Proc. Nat. Acad. Sci. USA
Vol. 71, No. 4, pp. 1169–1173, April 1974

Recombinant Rabbit Secretory Immunoglobulin Molecules: Alpha Chains with Maternal (Paternal) Variable-Region Allotypes and Paternal (Maternal) Constant-Region Allotypes

[somatic recombination/immunosorbents/F(ab)$_{2\alpha}$ fragments]

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Communicated by Herman N. Eisen, November 16, 1973

ABSTRACT **A population of IgA molecules having heavy chains coded by two parental chromosomes in *trans* position has been identified in rabbits heterozygous at both the V$_H$*a* locus, which controls allotypic specificities on the variable part of heavy chains, and the C$_\alpha$*g* locus, which controls allotypic specificities on the constant part of alpha chains. These recombinant molecules have alpha-chain allotypic specificities controlled by both the maternal V$_H$*a* gene and the paternal C$_\alpha$*g* gene or conversely, the paternal V$_H$*a* gene and the maternal C$_\alpha$*g* gene. These recombinant molecules were found in F(ab)$_{2\alpha}$ fractions obtained after passage of F(ab)$_{2\alpha}$ preparations through immunosorbent columns designed to remove one population of F(ab)$_{2\alpha}$ molecules, i.e., g74- or g75-type molecules. The effluent F(ab)$_{2\alpha}$ fractions were then examined by radioprecipitation methods for allotypic specificities controlled by the V$_H$*a* and C$_\alpha$*g* loci. About 40% of the g75 F(ab)$_{2\alpha}$ molecules from each of three rabbits with the a^1g^{74} and a^2g^{75} allogroups were a1g75 recombinants. These a1g75 recombinant molecules represented from 2.5–5.6% of the total unfractionated F(ab)$_{2\alpha}$ sample. The F(ab)$_{2\alpha}$ fractions from two rabbits with the a^1g^{75} and a^3g^{74} allogroups had from 1.8–8.2% recombinant molecules: some were a1g74 recombinants and some were a3g75 recombinants. Somatic recombination as a mechanism responsible for the synthesis of polypeptide chains in which part of the information is obtained from one chromosome and part from the homologous chromosome is discussed.**

Three alleles at the rabbit *a* locus control the allotypic specificities a1, a2, and a3 on the variable part of immunoglobulin (Ig) heavy chains (1, 2). The presence of these specificities on γ, α, μ, and ϵ chains (3, 4), each with its specific constant region, has led to the concept that separate genes control the variable (V$_H$) and constant (C$_H$) regions of heavy chains (5). Within this concept, a V$_H$ gene, e.g., a V$_H$*a* gene, may associate with any of the linked heavy-chain constant-region genes, C$_\gamma$, C$_\alpha$, C$_\mu$, or C$_\epsilon$, and thus lead to the formation of a complete γ, α, μ, or ϵ heavy chain. It is not clear whether the association between V$_H$ and C$_H$ genes is at the level of DNA, RNA, or protein (6).

The *f* locus controls the allotypic specificities f71, f72, and f73, which presumably reside within the constant part of alpha chains of one subclass of IgA (7). The *g* locus controls the allotypic specificities g74 and g75 on the constant part of alpha chains of a second subclass of IgA (8, 9). The g74 and g75 allotypic specificities are each comprised of multiple antigenic determinants, some of which are present on the Fc$_{2\alpha}$ fragment and others on the F(ab)$_{2\alpha}$ fragment of the "g" subclass of secretory IgA (9). The *f* and *g* loci, which are closely linked to each other and to the V$_H$*a* locus (10), can be designated C$_\alpha$*f* and C$_\alpha$*g*. Thus, individual α chains may have V$_H$*a* specificities and also C$_\alpha$*f* or C$_\alpha$*g* specificities.

The phenomenon of allelic exclusion (11), in which only one of the two alleles at a given locus is expressed, exists for several immunoglobulin loci at the molecular and cellular levels (11–14). Allelic exclusion is generally assumed to apply to the synthesis of all immunoglobulins, even the polymeric forms assembled within an individual cell (15). Thus, individual secretory IgA molecules (11S) from a g^{74}/g^{75} heterozygous rabbit have either the g74 or the g75 determinants but not both (unpublished data).

