*A "LEPORE" TYPE OF HYBRID γ GLOBULIN**

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Abstract and Summary.—No explanation has been available concerning the γ globulin defect in a unique family with one member whose serum was devoid of all the usual Gm genetic antigens. In the present study, it was found that this serum lacks ordinary γG1 and γG3 proteins and contains instead hybrid molecules of the type γG3-γG1. These were demonstrated most clearly by the precipitation of γG1 proteins with antisera specific for γG3 antigens. The analogy to the delta-beta chain hybrids, established for Lepore-type hemoglobins, was striking. An unequal homologous crossover involving mispairing of heavy chain cistrons would readily explain the deletion of genetic markers.

A large amount of evidence has been accumulated which indicates that the heavy chains of human γG globulin are determined by at least four closely linked cistrons corresponding to the four known subgroups.[1, 2] Similar conclusions have been reached for mouse γ globulin.[3, 4] A different interpretation, envisioning multiple alleles at a single genetic locus, has been put forward.[5, 6] Strong arguments against such a concept have been presented previously.[7] However, the recent finding of a unique family with one member whose serum is completely devoid of known heavy chain genetic antigens has been cited in favor of the single-locus hypothesis.[6] The simultaneous loss of genetic markers for three different subgroup genes would presumably be difficult to explain.

In the present study, this unusual serum was studied in considerable detail. In particular, the possibility of an unequal homologous crossover, similar to that known for hemoglobin Lepore,[8] leading to hybrid molecules with the elimination of multiple genetic antigens was considered. Such a concept was suggested by recent observations on the deletion of various γ globulin genes which could be best explained on a similar basis.[9]

Material and Methods.—Normal sera of different population groups were furnished by Drs. F. H. Allen and A. G. Bearn. Serum 2904 was provided by Dr. A. G. Steinberg. Myeloma proteins were isolated from the serum of patients with multiple myeloma by zone electrophoresis and chromatographic separation as described previously.[10] Fab and Fc fragments of isolated myeloma proteins were produced by papain splitting under the conditions recommended by Porter.[11] Proteins of the γG1 type were incubated for the usual 16 hr; γG3 proteins were incubated with papain for 1 hr only because of the further digestion of the Fc fragment of this type over longer periods.

Genetic typing was carried out by the hemagglutination inhibition method with the use of both rabbit and human antisera as described previously.[1] Subgroup typing was done by both precipitation and hemagglutination inhibition techniques.[10, 1] The latter procedure was adapted from that utilized for genetic typing. Selected anti-D coats were usually utilized for the γG1 and γG3 systems; myeloma proteins coupled to red cells by bisdiazotized benzidine (BDB) were used for the γG2 and γG4 systems as well as in confirmatory experiments in the γG1 and γG3 procedures.

Results.—Absence of specific subgroup antigens: Previous studies on serum 2904 indicated a relatively normal total γ globulin along with an elevated γG3

level.[6] All of the subgroups were thought to be present. In the present study, detailed investigations of subgroup concentrations were carried out. The total γ globulin was found to be 12.4 mg/ml, a level within the normal range. The γG2 concentration was 5.5 mg/ml, a value in the high normal region. The γG4 concentration was not determined quantitatively but was close to that found for various normal sera in hemagglutination inhibition assays. However, when determinations of the γG1 and γG3 levels were attempted, some major discrepancies from other sera were encountered. This was particularly striking for γG3, the subgroup for which specific antisera have been utilized most extensively.[10, 12] It is well known that two different types of γG3-specific antisera can be prepared: one that reacts with an antigen in the Fc fragment and one that reacts with an antigen in the Fab portion of the molecule.[10] Both types of antisera have been utilized for quantitation, and completely parallel results have been obtained in a study of a large number of sera from different populations. Serum 2904 differed strikingly from other sera in this respect. The Fab antisera showed markedly elevated levels of γG3, while the Fc antisera showed a complete absence of γG3 proteins.

Table 1 illustrates the results of one experiment with the γG3-Fc system as determined by the standard hemagglutination inhibition technique utilized for different γ globulin antigens.[1, 9] Serum 2904 failed to inhibit even in the undiluted state, while normal serum Ko inhibited at a dilution of 1:160. The test system was specific for γG3 proteins both of Gm (*g*) and Gm (*b*) type but reacted only with the Fc fragment, as shown by loss of inhibition on pepsin destruction of the Fc fragment (Table 1). Similar experiments with the γG3-Fab test system showed greater inhibiting capacity for serum 2904 than for normal sera, and quantitative analyses by radial diffusion with this system gave a level of 4.7 mg/ml γG3 protein. It thus appeared that the portion of the Fc fragment containing the usual γG3 antigens was absent, while γG3-Fab antigens were quantitatively increased.

