

SIMILARITIES IN THE LIGHT CHAINS OF ANTI- γ -GLOBULINS SHOWING CROSS-IDIOTYPIC SPECIFICITIES*

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(Received for publication 18 September 1973)

Recent studies have indicated the presence of cross-idiotypic determinants among monoclonal IgM proteins possessing anti- γ -globulin activity that were not found among IgM proteins lacking this activity (1). The pattern obtained was very similar to that previously described for the IgM cold agglutinins (2) with antigenic specificities in proteins from different individuals which appeared to relate to the combining specificity of these proteins toward their respective antigens. Evidence was obtained both for the cold agglutinins and the anti- γ -globulins that the antigen-combining site was involved in these specificities. Through the use of the idiotypic antisera which showed cross-specificity, it was possible to delineate two entirely separate groups of anti- γ -globulins (1). One, the major Wa group, made up approximately 60% of the anti- γ -globulins; the other, the minor Po group, included approximately 20% of the total; a few anti- γ -globulins could not be classified in either of these two groups.

The relative role of the H and L chains of these proteins in the cross-specificities was not readily determined because the antigenic determinants involved required the combination of the chains for expression. However, amino acid sequence studies on the minor Po group of anti- γ -globulins demonstrated an extraordinary similarity in H chain sequence through two hypervariable regions (3); the L chains were quite distinct. This suggested that heavy chain similarities were primarily involved in the cross-specificity determinants of the minor Po group. The present studies were carried out to define these relationships further by both antigenic and sequence analyses with special emphasis on the major Wa group of anti- γ -globulins. A marked similarity in the L chains of this group was found.

Materials and Methods

The IgM anti- γ -globulins utilized in this study were the same as those described in a previous report (1). They were initially purified in most instances as cryoglobulins from sera where they appeared as monoclonal bands; a combined procedure using Pevikon electrophoresis and Sephadex columns as described previously (1) was then utilized for final isolation. All of the proteins precipitated with aggregated Fr II γ -globulin and this was the primary method of

* This investigation has been supported by U.S. Public Health Service grant AM 09792.

initial screening and criterion for inclusion in the study. They also all agglutinated red blood cells coated with Fr II γ -globulin or isolated IgG 1 myeloma proteins. The coating here was carried out by the method of Kritzman (4).

The light and heavy chains were isolated primarily by standard procedures described previously (5). In some experiments a milder isolation procedure kindly furnished by Dr. Ralph Schrohenloher was employed. Various Bence Jones proteins studied were obtained from multiple sources and had been used for previous studies. A number when sequence data were available were obtained from Dr. Leroy Hood and Dr. Norbert Hilschmann. The latter supplied Bence Jones protein Ti which was used extensively in this study.

Hemagglutination and hemagglutination inhibition procedures were carried out as described previously (1). The VK III antigen¹ was measured utilizing antisera to known VK III proteins which were absorbed with VK I and VK II proteins. The VK III b antigen was measured with three antisera: one made against the light chains of the anti- γ -globulin Glo, another against the Bence Jones protein Ti and another obtained from Dr. Alan Solomon. These antisera were absorbed as above but with the additional absorption with VK III proteins that lacked the III b antigen. For many of the studies the Bence Jones protein Ti was used as the red cell coat.

RESULTS

Previous studies on the major Wa group of anti- γ -globulins had indicated that this was a very homogeneous group with strong idiotypic cross-reactions. As a supplement to sequence analyses, V region subgroup determination on the L chains were undertaken by the antigenic procedures developed by McLaughlin and Solomon (6). Antisera made to a number of kappa I and III proteins were absorbed with kappa III or kappa I proteins respectively along with the added absorption of kappa II proteins for all the antisera. Certain of these antisera gave selective positive reactions in agar analyses with proteins of the same V region subgroup to which the antiserum had been made. A large series of standard subgroup I and II proteins (not anti- γ -globulins) which had been classified on the basis of sequence analyses was available for these analyses. In view of previous success with hemagglutination inhibition procedures, the subgroup I and III systems were established with the same series of antisera and standard proteins which had been used for the agar plate analyses. It was possible to classify light chains, Bence Jones proteins, whole IgM, whole IgA and whole IgG proteins as belonging to kappa subgroup III with several different antisera and standard proteins as red cell coats. This was also true of the kappa subgroup I system although less work was directed to this group. These results will be reported separately but several conclusions were apparent in the analysis of the kappa III subgroup by sequence as compared to antigenicity. The antigenic analysis, if positive, always identified the subgroup obtained by sequence determinations. There were no false positives but a number of proteins that were classified as VK I or VK III by sequence analysis were negative in the antigenic determinations. In the VK III system 3 of 15 proteins classified as

¹ The word antigen is used here and elsewhere to define a given specificity although it is probable that multiple antigenic determinants are involved.

