum of patient GR are very similar, if not identical, when compared by their mobilities in polyacrylamide gels under a variety of pH and dissociating conditions, amino acid compositions, peptide maps, and amino acid sequence of the N-terminal 40 residues which included the first hypervariable region. In previous studies, one or two gel systems were employed as a measure of identical polypeptide chain mobility. These results may be misleading as shown for the light chain mobility for patient VN (Goldrosen et al., 1972). Under acidic conditions, both λ chains had identical mobilities, but when an alkaline pH was utilized, the chains showed distinctly different migrations. Therefore, we used six separate systems, including isoelectric focusing, to compare the κ chains from IgA and IgG. Under every condition employed, both polypeptides had identical mobility, suggesting that each had a similar molecular weight and charge. This identity was also reflected in their amino acid compositions. Further, the primary sequence of each L chain appeared to be identical by peptide mapping. The production of similar patterns by two different methods of protein digestion and peptide fractionation gave strong support to this conclusion. Moreover, each polypeptide had the same amino acid sequence for the N-terminal 40 residues. These data indicated that the L chains were identical for about 35% of the V region which belonged to the $V_\kappa I$ subgroup prototype. In addition, they contained unique residues not seen in any other V_L region (Gally, 1973), e.g. the arginyl residues at positions 27 and 39, and both L chains were identical throughout the highly substituted hypervariable region (Wu and Kabat, 1970). This suggests that both proteins are derived from a single unique clone.

A comparable analysis of the L chain from other biclonal immunoglobulins has been carried out with only one other patient, Til (Wang et al., 1969; Pink et al., 1971). In this case, the κ chains from monoclonal IgM and monoclonal IgG were indistinguishable when compared by electrophoretic mobilities in starch gel, amino acid composition, tryptic peptide maps, optical rotatory dispersion, circular dichroism, and the amino acid sequence analysis of the N-terminal 37 residues. Studies on other pairs of biclonal proteins suggest analogous results, but amino acid sequences extending through the first hypervariable region have not

