Brief Definitive Report

NONALLELIC BEHAVIOR OF RABBIT VARIABLE-REGION ALLOTYPES*

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Allotypes of immunoglobulins are inherited as codominant Mendelian alleles (1,2). However, deviations from expected allelic behavior have been observed for allotypes expressed by cells in culture (3), by transplanted lymphoid cells (4), by inbred mice (5), and by hyperimmune rabbits (6). For example, Bosma and Bosma (5) observed that a mouse C_H allotype was expressed in an unpredictable and transitory fashion in a congenic strain that had been bred to exclude this allotype. Strosberg et al. (6) have observed that antisera from a rabbit immunized with *Micrococcus lysodeickticus* simultaneously possessed immunoglobulins with three group a and three group b allotypes. These reports have prompted a reassessment of the assumption that immunoglobulin synthesis is directed by allelic structural genes.

By the use of sensitive radioimmune assays, low levels of group a allotypes not detected by qualitative typing or anticipated from breeding data were detected in about 50% of rabbit sera tested. In this report, "nominal allotypes" will refer to those allotypes determined by qualitative tests and breeding data, while "latent allotypes" will refer to those detectable only by sensitive radioimmune assays.

Materials and Methods

Rabbits used in this study were bled at 5 mo of age and group a and b allotypes were determined by the interfacial precipitin (ring) test using antiallotype sera prepared as previously described (7). One extended rabbit family was typed for H chain allotypes of groups d, f, g, n, x, and y by Dr. W. Carey Hanley (2,8). IgG was isolated and radioiodinated as previously described (7).

Allotypic markers were determined by radioimmune binding inhibition assays carried out as previously described (7). Inhibitor (100 μl) and 0.25 μg ^{125}I-labeled IgG antigen (50 μl) were added to a series of Beckman microfuge tubes. (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Antigen was diluted into saline buffered with phosphate (0.05 M, pH 7), which contained 1-2% carrier protein, 0.04 M dithiothreitol (DTT) and ^{22}Na as volume marker (40,000 cpm/50 μl) (9). The contents of the tubes were mixed and allowed to stand at room temperature for 30 min. 50 μl of HAS antiallotype serum (7) at a dilution sufficient to bind approximately 50% of the ^{125}I-labeled IgG was added with thorough mixing. The tubes were agitated for 3-4 h and stored overnight at 4°C. After centrifugation, a portion of the supernate was aspirated and discarded. and the remaining ^{125}I and ^{22}Na radioactivity determined. Percent binding was calculated according to Gotschlich (9) and percent inhibition as described by Kindt et al. (10). DTT was added to the assays at a final concentration of 0.01 M to minimize interference by 19S rheumatoid factors (10) in whole serum. At this concentration, DTT had minimal effect on binding reactions. Carrier proteins used to dilute antigens included 1% BSA and 10% (by vol) rabbit serum, which contained a 50- to 100-fold excess of all group a and b allotypes, except that being determined.

* Supported by U.S. Public Health Service Grants Al 11439 and Al 11995.

‡ Established Investigator of the American Heart Association.

Homologous inhibition curves differed slightly for each of the group a allotypes, but all had similar detection limits. Inhibition values less than 10% were considered insignificant, because values in this range were not reproducible in replicate assays. All concentrations are reported as μg/ml above the amount giving 10% inhibition. No significant inhibition of allotypic binding reactions was observed with a 400-fold excess of IgG of heterologous a allotypes. Reproducibility of replicate assays was improved when whole serum test samples were centrifuged and then filtered through a 0.45 μm Millipore before assay.