VK III by sequence were negative in the antigenic determination. No VK I or II proteins, classified by sequence, were positive in the VK III system.

Table I shows the results of VK III antigenic analyses on eight IgM anti- γ -globulins in which sequence studies on the light chains had been carried out. There was agreement between the sequence and antigenic method for seven of the eight proteins. One protein, Po, classified as subgroup III on the basis of sequence analysis (3) was negative in the antigenic determinations. It is of interest that this protein has several unusual substitution in the first ten amino terminal positions.

One of the antisera used for the VK III assays was made against the L chains isolated from the IgM protein, Glo, which belonged to the major Wa idiotypic group. This antiserum had the added property of subdividing the subgroup III proteins into two types. Such a subdivision had been described by Solomon and McLaughlin (6) and an exchange of proteins and antisera indicated that the same classification was involved. The Bence Jones protein, Ti, which had been sequenced by Hilschmann and associates (7) represented the prototype of the sub-subgroup of VK III proteins recognized by this antiserum and termed III b. Coating red cells with protein Ti and utilizing the anti-Glo light-chain antiserum, the III b proteins inhibited agglutination while the other subgroup III proteins (termed III a) did not. Two other III b antisera gave similar results. Table II shows the inhibition results in a typical experiment revealing a number of positive proteins. The latter were found in all Ig classes and in Bence Jones proteins. The kappa III b antigen was found only in proteins that were positive in the kappa III serological system. Protein Po which was an IgM anti- γ -globulin described previously (1) as belonging in the minor Po group with K III light chains by sequence analysis was completely negative both as the whole protein and the light chains.

TABLE I
Comparison between Antigenic VK Typing and Sequence Typing in Those Anti- γ -Globulins Where Sequence Data Were Available

Protein	VK type	
	Sequence	Antigenic
Wa	III	III*
Ge	III	III
Sie	III	III
Glo	III	III
Dr	III	III
Be	III	III
Lay	I	I†
Po	III	—§

* The proteins typed as III were also negative in the subgroup I system.

† The proteins typed as I were also negative in the subgroup III system.

§ Negative in the antigenic systems for III and I.

Table III illustrates the results of screening a large number of proteins with the III and the III b system. Those IgM proteins with anti- γ -globulin activity are listed separately. It is apparent that 92% of the anti- γ -globulins were subgroup III by this screening procedure compared to 27% for Ig's lacking this activity. The III b antigen was found in 60% of the anti- γ -globulins as compared to only 8% for random IgM proteins. Most striking however was the finding that all 10 of the proteins of the major Wa group were of the VK III and III b type. The antigenic typing was carried out on the whole protein in these assays. In four instances the isolated L chains of the anti- γ -globulins were also studied; complete agreement was obtained.

Idiotypic Specificity.—Several antisera, made to the isolated L chains of the major Wa group proteins, were studied with regard to idiotypic specificity for

TABLE II
Detection of Kappa III b Proteins by Hemagglutination Inhibition; Selected Anti- γ -globulins and Control Proteins Are Listed

	Inhibitor protein concn, mg/ml					
	0.25	0.06	0.015	0.004	0.001	0.00025
Ti (B.J.)	0	0	0	0	0	2
6 (B. J.)	0	0	0	0	1	2
Glo (L chains)*	0	0	0	0	0	2
Glo (IgM)*	0	0	0	0	2	2
Pa (IgG)	0	0	0	0	1	2
Wa (IgM)*	0	0	0	0	2	2
Ca (IgM)	1	2	2	2	2	2
Ha (B. J.)	2	2	2	2	2	2
Po (IgM)†	1	2	2	2	2	2

Red cell coat, B. J. Ti; antiserum anti Glo L chains absorbed.

* Anti- γ -globulins of the Wa group with K III b light chains.

† Anti- γ -globulin of the Po group with K III a light chains.

TABLE III
Comparison of the Incidence of Kappa III and Kappa III b V Region Subgroups among General Kappa Igs and Anti- γ -Globulins (Antigenic Typing)

	No.	Kappa III	Kappa IIIb
		%	%
Igs*	81	27	11
IgM†	26	31	8
Anti- γ -globs.	17	92	60
Anti- γ -globs. (Wa group)	10	100	100

* This overall group of Igs included 19 IgG, 10 IgA, 26 IgM, and 26 Bence Jones proteins. All categories contained III b-positive proteins.

† Exclusive of the IgM anti- γ -globulins.

the immunizing antigen using the latter as the red cell coat for hemagglutination as described previously (8) as well as cross-specificities for the anti- γ -globulin L chains. No such specificities could be demonstrated. The maximum specificity found was to the VK III b antigen which was shared with non-anti- γ -globulin light chains and Bence Jones proteins. Further studies are continuing since idiotypic antisera have been produced to many other light chains and Bence Jones proteins (8).