In general, allotypic specificities present on heavy chains of individual IgG molecules in rabbits heterozygous at the V$_H$*a* locus and also at the C$_\gamma$ locus [which controls allotypic specificities d11 and d12, and e14 and e15 on the constant portion of the γ chain (16, 17)] are controlled by either the maternal or the paternal chromosome; i.e., the V$_H$ and C$_H$ regions are controlled by the V$_H$*a* and C$_\gamma$ genes, which are *cis* to each other (17–19). Recently, however, Landucci-Tosi and Tosi (20), using IgG preparations fractionated on the basis of allotypic characteristics, have reported finding a1e15 and a2e14 recombinant IgG molecules from a^1e^{14}/a^2e^{15} rabbits. The recombinant molecules represented less than 2% of the total IgG and thus were not detected in previous studies with unfractionated preparations of IgG. Recombinant molecules have also been found by Pernis *et al.* (21) in plasma cells of rabbits.

The purpose of the present investigation was to determine if V$_H$-C$_H$ recombinant-type heavy chains of the "g" subclass were present in secretory IgA (sIgA) molecules.

MATERIALS AND METHODS

The Rabbits were bred in Dr. S. Dray's colony at the University of Illinois Medical Center. Their allotypes were determined by double-diffusion experiments as described (1). The allogroups of rabbits heterozygous at the *a* and *g* loci were determined by genetic studies.

Antisera. The anti-allotype antisera, anti-a1, anti-a2, anti-a3, anti-f71g75, anti-f72g74, and anti-g74, were prepared as described (1, 8). As a precautionary measure, to remove antibodies against other IgG allotypic specificities, both known,

Abbreviations: Ig, immunoglobulin; sIgA, secretory IgA.

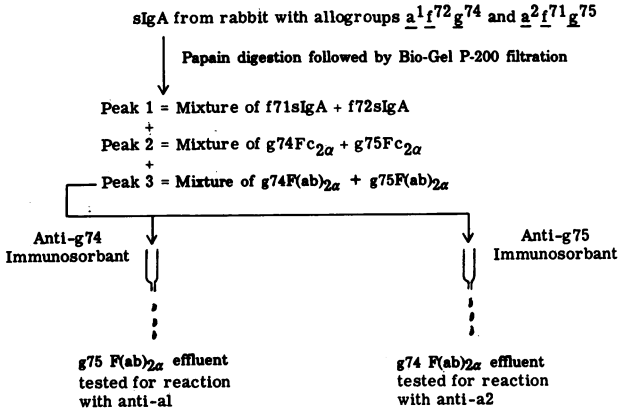

FIG. 1. Flow diagram illustrating the experimental procedures used to identify recombinant-type $F(ab)_{2\alpha}$ molecules.

e.g., anti-x32 and anti-y33 (22), and unknown IgG specificities, the anti-a1, anti-a2, and anti-a3 antisera were each passed through an immunosorbent column prepared by coupling IgG, obtained from a pool of a2 and a3, a1 and a3, or a1 and a2 sera, respectively, to Sepharose 4B (23). The anti-a1 should not have had contaminating anti-x32 or anti-y33 antibodies, since it was prepared in an a^2a^2 rabbit, and all a^2 rabbits in our colony have the allogroup $a^2x^{32}y^{33}$. The anti-f71g75 antiserum used for radioprecipitation analyses was passed through an immunosorbent column prepared by coupling secretory IgA obtained from an $f^{72}g^{74}$ and an $f^{73}g^{74}$ rabbit to Sepharose 4B (23). Anti-(rabbit) Fc_γ prepared in goat was prepared as described (7).

Immunosorption and Detection of Recombinant Molecules. IgG preparations of anti-f71g75 and anti-f72g74 antisera were prepared by sequential precipitations with sodium sulfate (18 and 14%) (24). The IgG-rich fractions were each coupled to Sepharose 4B (23), resulting in anti-f71g75 and anti-f72g74 immunosorbents, respectively (designated as anti-g75 and anti-g74 immunosorbents). The "g" type $F(ab)_{2\alpha}$ fragments, which were obtained after papain digestion of secretory IgA (7) from the five doubly heterozygous rabbits, were iodinated with ^{125}I (25). (The f subclass of molecules was readily separable by molecular sieving from the "g"-type $F(ab)_{2\alpha}$ due to the resistance of "f" molecules to papain digestion.) About 150 μg of these $F(ab)_{2\alpha}$ fragments, containing allotypic determinants controlled by the $C_\alpha g$ locus (9), were placed on the anti-g75 or the anti-g74 immunosorbent to remove the molecules with g75 or g74 determinants, respectively. The effluents (unbound materials) from the immunosorbents were examined by quantitative radioprecipitation methods (7) for the presence of the allotypic specificities controlled by the V_Ha and the $C_\alpha g$ loci. The radioprecipitation analyses were performed with 0.2 μg of ^{125}I-labeled $F(ab)_{2\alpha}$, an excess of anti-allotype antiserum, and a slight excess of anti-(rabbit) Fc_γ prepared in goat to ensure complete precipitation of the antigen–antibody complexes (7). The values reported are the average of the duplicate samples, which did not vary by more than 2%. The negative control with normal rabbit serum resulted in a maximum of 2% of the radioactivity precipitated.