Results

Quantitative allotypic determinations were carried out on nonimmune sera to test for the presence of latent allotypes. Serial dilutions of serum from rabbit 4120 were tested for all group a allotypes by the inhibition of a1, a2, and a3 binding reactions as depicted in Fig. 1. Although this rabbit was allotype a2 both by the ring test and from breeding information, serum 4120 inhibited binding of

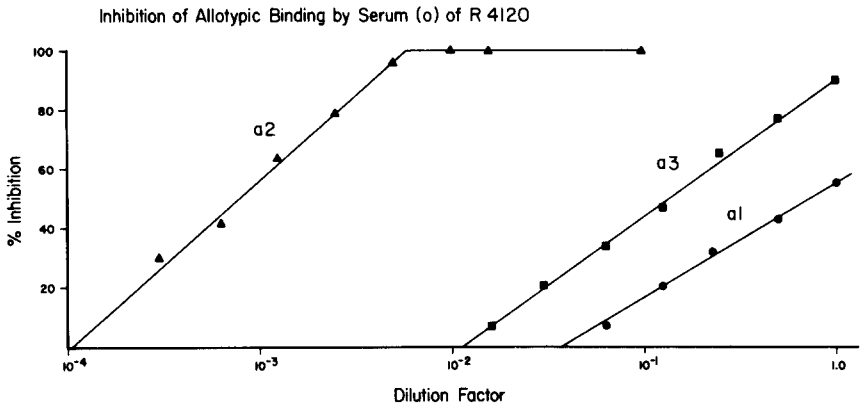

FIG. 1. Inhibition of binding of 0.25 μg of radiolabeled a1b4, a2b4, or a3b4 IgG to HAS anti-a1, anti-a2, or anti-a3 respectively, by dilutions of nonimmune serum from R4120. Carrier proteins used in these assays include an excess of immunoglobulins with all *a* and *b* allotypes except that being measured.

allotypes a1 and a3. The concentrations of immunoglobulins with the three group a allotypes in this serum, calculated from standard inhibition curves were: 5 mg/ml for the nominal allotype a2; 44 μg/ml for the latent a3; and 10 μg/ml for the latent a1. Nearly 100% inhibition of the a3 reaction could be achieved with serum 4120, a strong indication that this serum contains molecules identical to the radiolabeled a3 IgG antigen. The possibility that the a3 allotype present in serum 4120 was derived from maternal circulation is unlikely, because the mother was typed as an a1a2. Moreover, nine other rabbits expressed detectable levels of allotypes absent as nominal allotypes in either parent.

Serum samples from a large number of rabbits representing different populations were assayed to determine the frequency of latent allotype expression, and to learn if any particular allotypic combinations favor their occurrence. Fig. 2 shows the levels of latent group a allotype expression in 119 rabbits. Latent allotypes were expressed in rabbits with all nominal allotypic combinations, and there was no apparent correlation between allotype expression and age or sex. The frequency of latent allotype expression was greater for a1 and a3 than for a2,

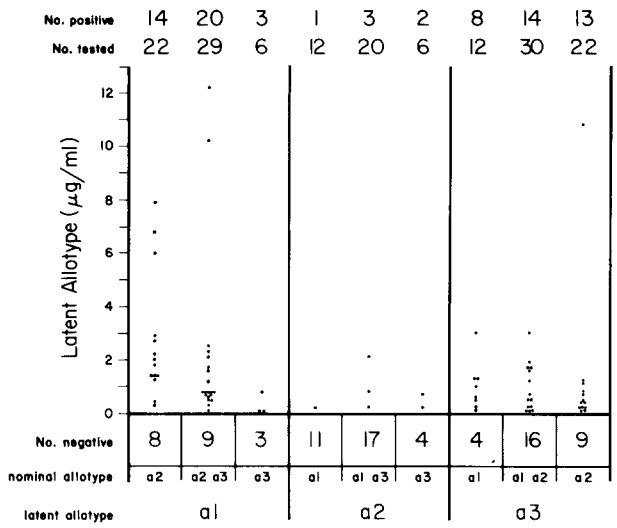

FIG. 2. Expression of latent allotypes in serum samples from 119 rabbits. Concentrations given are in μg/ml above that required to give 10% inhibition in the standard inhibition curves. Data for R4120 depicted in Fig. 1 are not represented in this summary.

although positive examples were found for each allotypic group. Nominal homozygotes expressing one latent allotype did not necessarily express the other. This lack of correlation precludes trivial causes for latent allotype detection that involve nonspecific inhibition of binding reactions.

Rabbits from one selectively bred family included in the survey expressed significantly higher levels of latent allotypes than those of the random population. 43% of the 73 rabbits in the related group had latent allotype levels of 1 μg/ml or above, whereas in the random group only 9% of 56 rabbits expressed this level. While the relevance of this observation has yet to be assessed, it is pertinent to mention that determination of the complete H chain allogroups of key rabbits in this family revealed no deviations from simple allelic behavior.

