Rabbit Immunoglobulin Lacking Group *a* Allotypic Specificities. III. Variable Region Structure and Genetic Control†

James W. Prahl,*‡ Brian F. Tack,‡ and Charles W. Todd

ABSTRACT: Rabbit IgG containing non-*a* γ chains was obtained by suppression of the group *a* allotypic markers. These γ chains were previously shown to differ in amino acid composition from the cognate γ chains bearing the *a* specificities, although no differences could be demonstrated in the carboxy-terminal halves of the chains. The present studies establish that the compositional variations reside in the N-terminal halves of the γ chains, as shown by comparing the C1 fragments obtained by cyanogen bromide cleavage. The N-terminal peptides of non-*a* γ chains lack the distinctive amino acid compositions previously correlated with allotypy. Indeed, the compositions of the N-terminal peptides of non-*a* chains resemble each other more closely than they resemble the corresponding peptides of *a* chains. Non-*a* γ chains are characterized by a predominant, if not unique, initiation sequence of <Glu-Glu-Gln, an initiating sequence normally seen in only a small proportion of γ chains from normal unsuppressed rabbits. The hypothesis of a genetic locus coding the variable region of the non-*a* γ chain, distinct from that encoding the variable region of γ chain expressing group *a* specificities, is discussed.

Group *a* allotypic variation in the heavy (H) chain of rabbit immunoglobulin G is characterized serologically and chemically. Wilkinson (1969) observed variations in the distribution of the N-terminal peptides, as isolated from the Pronase digests of the H chains of immunoglobulins from rabbits homozygous for each of the three group *a* alleles, *i.e.* *a*1, *a*2, and *a*3.[1] These variations appeared to correlate with allotypic specificity.

The presence of a methionine residue in position 33 from the N terminus of the γ chain, although nonintegral, has permitted the isolation of an N-terminal peptide from the cyanogen bromide cleavage products of the chain. Whether isolated from the γ chains of pooled IgG of rabbits homozygous at the group *a* locus (Prahl and Porter, 1968), or from their purified antibodies (Koshland, 1967), compositional variations again characterized the N-terminal peptides of each allele. Subsequently, the primary sequences of these peptides were also shown to be distinctive for each group *a* allele (Wilkinson, 1969; Fleischman, 1971). Mole *et al.* (1971) have extended these correlative variations of sequence to other areas within the variable region.

Allotypic suppression in homozygotes results in the expansion of the population of allotypically deficient immunoglobulins (Dubiski, 1967; David and Todd, 1969). A previous publication in this series (Tack *et al.*, 1973a) has reported compositional changes in the H chains of IgG deficient for the group *a* allotypes, as compared to their normal allotypic counterparts. Previous studies had failed to reveal these variations in the carboxyl-terminal halves of the heavy chains, *i.e.* the Fc γ (Prahl and Todd, 1971; Knight *et al.*, 1971). In addition, γ chains which are deficient for the group *a* allotypic specificity have been shown to retain the constant region allotypic specificity of group *d* (Tack *et al.*, 1973b) and group *e* (Landucci-Tosi *et al.*, 1972).

Evidence will be presented here verifying that the compositional differences between the allotype-bearing and allotype-deficient γ chains reside in the N-terminal halves of the chain. Further, it will be shown that the N-terminal region of allotypically deficient γ chains differs from that of its cognate allotype, implying the existence of a genetic locus distinct from that encoding the group *a* allotypic specificities.

Materials and Methods

Isolation of γ Chains. Isolation of IgG lacking group *a* allotypic markers from the sera of allotypically suppressed rabbits, the quantification of IgG and of group *a* and *b* specificities, and other serological methodology have been described previously (Tack *et al.*, 1973a). The reduction and radioalkylation of IgG and the subsequent isolation of the γ chain were discussed in detail by Tack *et al.* (1973b).

Isolation of the Cyanogen Bromide C1 Fragments. Cyanogen bromide cleavage of the respective γ chains was carried out according to Prahl *et al.* (1969). The lyophilized fragments were dissolved in 50 mM sodium formate (pH 3.2), 7 M with guanidinium chloride, and incubated for several hours at 37°. Fractionation of the fragments was accomplished by gel filtration through a column (2.5 × 100 cm) of Sephadex G-100 equilibrated in 50 mM sodium formate (pH 3.2), 6 M with urea. Fractions from the peak designated C1 were pooled, freed of urea by filtration through a column of Sephadex G-25 in 0.5 M acetic acid, and lyophilized.