Inhibition Assay. $F(ab)_{2\alpha}$ fragments of sIgA from three of the doubly heterozygous rabbits (a^1g^{74}/a^2g^{75}) were iodinated with ^{131}I (25), and 150 μg were passed through the anti-g74 immunosorbent column. The effluent molecules, bearing g75 determinants, were used to inhibit the reaction of anti-a1 with 0.1 μg of ^{125}I-labeled $F(ab)_{2\alpha}$ obtained from sIgA of an a^1/a^1 rabbit. The $F(ab)_{2\alpha}$ fragments used as inhibitors were labeled with ^{131}I rather than ^{125}I since the a1-type antigen in the inhibition assay was labeled with ^{125}I. The ^{131}I-labeled $F(ab)_{2\alpha}$ fragments were tested by quantitative radioprecipitation methods after passage through the anti-g74 immunosorbent to be certain that all g74-type molecules had been removed. In each experiment, anti-g74 precipitated less than 3% of the radioactivity in these $F(ab)_{2\alpha}$ fractions. All samples of the inhibition assay were counted on two channels in a well-type γ-spectrometer, and the counts per minute determined on the channel set for ^{125}I ($F(ab)_{2\alpha}$ antigen) were corrected for the contribution by ^{131}I ($F(ab)_{2\alpha}$ inhibitor). The amount of anti-a1 used for the inhibition assay was titrated to precipitate 41% of the radioactivity of the a1-type ^{125}I-labeled $F(ab)_{2\alpha}$ fraction in the absence of inhibitor. A standard inhibition curve was established with 0.1–4.0 μg of unlabeled $F(ab)_{2\alpha}$ inhibitor, obtained from sIgA of an a^1/a^1 rabbit; the $F(ab)_{2\alpha}$ fragment obtained from sIgA of an a^2/a^2 rabbit was a negative control. All reactions were run in duplicate, and the volume in each tube was kept constant.

RESULTS

The rationale used to identify recombinant sIgA molecules from rabbits heterozygous at the *a*, *f*, and *g* loci was as follows: the sIgA of one type with respect to the C_α allotypic specificities was isolated and examined for heavy-chain allotypic specificities (both C_α and V_H) by quantitative radioprecipitation methods. We chose to work with $F(ab)_{2\alpha}$ fragments of the "g" subclass of IgA rather than with whole sIgA molecules, due to ease of preparation of good yields of $F(ab)_{2\alpha}$ with a high degree of purity. Secretory IgA preparations from an $f^{71}g^{75}/f^{72}g^{74}$ heterozygous rabbit have four kinds of IgA molecules (with respect to the C_α): f71, f72, g74, and g75 molecules. The $F(ab)_{2\alpha}$ molecules isolated from papain digests of such preparations (Fig. 1) contained a mixture of only two types of molecules, with respect to the C_α locus: g74 $F(ab)_{2\alpha}$ and g75 $F(ab)_{2\alpha}$. This mixture was iodinated and separated by the use of anti-g74 or anti-g75 immunosorbents to remove either the g74 or the g75 molecules, leaving purified fractions of g75 $F(ab)_{2\alpha}$ and g74 $F(ab)_{2\alpha}$, respectively. If recombination events occurred in an a^1g^{74}/a^2g^{75} heterozygous rabbit, removal of the g74 molecules from the $F(ab)_{2\alpha}$ preparation (with an anti-g74 immunosorbent) would leave g75 molecules of two types: a normal a2g75 type and a recombinant a1g75 type. After removal of a selected population of $F(ab)_{2\alpha}$ molecules, quantitation of the recombinant molecules by precipitation methods with anti-a1, anti-a2, and anti-g75 should be more reliable. Similarly, a2g74 recombinant molecules could be identified by testing purified g74 $F(ab)_{2\alpha}$ molecules for the presence of a2.

