STRUCTURAL STUDIES OF MONOCLONAL HUMAN CRYOPRECIPITABLE IMMUNOGLOBULINS

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SUMMARY: The light chain type, immunoglobulin class and when possible, heavy chain subclass of eleven monoclonal human cryoglobulins were correlated with the variable region subgroup of their light chains. The variable region subgroups were assigned by determining the primary amino acid sequence for the first 15 amino-terminal residues of these light chains. 5/5 IgM cryoglobulins which react with human IgG had light chains of the variable region-III kappa chain subgroup (vK-III). 4/4 IgG and 2/2 IgM cryoglobulins with undefined antibody specificity had both lambda and kappa light chains none of which were vK-III. The data support the concept that there is marked restriction of the IgM anti-IgG antibody response to the IgG auto-antigen.

Certain human auto-antibodies with similar antigenic specificity may share related antibody combining site structures. This structural similarity includes a) relatedness of antigenic determinants attributed to the antibody combining site configuration(s) or idiotype(s) (1-3), b) sharing of variable region subgroups of light and heavy chains (2,4) and c) an unusual degree of primary amino acid sequence homology in hypervariable regions of antigen contact points of the combining sites of these antibodies (5). Two types of human auto-antibodies which have been shown to have these similarities are cold agglutinin (1) and anti-IgG (2-5) antibodies.

In order to extend these observations, a varied group of five monoclonal, cryoprecipitable immunoglobulin M proteins with anti-IgG activity, and six other types of cryoglobulins with undefined antigenic specificity were isolated, classified as to light and heavy chain class, light chain variable region subgroup, and the presence of anti-IgG activity.

The cryoglobulins studied were isolated from blood collected and clotted at 37° C, formed in serum incubated at 4° C for up to one week, collected by centrifugation at 4° C, and purified by multiple washes (3 or 4) with chilled 0.1 M borate buffered 0.15 M NaCl, pH 7.75 to 7.8 (BBS). Occasionally proteins were resolubilized at 37° C in BBS, reprecipitated by incubation at 4° C and rewashed as above.

The purity and immunoglobulin class composition were determined by immunodiffusion and immunoelectrophoresis in 1 percent agar gel using rabbit antisera specific for the human mu, alpha, and gamma heavy chain, and kappa and lambda light chain determinants, and a horse anti-whole human serum antiserum*. Cryoglobulins were either subjected to further purification or utilized directly, based on their immunologic composition and antigenic specificity. Those which were solely IgM or IgG and with undefined antigenic specificity were used directly since they were of a single light chain type.

"Mixed" IgM-IgG cryoproteins were solubilized at 37° C in 0.015 to 0.02 M phosphate pH 7.6, and placed on a DEAE-cellulose column packed and equilibrated at 37° C in the same buffer. The IgG was eluted and separated from IgM by washing the column with ten to twenty times the column volume of phosphate buffer. Generally, IgM was eluted with 0.4 M NaCl in 0.02 M phosphate pH 7.6. Both fractions were retested for immunologic purity and composition as above.

Prior to reduction and alkylation, and amino acid sequence determination, the charge restriction of all proteins was determined by isoelectric focusing on a standard 110 ml analytical, liquid ampholine column as previously described (6). Iso-focusing profiles were obtained which indicated restricted molecular charge and heterogeneity, and were comparable to profiles obtained for monoclonal IgG myeloma proteins and non-cryoprecipitating IgG and IgM anti-IgG globulins (6-8). These proteins were then felt to be sufficiently "clean" for chemical analysis.

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* All kindly prepared, characterized and donated by Dr. John P. Leddy.

Disulfide bonds of cryoglobulins were chemically reduced with 0.03 to 0.1 M dithiothreitol (DTT), and alkylated with a molar excess of iodoacetamide over DTT, in 0.2 M Tris-HCl buffer, pH 8.5 to 8.6, 6 to 8 M in urea or guanidine hydrochloride. Light and heavy chains were obtained from the reduced and alkylated proteins by column chromatography on Sephadex G-100 in 1 M propionic or 1 M acetic acid, 6 M in urea.