Isolation of N-Terminal Peptides of C1 Fragments (NTC1). The C1 fragments were dissolved in 0.5 M Tris-HCl buffer (pH 8.2), 2 mM with EDTA and 7 M with guanidinium chloride, at a protein concentration of 20–25 mg/ml. Solid dithio-

† From the Division of Biology, California Institute of Technology, Pasadena, California 91109, and the Department of Immunology of the City of Hope National Medical Center, Duarte, California 91010. *Received July 6, 1973.* This research was supported by grants from the National Institutes of Health (GM-06965, AI-07995, AI-10781, and AI-09981). Contribution No. 282 from the American National Red Cross Blood Research Laboratory.

‡ Present address: American National Red Cross Blood Research Laboratories, Bethesda, Md. 20014.

[1] The nomenclature employed for rabbit immunoglobulins, their chains, and fragments follows that recommended by a committee of the World Health Organization (1964).

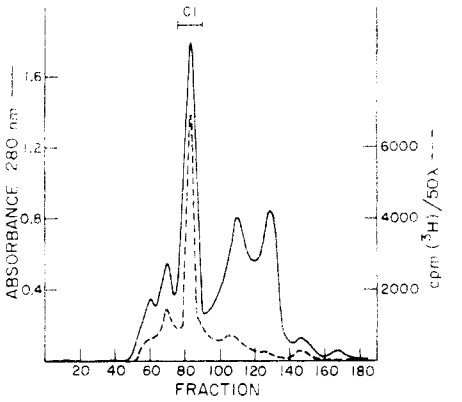

FIGURE 1: Gel filtration of the cyanogen bromide cleavage fragments of $a2,d12$ γ chain (100 mg) on a column (2.5 × 140 cm) of Sephadex G-100 in 50 mM sodium formate (pH 3.2), 6 M with urea: (—) absorbance at 280 nm; (---) radioactivity. The fractions pooled for the C1 fragment are indicated by the bars.

threitol was added to a final concentration of 10 mM, and the solution was incubated at 37° for 1 hr. Radioalkylation was accomplished by the addition of iodoacetamide to a final concentration of 20 mM, containing 50 μCi of [1-^{14}C]iodoacetamide. After 2 hr at room temperature, the reaction mixture was fractionated by gel filtration on a column (2.5 × 140 cm) of Sephadex G-75 in 50 mM sodium formate (pH 3.2), 6 M with urea. The fractions were monitored by extinction at 280 nm and by radioactivity. Fractions containing the NTC1 peptide were pooled, freed of urea by passage through a column of Sephadex G-25 in 50 mM NH_4OH, and lyophilized. The peptide was dissolved in 50 mM NH_4OH and refractionated on a column (1.5 × 220 cm) of Sephadex G-50 or G-75 in 50 mM NH_4OH. The eluate was monitored by extinction at 215 nm and by radioactivity. The NTC1 peptides were eluted at 44–54% of the total column volume on either gel.

Isolation of N-Terminal Peptides after Pronase Digestion. Pronase digestion of the heavy chain, the C1 fragment, or the NTC1 peptide was carried out according to Wilkinson (1969). The digest was applied to a column of Dowex 50-X2 (H^+ form) and eluted with water. The material unretarded by the Dowex 50 was lyophilized and dissolved in a known volume of water. An aliquot was removed for amino acid analysis. The residual peptide was redigested with Pronase and subjected to high voltage paper electrophoresis at pH 3.5.

Analytical Methods. Amino acid analysis was carried out as described by Tack *et al.* (1973a) and the determination of radioactivity was carried out as described by Tack *et al.* (1973b). The staining technique of Pan and Dutcher (1956), using hypochlorite–starch–iodide, was employed to detect peptides containing no free α-amino group.

Results

Proceeding from the N-terminal end of rabbit γ chain, the first methionine residue in the constant region of the $d12$ chain is found at position 250. A replacement of methionine for threonine is found at position 226 in chains typing $d11$ (see Figure 5, Tack *et al.*, 1973a). Cyanogen bromide cleavage of the γ chain therefore yields the fragment C1, which spans the variable regions of some 110–120 residues and approximately one-third of the constant region of the chain, *i.e.* CH_1.