In a study of the γG1 antigens an equally striking anomaly was encountered (Table 2). Here too, antisera for the Fc as well as the Fab portion of the molecules are available which give similar results with normal sera and γG1

TABLE 1. *γG3-Fc system: inhibition of agglutination by various isolated proteins or sera.*

	Mg of Protein or Serum Dilution Added			
	0.5	0.12	0.03	0.008
M*-γG1 (El.)	2	2	2	2
M-γG2 (Ne)	2	2	2	2
M-γG3 (Vi)	0	0	0	±
M-γG3 (Ji)	0	0	0	1
M-γG4 (Ge)	2	2	2	2
Fr II	0	0	0	1
Fr II (pepsin)	2	2	2	2
	1:10	1:40	1:160	1:640
Serum Ko	0	0	0	2
Serum 2904	2	2	2	2

Control agglutination without inhibition = 2+. Coat-anti Rh 4752; antiserum-rabbit anti-γG3 abs. with γG1 and γG2 proteins, and by pepsin-digested γG3 proteins.

* M = myaloma protein.

TABLE 2. *Results of inhibition assays for γG1 and γG3 determinants in serum 2904 compared to normal sera and isolated myeloma proteins.*

Inhibitor	Test for γG1 Fab k	γG1 Fc	γG3 Fab	γG3 Fc
Normal sera (Ko, Jo)	0.008*	0.008	0.03	0.03
Serum 2904	>10	0.008	0.002	>10
Myeloma proteins				
γG1 (Ke): Fab	0.015	>0.5	>0.5	>0.5
Fc	>0.5	0.008	0.5	>0.5
γG3 (Vi): Fab	>0.5	>0.5	0.002	0.5
Fc	>0.5	>0.5	>0.5	0.008
γG2 (Ne)	>0.5	>0.5	>0.5	>0.5
γG4 (Ge)	>0.5	>0.5	>0.5	>0.5

* Minimal concentration in mg of γ globulin per ml giving inhibition in the test system.

myeloma proteins in hemagglutination inhibition assay. Serum 2904 gave a completely negative inhibition of the γG1-Fab system, while the Fc system was inhibited to a degree comparable to a normal serum. Therefore, for the γG1 proteins, the usual Fab antigens were missing, while the Fc antigens were intact—the opposite result from that obtained for the γG3 analyses.

Precipitation of γG1 antigens with antisera specific for γG3 proteins: Both the γG1 and the γG3 specific antigens correspond to major structural differences distributed within various parts of both Fab and the Fc fragments.[7, 10] The findings thus strongly indicated that serum 2904 completely lacked γG3-Fc and γG1-Fab fragments but contained γG3-Fab and γG1-Fc in approximately the same quantity. These results raised the possibility of the presence of hybrid molecules consisting of a Fab portion that was γG3 and an Fc portion of γG1 type with a deletion of γG1-Fab and γG3-Fc. If this were the case, then a precipitating antiserum specific for the γG3-Fab region should cause the precipitation of the γG1-Fc portion. The γG3-Fab specific antisera described above represent good precipitating antisera about which considerable knowledge has been gathered. In control experiments with normal sera and artificial mixtures of γG3 and γG1 myeloma proteins, these antisera always failed to bring down γG1 proteins at different ratios of antigen to antibody. In the case of serum 2904 the γG1 antigens were readily precipitated by the γG3-Fab antisera. This was evident both from analyses of the precipitate as well as of the remaining supernatant. Incubation of the washed precipitate with the γG1 antiserum absorbed out the γG1 activity. Determination of γG1 levels in the supernate by hemagglutination inhibition indicated a marked fall after addition of the γG3 antiserum. This was dependent on the amount of antibody added, and reached a 32-fold decrease of the original γG1 level in the region of antibody excess. Assays on the precipitate indicated that it contained more than 95 per cent of the γG1 molecules. Two different γG3 antisera gave similar results in precipitating the γG1 antigens. Controls with dilutions of serum 2904 without addition of antiserum and with antiserum added to normal sera or mixtures of myeloma proteins were included in each of these experiments. Similar experiments in the γG2 Fab and Fc systems indicated normal γG2 molecules and failed to show any involvement of this subgroup in the hybrids.