Detection of Recombinant Molecules. The absorption of the ^{125}I-labeled $F(ab)_{2\alpha}$ preparation, from rabbit J50-2 (a^1g^{74}/a^2g^{75}), on the anti-g74 immunosorbent removed essentially all g74 molecules (3% of the radioactivity was precipitated by anti-g74), leaving predominantly g75 molecules (92% of the radioactivity was precipitated by anti-f71g75) (Table 1). Similarly, all g75 molecules were removed when the ^{125}I-labeled $F(ab)_{2\alpha}$ preparation was absorbed on the anti-g75 immunosorbent, leaving essentially all g74 molecules (98%) (Table 1).

TABLE 1. *Percent radioactivity of $F(ab)_{2\alpha}$ fragments (obtained from five rabbits) precipitated by anti-allotype antisera*

	Unabsorbed	Absorbed with anti-g74	Absorbed with anti-g75
J50-2 a^1g^{74}/a^2g^{75}			
anti-a1	66	**40**	65
anti-a2	3	10	**2**
anti-g74	83	3	98
anti-f71g75	14	92	1
nRS*	1	1	1
H192-6 a^1g^{74}/a^2g^{75}			
anti-a1	60	**36**	58
anti-a2	4	11	1
anti-g74	67	<1	93
anti-f71g75	13	83	1
nRS	1	<1	1
J312-2 a^1g^{74}/a^2g^{75}			
anti-a1	59	**41**	60
anti-a2	3	9	<1
anti-g74	82	2	97
anti-f71g75	10	84	<1
nRS	2	2	<1
G248-1 a^1g^{75}/a^3g^{74}			
anti-a1	41	53	**7**
anti-a3	59	**10**	77
anti-g74	56	1	95
anti-f71g75	37	92	<1
nRS	<1	<1	<1
K44-6 a^1g^{75}/a^3g^{74}			
anti-a1	28	65	**7**
anti-a3	45	**12**	65
anti-g74	55	2	95
anti-f71g75	36	91	1
nRS	1	1	1

Boldface numbers are values that could indicate recombinant-type molecules.

* Normal rabbit serum.

Since the allogroups of rabbit J50-2 are a^1g^{74} and a^2g^{75}, removal of the g74 molecules should also remove all a1 molecules if there were no a1g75 recombinants. In fact, however, 40% of the radioactivity of the molecules remaining after removal of the g74 molecules was precipitated by anti-a1.

Since only 92% of the radioactivity was precipitated by anti-f71g75 (Table 1), rather than 100%, as many as 8% of the molecules could be other than g75-type molecules bearing the a1 specificity. Even so, a minimum of 32% [40 − (100 − 92)] of the molecules must have both the a1 specificity and the g75 specificity and, thus, represents recombinant molecules. Since the g75 molecules represented 14% of the total population of $F(ab)_{2\alpha}$ molecules before passage through the immunosorbent (Table 1), the minimum percent of a1g75 recombinant molecules in the original $F(ab)_{2\alpha}$ population is 4.5%; the maximum is 5.6%, assuming 100% of the $F(ab)_{2\alpha}$ molecules have g75 specificities.

No a2g74 recombinant $F(ab)_{2\alpha}$ molecules from rabbit J50-2 could be identified. Passage of the ^{125}I-labeled $F(ab)_{2\alpha}$ preparation through the anti-g75 immunosorbent resulted in the removal of all g75 molecules, since anti-f71g75 precipitated less than 1% of the radioactivity of the absorbed $F(ab)_{2\alpha}$ sample (Table 1). Anti-g74 precipitated 98% of the radioactivity, but anti-a2 precipitated only 2% of the radioactivity. Thus, within experimental error, no a2g75 recombinant molecules were found.