In the variable region of rabbit γ chain, nonintegral methionine residues are found in significant yield in positions 33 and 79 (Mole *et al.*, 1971). A disulfide bridge spanning residue 21–91 (O'Donnell *et al.*, 1970) covalently links the NTC1 peptide to the rest of the C1 fragment in those chains containing a methionine in position 33 or 79. In that population of molecules in which a methionine residue is found in both positions, a peptide encompassing residues 34–79 will be lost upon subsequent fractionation. Bearing this limitation in mind, the C1 fragment has been considered representative of the N-terminal half of the rabbit γ chain.

TABLE I: Amino Acid Composition of C1 Fragments.[a]

Amino Acid	$a1$	$a1$	$a2$	$a2$	$a3$	$a3$	$a3^b$
Ala	12.6	14.0	14.7	13.7	15.4	13.7	14.0
Arg	7.9	7.9	6.8	6.8	7.4	7.4	6.1
Asp	15.2	16.1	19.2	17.2	14.5	16.9	14.7
$CysCH_2COOH$	3.6	4.5	2.6	4.0	3.5	4.4	3.9
Cys	6.1	6.5	5.6	5.8	4.6	4.8	4.1
Glu	16.5	18.5	17.6	16.8	15.9	15.9	14.1
Gly	21.7	25.3	20.1	24.1	22.0	23.2	21.0
His	1.9	2.0	1.3	1.5	2.2	2.0	0.9
Ile	7.2	5.6	7.2	5.5	5.6	5.7	4.1
Leu	19.8	19.4	19.9	20.1	20.1	19.6	17.5
Lys	12.6	11.5	11.5	11.8	12.9	12.2	9.7
Met	1.5	2.2	1.7	1.8	1.6	2.3	2.6
Phe	8.2	7.2	7.8	8.0	9.2	8.7	6.6
Pro	25.0	23.2	20.8	23.3	23.4	22.0	19.9
Ser	28.1	33.1	29.5	32.1	29.1	32.7	34.2
Thr	30.8	24.6	32.1	25.5	31.1	26.5	29.1
Tyr	8.3	8.5	9.0	8.7	8.7	8.9	6.4
Val	22.5	20.0	22.8	23.5	22.7	22.8	19.7

[a] Compositions are reported as amino acid residues per 250 residues, except for $a3,d11$ which is calculated for 226 residues. Cysteine and methionine were determined as cysteic acid and homoserine, respectively. The group a allotypes are shown at the head of each column.

The cyanogen bromide cleavage products of the γ chain were dissolved in 50 mM sodium formate (pH 3.2) 7 M with guanidinium chloride, and incubated for several hours at 37° before gel filtration on Sephadex G-100 using formate, 6 M with urea (Fruchter *et al.*, 1970). Such a fractionation is shown in Figure 1. As reported by Friedenson *et al.* (1972), 7 M guanidinium chloride appears to be far more effective than 6 M urea in dissociating aggregates of C1, although the latter is capable of maintaining the dissociation once it is achieved.

The amino acid compositions of C1 fragments isolated from a and non-a γ chains are given in Table I. The variations which had been observed when comparisons were made among the compositions of a and non-a chains (Tack *et al.*, 1973a) were seen to persist when similar comparisons were made among the corresponding C1 fragments. As had been reported by Koshland (1967), variations in amino acid composition in whole γ chain which appeared to be associated with group a allotype were found in the C1 fragments from those chains. The situation is also the same in the case of C1 fragments isolated from non-a γ chains, where variations observed in whole chains can be identified in their C1 fragments.

The tacit assumption that the compositions of the C1 fragments given are representative of the N-terminal halves of the chain is probably not realistic, at least not for those isolated from non-a γ chains, as was first suggested by the