In view of the above findings regarding the constant occurrence of the III b light chain antigen in the major Wa group of anti- γ -globulins, the idiotypic antisera made against the whole IgM proteins previously studied were reinvestigated. It was apparent that this antigen was not responsible for the cross-idiotypic specificity of these antisera. None of the III b proteins of the non-anti- γ -globulin group shown in Table III inhibited these systems (included in this group was one IgM protein with III b type light chains and blocked heavy chains). Further absorptions of the cross-specific antisera were also carried out with as much as 20 mg of Fr II added per cm³ of antiserum; these antisera remained highly specific for the Wa type of anti- γ -globulins and were not inhibited by any non-anti- γ -globulin protein and were inhibited very weakly by high concentrations of pooled γ -globulin.

Antigenic analyses were also carried out on isolated heavy chains of the anti- γ -globulins. This work failed to give a clear answer as to the presence of V regions antigens restricted to the anti- γ -globulins. However previous work has indicated that this presents special problems not encountered with the light chains. Idiotypic antisera made to the whole anti- γ -globulins were not inhibited by heavy chains when the whole protein was used as the red cell coat. When heavy chains were used as the coat, these antisera did show some specific relationships among the anti- γ -globulins. However, the pattern of relationship was not so clear as with the light chain and did not parallel the cross-idiotypic results for the Wa group. This work is continuing with new antisera made against the isolated heavy chains.

Sequence Analyses

Amino terminal sequence analyses were carried out on the isolated light chains of a number of the IgM anti- γ -globulins of the major Wa group and further studies are continuing. Protein Glo light chains were sequenced to position 41 and were very similar to the Bence Jones protein, Ti, differing by only two amino acids at positions 31 and 33. As mentioned above these two proteins both belonged in the VK III b antigenic sub-subgroup. The light chains of the other anti- γ -globulins of the major Wa group also appeared very similar both to each other and to Ti. However further studies are necessary particularly through the hypervariable regions to determine if these anti- γ -globulin light chains are more closely related to each other than to Ti and the other non-anti- γ -globulin proteins of the III b sub-group. The structural basis for the III b

antigen was not apparent in these studies because III a proteins were present which showed very similar sequences at least up to position 30. The substitution of glycine for alanine at position 9, postulated as differentiating the III b from the III a proteins (6), was ruled out and it is likely that the amino acid substitution or substitutions responsible for the III b antigen are located beyond residue 30.

The heavy chains of six of the anti- γ -globulins of the major Wa group were studied. They were blocked in all instances and in the three cases where the N-terminal peptides were isolated they proved to be of the V region subgroup II (9). This was in contrast to the two anti- γ -globulins of the Po group where both proteins studied were of the VH III subgroup. Thus there appeared to be a relationship among the Wa proteins at the heavy-chain level as well as the marked similarity among the light chains.

DISCUSSION

These studies on the light chains demonstrate that a striking restriction of the V region subgroups is observed in the majority of IgM proteins with anti- γ -globulin activity. This restriction extends from a gross kappa preponderance to the finer restriction involving the kappa III b sub-subgroup. All 17 of the anti- γ -globulins accumulated in the authors' laboratory for these studies were kappa in type. However, lambda anti- γ -globulins have been described by others (10) but apparently are rare. The major Wa group of anti- γ -globulins was recognized previously as being very homogeneous with marked similarities based on cross-idiotypic specificities (1). The present studies on the light chains indicate that all of the proteins of this group had VK III b light chains based on antigenic analysis.

The advantage of the antigenic analysis stemmed from the fact that it was possible to screen large numbers of non-anti- γ -globulin control proteins and thus gain a clearer picture of the restriction of the III and the III b antigens to proteins with this combining specificity. Considerable agreement was obtained from initial studies comparing the serological VK III subgroup typing with sequence data. In a few of the proteins determined by sequence analysis to be VK III the hemagglutination inhibition system gave negative results. However all serologically positive proteins were VK III by sequence. The serological method showed the VK III incidence to be 27% among a group of 81 non-anti- γ -globulin kappa proteins examined. This contrasted strikingly with a 92% incidence among the anti- γ -globulins. The VK III b antigen was recognized by McLaughlin and Solomon (6) and proved to be an important addition serological marker in the present study. It too showed a marked preponderance among the anti- γ -globulins as compared to an incidence of only 11% among 81 control proteins.