TABLE 2. *Minimum and maximum percent of $F(ab)_{2\alpha}$ recombinant molecules identified*

Rabbit	Allogroups from Parent 1	Allogroups from Parent 2	Recombinant types	Min (%)*	Max (%)*
J50-2	a^1g^{74}	a^2g^{75}	a1g75	4.5	5.6
			a2g74	—	—
H192-6	a^1g^{74}	a^2g^{75}	a1g75	2.5	4.7
			a2g74	—	—
J312-2	a^1g^{74}	a^2g^{75}	a1g75	2.5	4.1
			a2g74	—	—
G248-1	a^1g^{75}	a^3g^{74}	a1g74	1.1	3.9
			a3g75	0.7	3.7
K44-6	a^1g^{75}	a^3g^{74}	a1g74	1.1	3.9
			a3g75	1.1	4.3

* Sample calculation: Maximum percent of a1g75 recombinant molecules = a × b and the minimum percent of a1g75 recombinant molecules = a(b − (100 − c)) where a = percent radioactivity of unabsorbed $F(ab)_{2\alpha}$ precipitated by anti-f71g75; b = percent of $F(ab)_{2\alpha}$, absorbed with anti-g74 immunosorbent, precipitated by anti-a1; and c = percent $F(ab)_{2\alpha}$, absorbed with anti-g74 immunosorbent, precipitated by anti-f71g75.

The $F(ab)_{2\alpha}$ fragments from four other doubly heterozygous rabbits were similarly examined for recombinant molecules (Table 1). The a1g75 recombinants were identified in g75 $F(ab)_{2\alpha}$ fractions from rabbits H192-6 and J312-2. Thirty-six percent and 41% of the radioactivity of the g75 $F(ab)_{2\alpha}$ of H192-6 and J312-2, respectively, was precipitated by anti-a1. Since anti-f71g75 precipitated only 83 and 84% of the radioactivity, 17% (100–83) and 16% (100–84) of the molecules may not have been g75 molecules; thus, the minimum percent of recombinant molecules in the g75 $F(ab)_{2\alpha}$ preparation is 19% (36–17) and 25% (41–16), respectively. Based on the original population of $F(ab)_{2\alpha}$ molecules, a minimum of 2.5% (Table 2) of the $F(ab)_{2\alpha}$ molecules from each animal are a1g75 recombinants.

Essentially no a2g74 recombinant-type molecules were found in either rabbit H192-6 or rabbit J312-2. After passage of the ^{125}I-labeled $F(ab)_{2\alpha}$ preparations through the anti-g75 immunosorbent column, less than 1% of the radioactivity of the resulting g74 molecules was precipitated by anti-a2.

Rabbits G248-1 (a^1g^{75}/a^3g^{74}) and K44-6 (a^1g^{75}/a^3g^{74}) had both a3g75 and a1g74 recombinant $F(ab)_{2\alpha}$ molecules (Table 1). For rabbit G248-1, 10% of the radioactivity of the g75 $F(ab)_{2\alpha}$ molecules (obtained from the anti-g74 immunosorbent) was precipitated by anti-a3 and 7% of the radioactivity of g74 $F(ab)_{2\alpha}$ (obtained from the anti-g75 immunosorbent) was precipitated by anti-a1. Thus, from 0.7 to 3.7% of the $F(ab)_{2\alpha}$ molecules are a3g75 recombinants and from 1.1 to 3.9% are a1g74 recombinants (Table 2). Similarly, the a3g75 and a1g74 $F(ab)_{2\alpha}$ recombinant molecules of rabbit K44-6 represent from 1.1 to 4.3% and from 1.1 to 3.9% of the $F(ab)_{2\alpha}$ molecules (Table 2).

Inhibition Experiments. To ensure that the reaction of the a1g75 recombinant molecules of rabbits J50-2, H192-6, and J312-2 with anti-a1 was due to a1 determinants rather than to

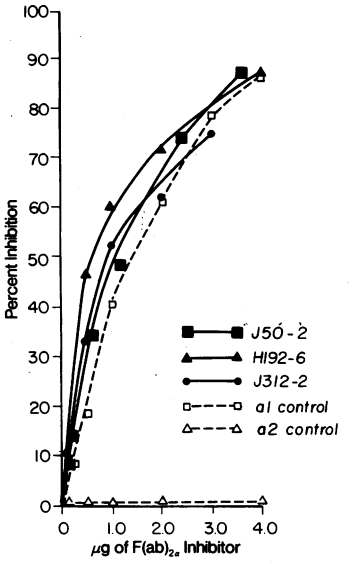

FIG. 2. Inhibition of precipitation of anti-a1 with ^{125}I-labeled F(ab)$_{2\alpha}$ obtained from an a^1/a^1 rabbit. Inhibitors are F(ab)$_{2\alpha}$ fractions [from rabbits J50-2, H192-6, and J312-2 (a^1g^{74}/a^2g^{75})] after passage through the anti-g74 immunosorbent. The standard inhibition curve (□- - -□) was determined with F(ab)$_{2\alpha}$ from an a^1/a^1 rabbit. The a2 control = F(ab)$_{2\alpha}$ from an a^2/a^2 rabbit.