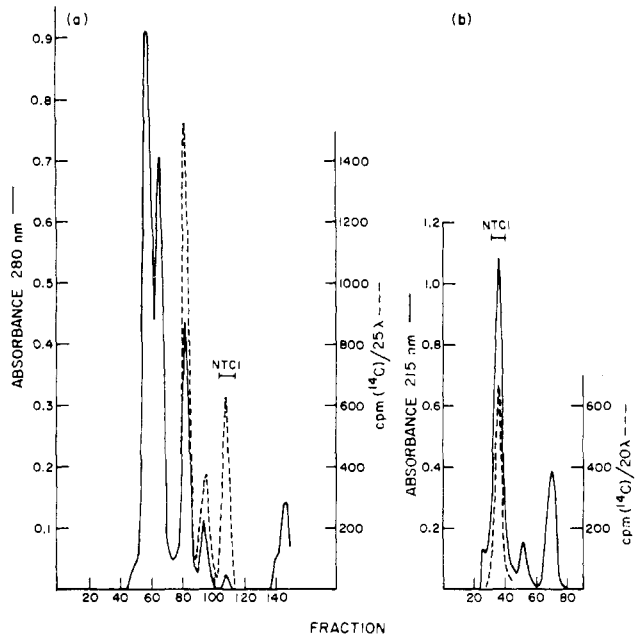

FIGURE 2: (a) Gel filtration of the totally reduced and alkylated C1 fragment (64 mg) of $a\bar{2}$,d12 chain on a column (2.5 × 140 cm) of Sephadex G-75 in 50 mM sodium formate (pH 3.2), 6 M with urea: (—) absorbance at 280 nm; (---) radioactivity. (b) Rechromatography of the NTC1 peptide on a column (1.5 × 220 cm) of Sephadex G-75 in 50 mM ammonium hydroxide. The absorbance was monitored at 215 nm. In both cases, the fractions pooled for the NTC1 peptide are indicated by the bars.

high content of homoserine observed in those fragments. One homoserine residue is contributed by the methionine at position 250. A second residue of homoserine could be contributed by the methionine originally present in positions 33 or 79. Since those chains possessing methionines in both positions would lose the peptide encompassing residues 34–79, a maximal theoretical yield of homoserine could approach two, unless additional methionine positions in substantial yield are postulated. No evidence in these studies indicated this was the case. However, the peptide encompassing residues 34–79 has been found in substantial yield among the CNBr fragments of non-a γ chains (Figure 1, fractions 140–160) in yields approaching 0.3 mol of peptide/mol of C1 fragment.

The compositions shown in Table I are calculated on the basis of 250 residues, which is a valid assumption for only 70% of the sample at most. The result of this assumptive error is reflected in the spuriously high homoserine values reported. Attempts to compensate for the lost peptide were complicated by the uncertainties of recovery and so were abandoned. Nonetheless, even with this limitation, the compositions of C1 fragments isolated from non-a γ chains strongly imply the variations observed earlier in the intact chains persist in these fragments.

Upon total reduction and radioalkylation of the C1 fragment and subsequent fractionation of the peptides by gel filtration, an N-terminal peptide (NTC1; see Figure 5 of Tack *et al.*, 1973a) can be isolated and purified (Figures 2a and 2b). The amino acid compositions of the NTC1 peptides of C1 fragments from a and non-a γ chains are given in Table II. Here again, distinctive differences in composition are noted on comparison of the non-a NTC1 peptides with the corresponding a peptides. Elevated glycine and glutamic acid and decreased threonine contents are seen in the peptides from a-negative chains. Once again the compositional characteristics

TABLE II: Amino Acid Compositions of NTC1 Peptides.[a]

Amino Acid	$a1$	$a\bar{1}$	$a2$	$a\bar{2}$	$a3$	$a\bar{3}$[b]	$a\bar{3}$[c]
Ala	0.91	1.77	1.25	1.44	1.82	1.28	1.16
Arg	1.04	0.22	0.22	0.15	0.27	0.18	0.12
Asp	1.18	0.99	2.21	0.90	1.61	1.25	1.34
Cys	0.77	0.69	0.59	0.52	0.77	0.82	0.76
Glu	2.93	4.99	3.45	4.80	4.00	4.85	4.90
Gly	4.56	6.74	4.42	7.21	4.87	6.16	6.11
His	0.15	0.13	0.25	0.08	0.18	0.16	0.08
Ile	0.52	0.34	0.69	0.18	0.37	0.45	0.40
Leu	3.98	4.03	3.55	4.12	3.59	4.12	4.15
Lys	0.35	0.31	1.57	1.54	0.94	1.46	1.58
Met	0.98	0.66	0.87	0.84	0.95	1.00	0.97
Phe	0.78	1.09	1.42	1.35	1.24	1.40	1.48
Pro	2.18	1.13	1.39	0.94	1.37	1.13	1.14
Ser	4.55	5.21	4.67	5.13	4.82	4.29	4.37
Thr	4.73	1.08	3.98	0.84	3.29	2.54	2.37
Trp	n.o.	0.56	n.o.	0.84	n.o.	n.o.	n.o.
Tyr	0.93	0.87	0.24	1.04	1.32	1.41	1.19
Val	2.48	1.20	2.21	1.10	1.67	1.40	1.57
Recovery (%)	36	31	13	14	20	54	42