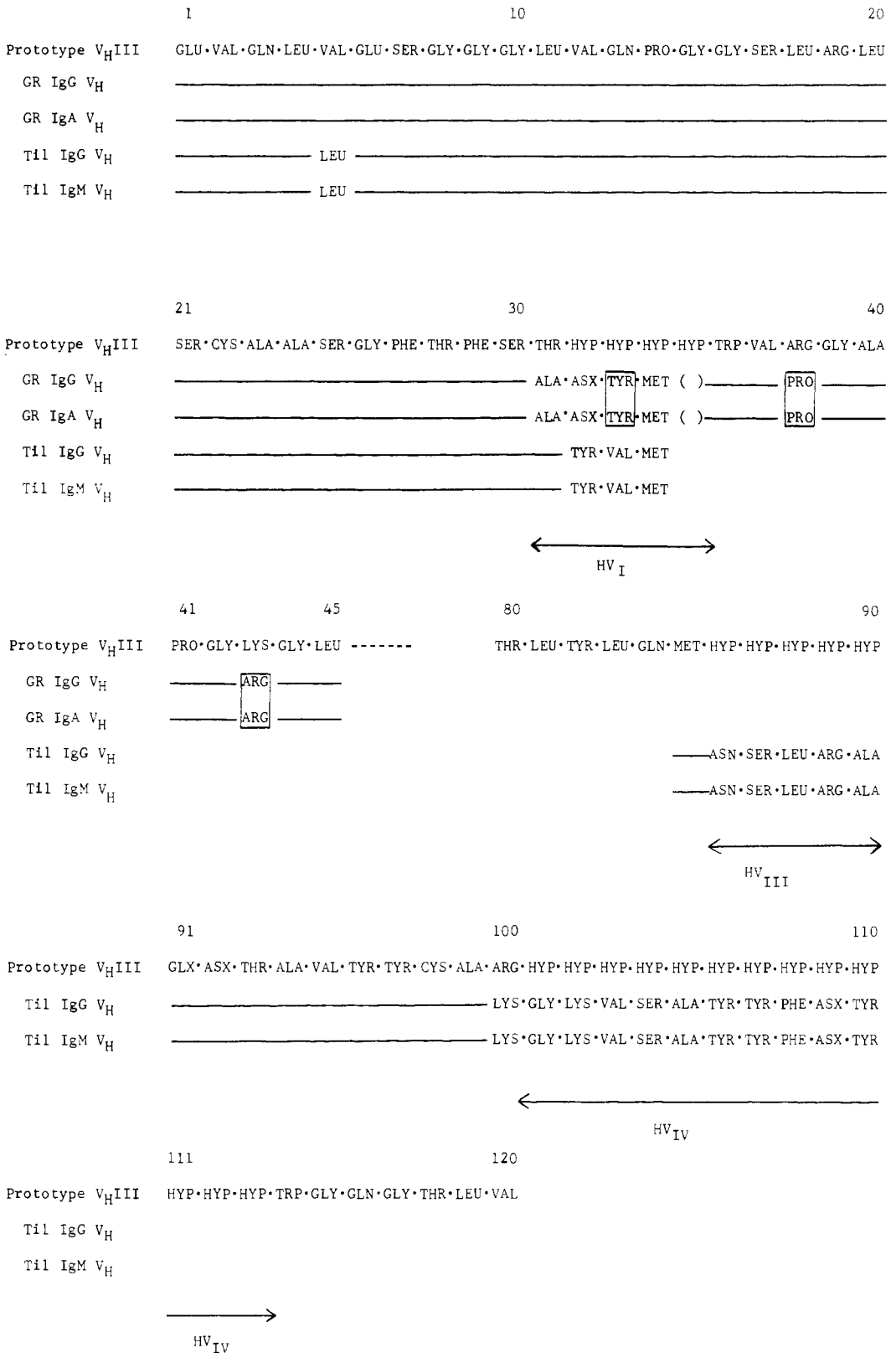

FIGURE 6: The N-terminal sequence of human heavy chains from biclonal immunoglobulins from patient GR and that from patient Til (Wang et al., 1973) compared with a human prototype V_HIII sequence. The numerical values corresponding to specific amino acid residues may differ from those reported in the literature due to insertion and deletion adjustments of the prototype sequence. For an explanation of the symbols, see Figure 5.

been reported (Seon et al., 1973a; Wolfenstein-Todel et al., 1974). Our data and those reported above lend strong support to the hypothesis that many pairs of biclonal immunoglobulins originate from a common stem cell and share the same V_L region gene as well as the same C_L region gene.

Unequivocal demonstration of the association of one V_H region with two or more C_H regions is required to support the postulated development of biclonal myeloma immunoglobulins from a common stem cell. The data on patient GR indicated that the N-terminal 45 amino acid residues of the

α and γ chains were indistinguishable. This primary sequence represents about 40% of the V region and included the first hypervariable region as defined by Capra and Kehoe (1974) for the H chain subgroup prototype gene V_HIII. Identical amino acid sequences of the V region residues 1–34 and 83–108 (Wang et al., 1970, 1973; Pink et al., 1971), which includes sequences of three hypervariable regions, of the γ and μ chains from patient Til also provide evidence in support of this requirement. However, these include only a partial sequence for the first and fourth H-chain hypervariable regions which are involved in the antigen binding site (Amzel et al., 1974), and a complete sequence of the third hypervariable region which does not function in the antibody-combining site (Figure 6). Additional support of this supposition from other biclonal proteins is meager (Seon et al., 1973b; Wolfenstein-Todel et al., 1974).

Attempts to extrapolate V region identity from partial amino acid sequences identical through the first hypervariable region may be hazardous because examples are known where changes can occur in amino acid positions following this hypervariable region (see Figure 6 in Hood et al., 1975). Obviously, it is critical to carry out a complete amino sequence analysis of the entire V_H region from two biclonal proteins. However, the first hypervariable regions of the GR α and γ chains are identical, and the residues at positions 33, 38, and 43 are unique as they have not been observed in any other human V_H region. Since both the IgA and IgG appear to share identical idiotypic determinants (Fair et al., 1974a), it is likely that the V_H regions from IgA and IgG are very similar, if not identical. Hence, these data are consistent with the notion that the cells producing the GR proteins were derived from a common stem cell.