other unidentified determinants that could possibly be present in the anti-a1 antiserum, the F(ab)$_{2\alpha}$ fractions containing "recombinant molecules" were used to inhibit the reaction of anti-a1 with ^{125}I-labeled F(ab)$_{2\alpha}$ obtained from an a^1/a^1 rabbit. In each of three experiments, the F(ab)$_{2\alpha}$ molecules obtained from the anti-g74 immunosorbent inhibited the reaction of a1 and anti-a1 as well as the a1-type F(ab)$_{2\alpha}$ used in the control (Fig. 2). Four micrograms of F(ab)$_{2\alpha}$ inhibitor (which contained 58% a1 molecules) used for the standard curve resulted in approximately a 90% decrease in the radioactivity precipitated by the reaction of ^{125}I-labeled a1-type F(ab)$_{2\alpha}$ with anti-a1. Similar amounts of F(ab)$_{2\alpha}$ from the anti-g74 immunosorbent (containing a1g75 "recombinant-type molecules") also resulted in 90% inhibition of the reaction of ^{125}I-labeled F(ab)$_{2\alpha}$ with anti-a1. No inhibition occurred with F(ab)$_{2\alpha}$ obtained from an a2 rabbit, as expected.

DISCUSSION

The $C_{\alpha}g$ locus controls allotypic specificities on the constant region of alpha heavy chains (on both the Fc and Fd portions), and the V_Ha locus controls allotypic specificities on the variable region of the alpha chain (2, 8, 9). If no recombinant IgA molecules exist, rabbits heterozygous at the V_Ha and $C_{\alpha}g$ loci would be expected to have only two types of IgA molecules (with respect to these two loci). For example, a rabbit with the allogroups a^1g^{74} and a^2g^{75} would have a1g74 and a2g75 α-chain type molecules, excluding other V_H subgroup loci, e.g., V_Hx and V_Hy (22). If, however, an association (at the DNA, RNA, or protein level) could occur between the V_H gene on one chromosome and a C_H gene on the homologous chromosome (i.e., V_H and C_H, which are *trans* to each other), a1g75 and a2g74 recombinant molecules might be found. Radioprecipitation studies on the g75 F(ab)$_{2\alpha}$ preparations, obtained from the anti-g74 immunosorbent, for each of three rabbits with the genotype a^1g^{74}/a^2g^{75} revealed that about 40% of the g75 molecules were recombinants, i.e., they had a1 allotypic specificities. Since the g75 molecules constituted from 10 to 14% of the unabsorbed F(ab)$_{2\alpha}$ mixture, such a1g75 recombinant-type molecules constituted from 2.5 to 5.6% of the molecules in the original unabsorbed F(ab)$_{2\alpha}$ preparation; this represents from 4.2 to 8.5% of the radioactivity of the unabsorbed F(ab)$_{2\alpha}$ precipitated by anti-a1, which precipitated about 60% (66, 60, and 59%) of the total sIgA radioactivity. Thus, from 4.2 to 8.5% of the F(ab)$_{2\alpha}$ molecules having variable regions controlled by the V_Ha gene have the constant region controlled by a $C_{\alpha}g$ gene in the *trans* position.

In the data presented, the unequal distribution of a1 and a2 molecules may have aided us in the identification of a1g75 recombinant-type molecules, but at the same time may have precluded identification of a2g74 recombinant-type molecules. Consider, for example, that balanced recombination has occurred within 3% of the cells, i.e., 3% of the cells have a^2 and g^{74} *cis* to each other and a^1 and g^{75} *cis* to each other, but that the chromosome with the a^1 gene is expressed 15 to 20 times more often than the chromosome with the a^2 gene; most of the recombinant molecules would be a1g75 and less than 10% would be a2g74 recombinants. Since g75 molecules represent less than 15% of the unfractionated molecules, the a1g75 recombinant molecules would be a significant proportion of these g75 molecules. The a2g74 recombinants, however, would not represent a significant proportion of the g74 molecules, which constitute from 67 to 83% of the unfractionated sample. The near lack of a2 determinants in F(ab)$_{2\alpha}$ preparations from the three a^1g^{74}/a^2g^{75} rabbits may suggest that the genotypes designated were incorrect. The genotypes, however, were determined by family studies, and it is clear that each of these three rabbits did indeed have a a^2 gene.