[a] Reported as residues per mole of peptide based on a total of 33 residues. The allotype of the donor rabbits is shown at the top of the respective column. [b] Allotype is $a\bar{3}$,d11. [c] Allotype is $a\bar{3}$,d12. Methionine was determined as homoserine and cysteine as the *S*-carboxymethyl derivative; n.o., nonobserved.

which distinguished the peptides of different group a alleles are lost (Wilkinson, 1969). For example, the integral arginine, representative of that in position 9 of the NTC1 peptide of the a1 allotype, is not observed upon analysis of the NTC1 peptide of $a\bar{1}$, although a fractional content of arginine is seen. Tryptic digestion of the $a\bar{1}$ NTC1 peptide revealed the fractional residue of arginine observed was not found in position 9. Analyses of the NTC1 peptides isolated from $a\bar{3}$,d11 and $a\bar{3}$,d12 show marked similarity, but some variation is seen when these peptides are compared with those of $a\bar{1}$ and $a\bar{2}$ chains. The cause for this variation within non-a NTC1 peptides is not understood at this time, but may be related to further allelism within the non-a variable region. Interestingly, tryptophan has been identified in the hydrolysates of the NTC1 peptides obtained from $a\bar{1}$ and $a\bar{2}$, but not $a\bar{3}$ chains, further emphasizing the difference between the two groups.

The H chains of rabbit immunoglobulin are characteristically blocked by cyclization of the N-terminal glutamyl residue to the L-pyrrolidine-5-carboxyl derivative. After Pronase digestion of the H chain, the C1 fragment, or its NTC1 peptide to smaller peptides and subsequent fractionation, the N-terminal tri- to pentapeptides have been isolated (Wilkinson, 1969). Shown in Table III are the compositions of the pool of known tripeptides, as constructed from the analysis of the peptide material unretarded by Dowex 50. This material is contaminated by glycopeptides and acidic peptides, which are not retained by the ion exchange resin when H chains or C1 fragments are used for the Pronase digestion. In those cases, purification was attempted by paper electrophoresis at pH 3.5 after redigestion with Pronase.

TABLE III: N-Terminal Pronase Peptides of γ Chain.[a]

	a1			*a3*		
	Normal[b]	Control[c]	Supp	Normal[b]	Control[c]	Supp
<Glu-Ser-Val-Glu	0.72	0.67	0.05	0.11	0.16	0.03
<Glu-Ser-Leu-Glu	0.04	0.06	0.03	0.58	0.63	0.06
<Glu-Glu-Gln	0.08	0.10	0.77	0.13	0.09	0.67

[a] Reported as moles of peptide per mole of heavy chain (calculated per Wilkinson, 1969). The electrophoretic mobilities of <Glu-Ser and <Glu-Glu-Gln were -0.71 to -0.73 and -0.63 to -0.68 relative to pyrrolidonecarboxylic acid, respectively, at pH 3.5. The larger <Glu-Ser peptides moved with a mobility of -0.41 to -0.45 relative to pyrrolidonecarboxylic acid. [b] γ chain isolated from normal adult rabbits homozygous for the *a* allotype indicated. [c] γ chain isolated from unsuppressed rabbits of the same age and allotype as their suppressed counterparts.

TABLE IV: N-Terminal Pronase Peptides of *a2* γ Chains.[a]

	Normal	Supp[b]	Desupp[c]
<Glu-Ser-Val-Glu	0.38	0.15	0.31
<Glu-Ser-Leu-Glu	0.34	0.09	0.29
<Glu-Glu-Gln	0.17	0.69	0.36

[a] Reported as moles of peptide per mole of heavy chain (calculated as per Wilkinson, 1969). [b] The IgG of suppressed rabbits contained less than 2% of *a2*-bearing molecules as determined by interfacial precipitation reactions. [c] By 16 weeks of age, the IgG of the previously suppressed rabbits now was estimated to contain approximately 40% of *a2*-bearing molecules.