One possible explanation for the occurrence of latent allotypes in only 50% of the rabbit sera is that their expression is under some form of regulatory control. To explore this possibility the expression of latent allotypes as a function of time was determined for 12 nonimmune rabbits. Allotype levels of sera taken at 1-wk intervals for 5 wk are shown in Table I. The latent allotypes did not persist throughout the observation period, but occurred sporadically in 7 of the 12 rabbits tested. Such results indicate that repeated observations might increase the frequency of latent allotype detection among groups of rabbits.

Discussion

These studies have shown that a high percentage (50%) of normal rabbit sera express group a allotypes not anticipated by qualitative typing or breeding data. Immunoglobulins possessing these allotypes occurred in nonimmune sera at levels between 0.1 and 44.0 μg/ml. Latent allotype expression in individual rabbits occurred in a sporadic and transitory fashion. This result concurs with

TABLE I
Expression of Latent Allotypes over a 6-wk Period

Rabbit	Allotype		μg/ml* of latent allotype at week:					
	Nominal	Latent	0	1	2	3	4	5
1	a1	a3	—‡	—	—	0.4	—	0.1
2	a1	a3	—	—	—	0.8	—	0.2
3	a2	a3	—	—	0.5	0.5	3.8	—
4	a3	a2	—	0.2	—	—	—	0.7
5	a1,2	a3	—	—	—	3.5	—	—
6	a1,2	a3	—	2.0	—	—	0.6	—
7	a1,3	a2	0.6	0.7	0.6	0.1	1.0	—

* Concentrations are expressed as μg/ml above the level required to give 10% inhibition of binding.
‡ Level is below the limit of detection.

observations of Bosma and Bosma (5) and suggests that latent allotype expression may be a universal phenomenon. Moreover, the presence of latent allotypes has been demonstrated in a number of isolated IgG fractions, indicating that latent allotypes are present on normal IgG molecules, including induced antibodies (Ref. 6; B. Fraser, M. Mudgett, and T. J. Kindt, unpublished data).

Several explanations may be advanced for the observed deviations of allotypes from allelic behavior. Certain explanations such as cross-reactions among the allotypic specificities and carry over from maternal circulation can be eliminated on the basis of data reported here and by others (6). The possibility of cellular chimerism, resulting from the incorporation by the fetus of allotypically different lymphoid cells from the mother or from littermates, is also minimized by the present results. Latent allotypes in progeny need not be present as nominal allotypes in either parent.

On the other hand, the small fraction of immunoglobulin expressing latent allotypes may result from the presence of small numbers of allotypically different V-region genes. V-region gene complexes with minor allotypic heterogeneity could result from crossovers among multiple germ line genes. While such a model can be easily postulated for the group a allotypes of the V_H region, it does not provide a satisfactory explanation for the reported data showing unexpected expression of C-region allotypes (5, 6).

While additional data are needed to rule out alternative models, the reports of nonallelic behavior of allotypes are consistent with the presence of structural genes for all allotypes in each individual. Expression of nominal allotypes would then be governed by allelic regulator genes, and environmental, metabolic, or experimental perturbations could cause the sporadic appearance of latent allotypes. The basis for selective gene expression in mammals is as yet poorly understood. The latent allotype phenomenon may provide a probe to investigate mechanisms of selective gene expression in immunoglobulin synthesis.

Summary

Group a allotypes not detected by qualitative typing or anticipated from breeding data (latent allotypes) were detected at low levels in 50% of normal rabbit sera tested. The latent allotypes, which were serologically identical to allotypes of pooled IgG, were detected in sera from rabbits with all possible

combinations of group a allotypes and their occurrence in individual rabbits was transitory and sporadic. These findings give reason to question the assumption that the synthesis of immunoglobulin allotypes is directed by allelic structural genes.

The authors thank Dr. W. Carey Hanly for H chain allogroup determinations and Dr. Richard M. Krause for helpful suggestions in preparation of this manuscript.

Received for publication 31 March 1975.

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