Studies on biclonal immunoglobulins could shed light on the pathway of differentiation of antibody-producing cells if the following is assumed: (1) the immunoglobulin-producing myeloma cells represent "frozen" stages of maturation, i.e. cells producing IgG will not subsequently produce other immunoglobulins; (2) the intermediate steps in differentiations are not skipped during the neoplastic event. Immunosuppression of lymphoid cells by anti-μ chain antisera early in development inhibited the production of IgM, IgG, and IgA antibody-producing cells (Kincade et al., 1970; Lawton et al., 1972; Pierce et al., 1972; Herrod and Warner, 1972; Martin and Leslie, 1974; Manning, 1974). These studies indicated that IgG and IgA-forming cells are derived from IgM-producing precursors (Lawton and Cooper, 1973; Martin and Leslie, 1974; Manning, 1974). From these data, two models on the differentiation of antibody-producing cells were formed. In the first, a sequential and linear development of IgM $\rightarrow$ IgG $\rightarrow$ IgA-forming cells was postulated (Cooper et al., 1972), whereas in the second, a multipotential IgM precursor cell may give rise to all the immunoglobulin classes by separate pathways (Manning, 1974; Warner, 1974). There are four combinations of biclonal immunoglobulins of different H chain classes and these are: IgM + IgG, IgG + IgA, IgM + IgA, and IgD + IgG. These data suggest that B cell differentiation is more complex than either of the previous models. For example, the linear sequential model does not explain IgA + IgM biclonals, nor does the multipotential model explain IgA + IgG biclonals.

Whatever the pathway of antibody-forming cell differentiation is, there are two possible explanations for the association of one V_H gene with two or more C_H genes. First, Sledge et al. (1975) have suggested that multiple copies of a given V_H gene could be made and inserted simultaneously next to all of the C_H region genes. The expression of any one V_HC_H gene combination would then be regulated by normal developmental mechanisms. A second mechanism of gene expression has been suggested by Wang et al. (1970). In this case, a single V_H gene is translocated from one C_H gene to another during a clonal development with each cell expressing only a single V_HC_H gene product. Immunofluorescent studies on bone marrow in cells from individuals with biclonal proteins indicate that one immunoglobulin class is synthesized from a single population of cells (Wang et al., 1969; Levin et al., 1971; Silverman et al., 1973; Bihrer et al., 1974). However, other investigators have suggested that biclonal immunoglobulins may originate from single cells (Costea et al., 1967; Sanders et al., 1969; Rudders et al., 1973; Bouvet et al., 1974). If any antibody-producing cell can produce two different classes of immunoglobulins with identical V_H regions over a long period of time, the simultaneous insertion model described above would be strongly favored (see Sledge et al., 1975).

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Biosynthesis of the Polyoxins, Nucleoside Peptide Antibiotics: Glutamate as an Origin of 2-Amino-2-deoxy-L-xylonic Acid (Polyoxamic acid)†

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ABSTRACT: The biosynthetic origin of the carbon skeleton of 2-amino-2-deoxy-L-xylonic acid (polyoxamic acid) is described. This aminoaldonic acid is the N terminus of the nucleoside peptide antibiotics, the polyoxins, produced by *Streptomyces cacaoi* var. *asoensis*. In vivo experiments concerning incorporation and distribution of radioactivity from a number of ^{14}C-labeled compounds have clearly shown that the carbon skeleton of glutamate is a precursor for this aminoaldonic acid and sugars are incorporated only after their conversion into glutamate through the glycolytic and the tricarboxylic acid cycle pathways. Experiments utilizing [^{14}C]acetate and succinate have also indicated multiple passages through the Krebs cycle are operating before their incorporation into polyoxamic acid via glutamate. The distribution of ^{14}C between C-1 and C-5 of polyoxamic acid from the [5-^{14}C]glutamate experiment has indicated that 40% of glutamate incorporated into polyoxamic acid has been incorporated only after the reversible conversion into α-ketoglutarate followed by the passage through the Krebs cycle. Lack of incorporation of ^{3}H in the [1-^{14}C;2-^{3}H]- and [5-^{14}C;2-^{3}H]glutamate experiments is discussed in terms of a reaction(s) between glutamate and polyoxamic acid.

In our last paper (Isono et al., 1975), we reported a new metabolic role for L-isoleucine as a precursor of 3-ethylidene-L-azetidine-2-carboxylic acid (polyoximic acid). This amino acid is a C terminal amino acid of the nucleoside peptide antibiotics, the polyoxins (Chart I) (Isono et al., 1969), produced by *Streptomyces cacaoi* var. *asoensis* (Isono et al., 1965). Another unusual amino acid, 2-amino-2-deoxy-L-xylonic acid (polyoxamic acid) (I) or its 3-deoxy analogue (II) constitutes the N terminus of the biologically active polyoxins. This amino acid bears a carbamoyl group on the C-5 hydroxy-oxygen (Isono et al., 1969) (Chart I). Chemical replacement of this amino acid with a variety of

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