Allotype-deficient γ chains were found to be characterized by the predominant N-terminal sequences of <Glu-Glu-Gln when examined in this manner. This sequence has been reported by Wilkinson (1969) as only a minor component in allotype bearing chains. That this change in distribution was indeed related to allotypic deficiency is shown in Table IV. It can be seen that appearance of the *a2* marker to the level of 40% at 16 weeks of age was accompanied by reappearance of the <Glu-Ser-Leu and <Glu-Ser-Val sequences, with a concomitant decrease in the <Glu-Glu-Gln sequence. Pronase digestion of the NTC1 peptides of $a\bar{1}$ and $a\bar{3}$ chains yielded only <Glu-Glu-Gln, the recoveries of which ranged from 0.93 to 0.96 mol/mol of peptide. No <Glu-Ser sequences were identified. As the NTC1 peptides are not obtained in integral yield relative to the C1 fragment, it is not clear at this time if the initiating sequence <Glu-Glu-Gln is unique to all allotypically deficient γ chains.

Discussion

The population of γ chains deficient for each of the group *a* allotypes has been expanded by allotype suppression. The deficient chains showed differences in amino acid composition when compared with allotype bearing chains obtained from unsuppressed animals of the same allotype and age group (Tack *et al.*, 1973a). Amino acid compositions and tryptic fingerprints of the Fc γ fragments and cyanogen bromide cleavage patterns of these deficient chains revealed no differences between them and allotype bearing chains (Prahl and Todd, 1971; Tack *et al.*, 1973a). Additionally, non-*a* γ chains retained the constant region allotypes *d*11 and *d*12 (Tack *et al.*, 1973b). Similar observations have been reported for the γ chain of a suppressed *a2* homozygote and the *e* allotypes (Knight *et al.*, 1971; Landucci-Tosi *et al.*, 1972).

These observations indicate that the compositional differences seen upon comparison of allotypically deficient and allotype-bearing γ chains do not reside in the carboxyl-terminal halves of the chains. Consistent with these observations, the present studies demonstrate that the variations in amino acid composition persist when comparing the N-terminal halves of the chains, *i.e.* the C1 fragments. Compositional and sequence variations in the N-terminal 33 residues (Wilkinson, 1969) and throughout the variable region of the γ chain (Mole *et al.*, 1971) have been shown previously to correlate with group *a* allotype. As shown here, many of these correlations do not appear to persist in the C1 fragments of allotype-deficient γ chains. The NTC1 peptide obtained from $a\bar{1}$ does not have the arginine residue in position 9 unique to *a*1 and contains less threonine and valine, but more glutamic acid, glycine, and lysine than the NTC1 peptide obtained from the *a*1 γ chain. Similarly, the NTC1 peptide from the *a*3 γ chain also contains more glutamic acid and glycine and less threonine than that from *a*3, among other minor differences. Thus, it would appear that at least some of the compositional variations seen in the C1 fragments of allotypically deficient γ chains reside in the variable regions of these chains. Whether some of these variations are to be found in the constant regions of the fragment (*i.e.* the CH_1 region) is as yet unanswered, but on the basis of serological, allotypic, and genetic studies to date, there is no reason to believe we are dealing with a new subclass of γ chain.

The present investigations were undertaken originally as an approach to the elucidation of the structural determinants of allotypic specificities (Todd, 1972). If suppression merely selected against those molecules bearing serologically recognizable allotypic conformations, it would not have been unexpected to find the allotypically negative chains were not distinguishable from *a* bearing chains, other than by the presence or absence of those sequences directly involved in generating the determinants of *a* specificities. Indeed this does not seem to be the case. Not only do the NTC1 peptides differ compositionally, but the predominant, if not unique, initiating sequence is <Glu-Glu-Gln. This initiating sequence has also been found as a minor sequence in the γ chain from pooled IgG of unsuppressed homozygous rabbits (Wilkinson, 1969). Unfortunately, the content of allotype bearing molecules in these pools was not known, but it seems probable that the <Glu-Glu-Gln sequence reported represents the normal contribution of allotypically deficient molecules (Bornstein and Oudin, 1964). Upon allotypic quantification of normal IgG pools from group *a* homozygous rabbits, a varying content of allotypically deficient molecules is almost always seen, reaching close to 20% in the case of the control *a2* pool (Tack *et al.*, 1973a).