Both a1g74 and a3g75 recombinant molecules were identified in the two rabbits (genotype a^1g^{75}/a^3g^{74}) that had approximately equal amounts of a1 and of a3 molecules, but the data were less striking, i.e., from 7 to 12% of the radioactivity in the g74 F(ab)$_{2\alpha}$ and the g75 F(ab)$_{2\alpha}$ preparations were precipitated by the anti-a1 or the anti-a3 reagents, respectively. In terms of the entire F(ab)$_{2\alpha}$ fraction, however, the percentage of recombinant molecules in these animals was similar to that in the a^1g^{74}/a^2g^{75} rabbits; the total a1g74 and a3g75 recombinant-type F(ab)$_{2\alpha}$ molecules in each animal was from 1.8 to 7.6% and from 2.2 to 8.2%, respectively.

The validity of the identification of a1g75 recombinant molecules in the three a^1g^{74}/a^2g^{75} rabbits relies predominantly on the specificity of the anti-a1 antiserum. To ensure that the reaction of anti-a1 with the a1g75 "recombinant molecules" was indeed due to a1 determinants, the absorbed F(ab)$_{2\alpha}$ fractions containing these "recombinant molecules" were used to inhibit the reaction between anti-a1 and F(ab)$_{2\alpha}$ obtained from an a^1/a^1 rabbit. If the anti-a1 was identifying a determinant other than a1 on the a1g75 "recombinant molecules," these "recombinant molecules" would not be expected to completely inhibit the reaction of anti-a1 with a1-type F(ab)$_{2\alpha}$ molecules. In fact, however, the a1g75 "recombinant molecules" from the three a^1g^{74}/a^2g^{75} rabbits inhibited the reaction nearly as well as the a1-type F(ab)$_{2\alpha}$ used to obtain the standard inhibition curve. Thus, a1 allotypic specificities are indeed present on the a1g75 recombinant molecules.

We have identified recombinant-type sIgA molecules in which the V_H and C_H regions of the alpha chain are controlled by V_Ha and $C_{\alpha}g$ loci that are *trans* to each other in the zygote. This observation can be accounted for by somatic

recombination (26), which has also been suggested as a mechanism to account for antibody variability (6, 27). This recombination could occur at the DNA level, or at the levels of transcription or translation. Although some data indicate that immunoglobulin chains are synthesized as a continuous chain (6), it is still conceivable that the constant and variable regions are synthesized separately and the respective peptides joined enzymatically. In fact, Apte and Zipser have recently observed the synthesis of one polypeptide chain from two independently controlled genes and suggest that the "splicing" occurs at the level of the polypeptide chain (28). If this mechanism is active in immunoglobulin-producing cells, the recombinant molecules described herein could be explained by having the V_H region of one chromosome and the $C_{\alpha}g$ region of the *trans* chromosome active within an individual cell. The polypeptide products of each of these genes (the variable and constant region of α chains) would then be joined, forming a complete α chain.

No evidence for recombination at the level of mRNA is available. It is, however, possible that somatic recombination occurs at the level of DNA during mitosis, and in fact, homologous chromosomes have been shown to be in close proximity to each other during mitotic metaphase in the Indian deer, *Muntiacus muntjack* (29). Gally and Edelman (6) have proposed a translocation hypothesis whereby a variable-region gene can be associated with one of several closely linked constant-region genes, giving rise to a single mRNA which codes for the entire polypeptide chain. The recombinant molecules we have identified can be accounted for by a balanced somatic crossover between homologous chromosomes as discussed above, or by an extension of the translocation hypothesis. Whereas variable-region genes usually associate with one of several closely linked constant-region genes in the *cis* position, on occasion they may associate with a constant-region gene in the *trans* position, giving rise to recombinant-type molecules.

This work was supported by grants from the National Institutes of Health (AI 11234 and AM 11914). K. L. K. is the recipient of a U.S. Public Health Service Research Career Development Award, AI 28687.

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