The cross-idiotypic specificities described previously for the anti- γ -globulins indicated that these proteins fell into several different groups two of which

could be clearly delineated. The minor Po group showed L chains of different V region subgroups but H chains that were very similar by sequence analyses even through two hypervariable areas (3). The present studies indicate that for the major Wa group the L-chain similarities must play an important role in the idiotypic classification since all of the proteins of this group were VK III b and this type was not found for the L chains of any of the proteins of the other groups. It was clear, however, that the VK III b antigen was not in itself responsible for the idiotypic specificity leading to the classification of the Wa group. Other VK III b proteins, including one IgM protein without anti- γ -globulin activity, completely failed to react with the idiotypic antisera. In view of this absolute correlation, it appears probable that the light chains of the Wa group are in large part responsible for the cross-idiotypic specificity and that other similarities will be apparent in further sequence studies. Antisera produced thus far to the L chains did not show idiotypic specificity but only expressed selectivity with respect to the K III b antigen. It may well be that more selective light-chain antisera will be obtained with further immunization that will show cross-idiotypic reactions. The idiotypic antisera made to the whole proteins might reflect such postulated light-chain similarities when combined with heavy chains. Another possibility that requires consideration is that unique similarities also exist for the heavy chains of this group of anti- γ -globulins. Previous sequence studies (9) have shown that all of six proteins of this group studied had blocked heavy chains and in the three instances in which further studies were carried out all were of the VH II subgroup. Antigenic studies of the V regions of the heavy chains are currently underway. The results to date indicate antigenic heterogeneity within the heavy chains of the Wa group and at present it appears less likely that the heavy chains are primarily involved. It would be informative to carry out recombination experiments of non-anti- γ -globulin VH II heavy chains with the light chains of this group of anti- γ -globulins but these have been unsuccessful thus far for a variety of technical reasons.

The present work extends previous studies from these laboratories relating to selective V region patterns for monoclonal antibodies of similar specificity from unrelated individuals. The most extreme situation was that for the L chains of two IgG anti- γ -globulins (3) which showed sequence identity for 40 residues and identical peptide maps; these were of the VK I subgroup. For some reason, that is not evident at present, the IgG anti- γ -globulins appear quite different from the IgM types described above with respect to specific V region antigens; none that were analogous to those of the major Wa group were encountered. Sequence similarity but not identity was found in the limited number of IgM anti- γ -globulin light chains studied. Previous studies also indicated that the cold agglutinins showed a restricted type of light chains ranging from kappa to VK III predominance (11). However, this was not as striking a restriction as for the anti- γ -globulins in the present study. It thus far

appears that heavy-chain similarities are primarily involved in the very evident cross-idiotypic specificities of the I and i types of cold agglutinins. Recently Pr type cold agglutinins have been demonstrated to have a separate category of cross-idiotypic specificities (12). In these the light chains may be primarily involved (13).

Thus recent experimental work is providing increasing evidence that among groups of monoclonal proteins with similar combining activity, in some instances, specific light chain V region subgroup similarities are dominant, in other instances, heavy chain similarities are primarily manifest and finally in certain instances similarities in both chains are evident. The picture that is emerging indicates that, for similar antibodies, the hypervariable areas, as determined by idiotypic specificity along with more limited sequence data, are associated with specific V region subgroups. Thus, for the majority of IgM anti- γ -globulins the light chains are kappa in type, K III in subgroup, K III b in sub-subgroup and perhaps K III b' to account for the cross-idiotypy that is observed. The evidence appears to indicate that specific patterns in the hypervariable areas of the Ig chains only occur in a given setting involving the less variable portions of the V region. However, it should be stressed that such an interpretation might only apply to the situation in outbred individuals as studied here. It appears highly probable that another very significant component of idiotypic systems is only observed in closely related individuals and highly inbred strains of animals. This point was discussed in greater detail previously (1).

SUMMARY

A marked homogeneity of the light chains was observed in an analysis of 17 IgM proteins with anti- γ -globulin activity. The V region subgroups of the light chains were determined by both sequence and antigenic analysis. The latter procedure permitted large scale screening for comparisons with control proteins. The two methods showed general agreement in the determination of Kappa III proteins; all proteins positive by antigenic analysis were also positive by sequence but exceptions were noted in the opposite direction. The anti- γ -globulins showed by antigenic analysis a 92% incidence of VK III light chains as compared to an incidence of 27% among 81 control proteins without this activity. A similar selection was observed for an antigen (VK III b) which subdivided the kappa III proteins.

The major Wa group of anti- γ -globulins which had been delineated previously on the basis of cross-idiotypic specificity was primarily responsible for the special light-chain selection. All the proteins of this group contain VK III light chains and all were of the VK III b type. Current evidence indicates that additional light-chain similarities were involved in the cross-idiotypic specificity of the Wa group. It thus appears that for the anti- γ -globulins various levels of selection of light chains are manifest ranging from a preponderance of kappa

type, of kappa III subgroup, of kappa III b sub-subgroup and perhaps of still further subdivisions to account for the cross-idiotypic specificity.

The authors are indebted to Doctors Gerald Penn, Ralph Schrohenloher, and Marvin Stone for certain of the sera utilized in this study and to Dr. Alan Solomon for exchanging subgroup proteins and antisera.

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