It is difficult to envisage all the structural variations observed as being directly involved in the generation of group *a* determinants. The present data are more simply explained by proposing the existence of at least two structural genes or gene groups encoding the H-chain variable regions of which

one genetic locus can be visualized as responsible for the variable regions bearing group *a* allotypes. The products of this gene or gene group would contain the initiating sequence <Glu-Ser-Leu/Val and the associated structural correlates. The second gene or gene group would code the variable regions lacking group *a* allotypes, which would in turn contain the initiating sequence <Glu-Glu-Gln and different structural correlates. Such a hypothesis requires that two structural criteria be firmly established. First, all allotype bearing molecules should contain the initiating sequences <Glu-Ser-Leu/Val and no <Glu-Glu-Gln. These data are accessible from pools which have been carefully quantified for allotypy. Conversely, the allotypically deficient pools should contain only the initiating sequence <Glu-Glu-Gln. This criterion appears to be fulfilled by the NTC1 peptides investigated in the present study, but the data for the entire γ chains or the C1 fragments themselves are less clear. Work is in progress to clarify this issue.

Recently Kim and Dray (1972) have reported the existence of two serological markers, *x*32 and *y*33, associated with the γ chain of *a*-negative immunoglobulins. These markers behave as if controlled by closely linked, nonallelic genes. Their presence on the N-terminal half of the γ chain as well as on α and μ chains suggests strongly that they are identifying determinants on the variable region of the chain, as appears to be the case for the group *a* allotypes. The conclusion that there are, therefore, at least three genetic loci, V_{Ha}, V_{Hx}, and V_{Hy}, encoding the variable region of rabbit immunoglobulin, is in agreement with the conclusions reached in the present study based on structual studies of γ chains deficient for the group *a* allotypes. The data presented here do not contribute to the identification of more than one non-*a* locus. This possibility, however, may account for the source of variability seen upon comparison of the amino acid compositions of the NTC1 peptides of *a*1 and *a*2 with those of *a*3 chains.

The use of the term "subgroup" to define the product of each of the two postulated structural genes or gene groups has been purposely avoided here because of the ambiguity which has arisen in its application. As originally defined, "subgroup" related to the ability to recognize discrete sets of sequences of V regions based upon structural characteristics (World Health Organization, 1969). Subsequently, the argument has been put forth "that a separate germline gene (or family of genes) must encode each V-region subgroup" (Smith *et al.*, 1971). The data presented here, in conjunction with the genetic analyses of Kim and Dray (1972), would permit the proposal of two subgroups, *i.e.* <Glu-Glu-Gln and <Glu-Ser-Leu/Val, which fulfills both these usages of the term. No genetic data are available at present which permit us to decide if <Glu-Ser-Leu and <Glu-Ser-Val represent two further subgroups, and hence separate germline genes encoding group *a* allotype, or if they represent alternate expressions of a single structure gene. We have preferred to remain noncommittal.

A regulatory phenomenon must be elicited to explain why allotype-deficient immunoglobulins are normally a minor component in the serum. This same general phenomenon has already been seen in the hemopoietic system involving hemoglobin A_2 (HbA_2). HbA_2 ($\alpha_2^A\delta_2^A$) contains an α chain which is identical with the α chain of HbA ($\alpha_2^A\beta_2^B$), the major hemoglobin of man. The second chain, the δ chain, differs from the β chain of HbA by ten amino acid substitutions (Huehns and Shooter, 1965) and is considered to represent a second structural gene. Normally, HbA_2 makes up only 1.5–3% of the total adult hemoglobin, although this proportion can be greatly increased in hemoglobinophathies involving mutational events on the β chain of HbA, as in sickle cell anemia.

The structural studies of the variable regions of allotype-deficient γ chains are not sufficiently advanced at this time to contribute to our understanding of the evolutionary path in the emergence of a second genetic locus. Should the structural studies under way confirm the marked similarity in non-*a* γ chains as suggested by the compositional data presented here, it would imply the emergence of the two postulated loci before that of the group *a* allelism seen at the one locus. It is difficult to envision the locus of *a*1, *a*2, and *a*3 each independently giving rise to a second locus (presumably by gene duplication) with subsequent convergence of the second locus to a high level of identity.

Allotype-deficient immunoglobulin appears capable of fulfilling at least some of the biological functions of allotype-bearing immunoglobin as evidenced by the survival and normal growth of suppressed newborn rabbits. In addition, a "restricted" antistreptococcal carbohydrate C IgG antibody, which lacks group *a* allotypic markers, has been obtained by hyperimmunization of an adult homozygous *a*1 rabbit (Kindt *et al.*, 1970). This antibody also fulfills the structural criteria set forth here (J. Prahl and R. Krause, unpublished observations). These observations and the presence of minor amounts of allotypically deficient IgG in normal sera imply at least the potential, if not active, functionality of the products of these genes.