Two to five mg of the purified light or heavy chains from these proteins were then subjected to primary amino acid sequence analysis in an updated Beckman 890 B sequenator with undercut cup, using the standard "rapid-protein", double-cleavage program and quadrol buffer. Amino acid residues were converted to the PTH form in 0.1 N HCl at 83° C for 10 minutes, extracted in ethyl acetate and analyzed. All PTH amino acid residues were first identified in a preliminary manner by thin layer chromatography in heptane, propionic acid, ethylene dichloride (58:17:25). Positive or confirmatory identification was then made by gas-liquid chromatography. Amino acid residues were identified up to residue fifteen which allowed classification of all light chains into their variable region subgroups. The heavy chains subjected to sequence analysis all had blocked amino terminal residues.

Table #1 lists the cryoglobulins studied, classified as to immunoglobulin class and antigenic specificity, i.e. IgM or IgG monoclonal cryoglobulins without defined antibody activity or IgM anti-IgG globulins isolated from "mixed" IgM-IgG cryoglobulins. The gamma chain subclasses of the IgG cryoglobulins were determined by immunodiffusion in agar gel by Dr. John P. Leddy. Light chain variable region subgroups are assigned by amino acid sequence and follow the classification of Wu and Kabat (9). Despite the fact that in some proteins no amino terminal residue was obtained, sequenator analysis was continued for five residues. In no instance, did any amino acid appear. It is assumed that a pyrolidone carboxylic acid residue occupies the amino terminal position of these "blocked" polypeptide chains.

The data show that all IgM anti-IgG antibodies contain type kappa light

TABLE 1

Protein	Heavy chain: Ig-class	Heavy chain: sub-class	Heavy chain: amino-terminus	V_L	Light chain amino terminal sequence
Bak	IgG	γ_1	ND*	vλ? ‡	Blocked
Pie	IgG	γ_1	ND*	vλ? ‡	Blocked
Web	IgG	γ_{2-3}	Block	vKI	ASP ILE GLN MET THR GLN SER PRO SER THR VAL SER ALA SER VAL
Lon	IgG	γ_4	ND	vλIII	TYR SER LEU THR GLN PRO PRO SER VAL SER VAL SER PRO – –
Hoe	IgM	-	ND	vKI	ASP ILE GLN MET THR GLN SER PRO SER THR LEU SER ALA SER VAL
Lod	IgM	-	Block	vKI	_____ ? _____ ? _____ ? ___
Anti-IgG					
Teh	IgM	-	Block	vKIII	GLU ILE VAL LEU THR GLN SER PRO GLY THR LEU SER LEU SER PRO
Cra	IgM	-	ND	vKIII	GLU _____
Pla	IgM	-	Block	vKIII	GLU _____
Glo	IgM	-	ND	vKIII	GLU _____
Pin	IgM	-	Block	vKIII	GLU _____
All§	IgG	γ_1	Block	vKIII	GLU _____

* ND = Not Done

‡ ? = V region subgroup not able to be assigned.

§ All = Previously reported myeloma protein which is utilized as the standard vKIII sequences in this lab; antigenic specificity unknown.

chains confined to variable-region subgroup III (vK-III). None of the 4 IgG or 2 IgM cryoglobulins without anti-IgG activity contains this light chain variable region group. When these data are pooled with those in which only cryoprecipitating IgM anti-IgG proteins have been similarly studied (10), 7/8 have light chains of the vK-III subgroup. The one remaining contains lambda light chains of variable region subgroup V. When this analysis is extended to include all non-cryoprecipitating and cryoglobulin IgM anti-IgG antibodies which have been subjected to primary amino acid sequence determination, 13/15 of these are contained within the same vK-III region subgroup (4).

These data contrast sharply with those obtained by study of the cryoproteins of the IgG and IgM classes without defined antibody activity. These cryoglobulins included both type lambda and kappa light chains and exhibit a diverse distribution among the other light chain variable region subgroups.

The IgM anti-IgGs described here have been previously reported as having similar average intrinsic association constants (K_o: 1-2 x 10^5 L/M) for an IgG-1 Fc fragment (11). Further, identical antigenic specificity patterns for a determinant present of the Fc region of select subclasses of IgG have also been demonstrated (11). Thus these data suggest a marked limitation of the human IgM anti-IgG auto-antibody response. This restriction may be characteristic of many naturally occurring auto-antibodies and this is presently under investigation.

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