Additional studies: Studies of the major genetic markers of the Gm system confirmed the previous findings of their complete absence in this serum. Serum 2904 was Gm (*a-z-f-g-b-n-*) when tested in high concentration with very sensitive hemagglutination inhibition assays. This included a series of the closely associated Gm (*b*) antigens which were also negative. With the evidence obtained above for γG3-γG1 hybrid molecules, a model could readily be constructed which would account for the deletion of all known genetic markers. Figure 1

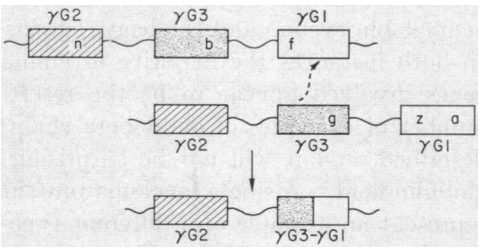

FIG. 1.—Diagram of the consequence of mispairing of γ globulin genes at meiosis followed by intracistronic crossover leading to a γG3-γG1 hybrid gene. The approximate positions of the known genetic markers which would be deleted are indicated.

illustrates diagrammatically the two major gene complexes found in Caucasians with the γG2, γG3, and γG1 cistrons placed in the order determined in previous studies.[7] A mispairing of the γG1 and γG3 cistrons at meiosis followed by an intracistronic crossover would result in a type of gamete with the genotype indicated. Such mispairing might well occur, in view of the many known homologies of the γG1 and γG3 cistrons. Also indicated in Figure 1 are the approximate positions of the known genetic markers. It can be seen that with the one single crossover depicted and the resultant hybrid cistron there would be a complete loss of all markers. The areas that are retained happen to be just the ones without known markers. The two chromosome areas shown in Figure 1 are common types found in Caucasians. A similar product could be obtained if the lower type were the same as the upper except that the γG2 cistron lacked the Gm (*n*) marker. This Gm^{n-}-Gm^{b}-Gm^{f}-gene complex is also frequently found among Caucasians.

Supportive evidence for the presence of hybrid γG3-γG1 heavy chains was obtained through analyses of serum 2904 for a number of other antigenic determinants of the γ globulin subgroups. "Non-*a*" and "non-*g*," two useful new antigenic markers, were found in normal concentrations; this was completely compatible with the concept described above. The details of these studies have been described separately.[13] Two other antigens also shared by certain of the subgroups furnished added evidence for γG1-Fc antigens. In addition, a different γG3-Fab antigen that is only revealed by pepsin splitting[14] was found in serum 2904 after enzyme treatment, while the corresponding γG1-Fab antigen was absent.

Discussion.—The current study was possible primarily because qualitative and quantitative methods have become available for the determination of a wide assortment of antigenic determinants of different γG globulin heavy chains. Hemagglutination inhibition techniques could be utilized for determination of all the subgroups, and, in most instances, subgroup antigens in different areas of the molecule could be detected. Previous experience has demonstrated[7] that

such antigenic determinants reflect specific amino acid sequences. It will be of interest in future work to establish these sequences for the hybrid molecules. At present, this is not entirely feasible for two reasons: First, problems have been encountered in the isolation of the hybrid protein; the serum has a high concentration of γG2 proteins which have proved difficult to remove completely. Second, the sequence differences between γG1 and γG3 proteins are only available for limited areas of the heavy chains.[15]

Many analogies exist between the beta and delta chain relationship in the hemoglobin system and the heavy chains of the four subgroups of γG globulin. In both instances the majority of amino acid sequences are identical and the genes involved appear to be the result of relatively recent duplications. A number of examples of delta–beta chain hybrids of the Lepore type have been described, and it will not be surprising to find additional examples in the γ globulin field. A single myeloma protein has been observed in mice which may represent an example of a different type of hybrid protein.[16] In patient 2904, no normal γG1 or γG3 protein was detectable; only the γG3-γG1 hybrid was found. This individual was probably homozygous for the hybrid gene, since other individuals were described in the family with a similar "silent allele" in the heterozygous state.[6] It appeared that in the hybrid protein a portion of the heavy chain toward the N-terminal end was γG3 and the C-terminal portion was γG1. No defects in the light chains were detected. An interesting point that requires further evaluation concerns the question whether the hybrid molecules function as antibodies. Preliminary evidence was obtained that this is the case for certain red cell antibodies found in serum 2904 where γG3 and γG1 determinants were detected. It would thus appear that whatever the mechanism is for generation of diversity of antibodies, it was not affected by the alteration described.

The concentration of the hybrid protein was far higher than the usual γG3 level but somewhat below the normal mean for γG1, suggesting that the rate of synthesis was influenced both by the parts of the gene coding for Fab-γG3 and the Fc-γG1. The observations on the hybrid gene indicate that there is normally only one cistron for the γG1 and one for the γG3 subgroups of γ globulin, since no normal molecules for these subgroups were found in serum 2904. Finally, the findings offer further evidence for the order and direction of the three subgroup cistrons, namely γG2-γG3-γG1.[7]

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