The mechanism of the generation of antibody diversity remains an issue of contention at this time. But the ability of the genetic locus of allotypically deficient γ chains to give rise to functional immunoglobulins requires that the mechanisms involved in producing antibody diversity be operative on both the *a* and non-*a* loci. If a germline hypothesis is proposed, it must accordingly incorporate sufficient reduplication of the variable-region library at both loci to provide adequate diversity for survival and function of suppressed individuals. The necessity for reduplication of the diversity region may be avoided, however, by postulating the existence of three separate genes encoding a diversity region, an allotypy region, and a constant region, respectively.

Suppression thus appears to be a valuable adjunct for exploring the genotype of eukaryotes, by allowing the expansion of gene products which are normally overlooked or seen as minor components. Similar observations have been made by Maniatis *et al.* (1969) in suppression of the appearance of adult hemoglobin in the metamorphosis of tadpole to adult frog, its replacement hemoglobin having been shown to be electrophoretically and immunologically distinct from the adult hemoglobin. The molecular basis of suppression is not understood at this time. Initiation of suppression may be postulated to occur by deviation during the formation of differentiated progenitor cells, clonal destruction of differentiated progenitor cells, or alteration of genetic expression of differentiated cells.

Maintenance of chronic suppression appears to be an active process, as demonstrated by cell transfer studies in mice suppressed for immunoglobulin allotype (Jacobson and Herzenberg, 1972; Jacobson *et al.*, 1972).

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Structure of Immunoglobulin A. Cysteine-Containing Peptides of the α Chain of an Immunoglobulin A1 Myeloma Protein†

Enrique Mendez,* Blas Frangione, and Edward C. Franklin

ABSTRACT: Studies of the cysteine-containing peptides of a completely reduced and alkylated heavy chain from a polymeric IgA1(κ) myeloma protein revealed an unusually high content of cysteine residues. At least 17 different radioactive cysteine residues were identified; since one additional cysteine-containing peptide may have been missed in this study, 18 is the minimum number of cysteine residues in the α chain. One of the cysteine residues formed the heavy–light disulfide bridge. There were at least two interchain heavy–heavy bridges, one involving a cysteine in the hinge and the other, a cysteine in a peptide disulfide bridged to the hinge. Two additional cysteine residues in the hinge form intrachain bridges with cysteine residues in the Fc and Fd fragments, respectively. While most of the remaining cysteine residues appear to be involved in intrachain bridges, it seems likely that some may be bridged to the secretory piece and J chain.

The IgA[1] fraction has several unusual characteristics: (1) unlike most other immunoglobulins, it frequently exists as a polymer linked to an unusual structural unit, the J chain by disulfide bridges (Morrison and Koshland, 1972); (2) in external secretions, IgA is bound to a unique subunit, the secretory piece. Here, too, in man, the bond is formed by disulfide bridges (Lamm and Greenberg, 1972); (3) the hinge region, while resembling that in most other classes of Ig in being rich in proline and cysteine residues, has several unusual properties which have been briefly described and will be elaborated in this and the accompanying report (Wolfenstein-Todel *et al.*, 1973). To try to understand some of these unusual characteristics of IgA, it seemed advisable to attempt to identify and localize all of the disulfide bridges of the molecule.

In recent years several studies have provided sequence data on a limited number of cysteine-containing peptides of the α chain. These include the carboxy-terminal peptide (Prahl *et al.*, 1971; Wolfenstein *et al.*, 1971; Kehoe *et al.*, 1973; Mendez *et al.*, 1973b), the hinge (Frangione and Wolfenstein-Todel, 1972), and a number of additional intra- and interchain cysteine-containing peptides (Wolfenstein *et al.*, 1971). More recently, Moore and Putnam (1973) have extended these studies and have described three additional cysteine-containing pep-

† From the Irvington House Institute and the Rheumatic Diseases Study Group, New York University Medical Center, New York, New York 10016. *Received July 5, 1973.* This work was supported by U. S. Public Health Service Grants No. AM 02594 and AM 01431, The Damon Runyon Memorial Fund for Cancer Research, and the Helen and Michael Schaffer Fund.

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