ROLE OF IMMUNE RECOGNITION IN LATENT ALLOTYPE INDUCTION AND CLEARANCE

Evidence for an Allotypic Network

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Recent descriptions of genetically unexpected immunoglobulins (Ig) (latent allotypes) in several species (1–5) suggest that the Ig gene complexes of these species contain more genetic information than is normally expressed. The presence of latent genetic information requires a considerable revision in current concepts of the genetic control of Ig expression, and for this reason, alternative interpretations of the data have been sought. However, as characterization has proceeded on latent allotypes, the alternative explanations that have been advanced have become less tenable (6). The most detailed studies have been in the rabbit, where latent allotypes have been firmly established by several laboratories to be serologically and structurally indistinguishable from the nominal allotypes with which they correspond (3, 7–11).

However, in spite of the extensive studies on serum latent allotypes, there are virtually no data available on the physiological aspects of latent allotype expression. For example, nothing is known about the proximal causes of latent allotype synthesis or about the reasons for the rapid, aperiodic fluctuations in serum latent allotype levels that have been noted in many studies.

The present report documents an attempt to determine what factors control the rapid disappearance and reappearance of latent allotypes. In the first series of experiments, clearance rates of passively administered IgG of different allotypes were measured in a number of rabbits. Using a paired label technique, it was shown that foreign allotypes were cleared more rapidly than self allotypes in a few of the rabbits tested, although the rest cleared self and foreign allotypes at identical rates. Rapid clearance of a foreign allotype correlated with a history of expression of that allotype as a latent allotype in the rabbit tested. In the second set of experiments, immunization against antiallotype antibodies markedly increased serum levels of latent allotypes. The data obtained suggest that an allotypic network, similar to an idiotypic network, may exist in rabbits to activate and/or to remove latent allotypes.

Materials and Methods

Rabbits. Most of the rabbits used in this study were bred in the Laboratory of Immunogenetics colony at National Institutes of Health, Bethesda, Md. Randomly bred rabbits, when used, were purchased from Dutchland Laboratories Inc. (Denver, Pa.).

Streptococcal Immunization. Immunization with group C streptoccoccal vaccine was performed according to the procedure described by Krause (12). In a few cases as noted, immunizations

were reduced from three per week to one per week because of adverse reactions to the immunization.

Determination of Allotypes. Group a and b allotypes of rabbits used in this study were determined by an inhibition of binding radioimmunoassay (RIA)[1] using published methodology (13). Latent allotypes were determined by both RIA and hemagglutination as previously described (7).

Isolation and Radioiodination of IgG. IgG fractions were obtained from nonimmune serum by ion exchange chromatography on DEAE-cellulose in 0.02 M potassium phosphate buffer, pH 7.0. Samples to be radioiodinated were dialyzed into glycine-NaOH buffer, pH 8.5, and labeled with either ^{125}I or ^{131}I by the ICl method of McFarlane (14), as follows: labeling solution was prepared by equilibrating ICl in dilute HCl with freshly received ^{125}I or ^{131}I (carrier-free; Amersham Corp., Arlington Heights, Ill.). Labeling solution was added dropwise with vortexing to the IgG solution in an amount sufficient to give equimolar amounts of IgG and ICl.

Labeled samples were immediately desalted on columns of Sephadex G-10 equilibrated in 0.01 M phosphate-buffered saline, pH 7.0, and then ultracentrifuged for 2 h at 35,000 rpm using a Beckman 50 Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.) The uppermost two-thirds of the centrifuged preparation was transferred to a new tube and centrifuged again under the same conditions. The uppermost two-thirds of the solution after the second spin was used immediately for the clearance studies. The absence of high molecular weight complexes in the final supernate was verified by gel permeation chromatography. For each radiolabeled preparation, only a single (7S) peak was obtained after chromatography on Sephadex G-200.

Determination of Rates of Clearance for Radioiodinated IgG. Rabbits to be used in the clearance studies were housed in metabolic cages in a room maintained at 70°F and given drinking water containing NaI for 1 wk before and throughout the course of the experiment. All animals included in this report were in good health throughout the experiment as judged by general appearance, appropriate consumption of food and water, constant weight, and normal values for urinary output, hematocrit, and total serum protein concentration. An air-conditioning failure occurred during experiment 1, resulting in fluctuating temperatures of 80–90°F during the experiment. The stress of these conditions produced only transient changes in the physical condition of most of the rabbits, but was reflected in a decreased rate of clearance (Results). Rabbits that were more seriously affected were removed from the study.

Each rabbit received ^{125}I-IgG of one allotype and ^{131}I-IgG of another allotype. One of the IgG preparations was fully matched to the allotypes of the recipient whereas the other was unmatched in either the group a or group b allotype, but not in both. In experiment 1, four a1a3 heterozygous rabbits received paired a1 and a3 IgG preparations as a control.

For each experiment, the radiolabeled IgG preparations were mixed, and an amount containing 500 μg of each preparation was injected into each recipient via the left marginal ear vein. A small bleeding (1–2 ml) was obtained 10 min later from the right ear to determine the zero-time level of radioactivity. Subsequent bleedings were obtained every 48 h for 2 wk. Serum was isolated from the blood samples, and ^{125}I and ^{131}I were determined in a Beckman Gamma 9000 spectrometer (Beckman Instruments, Inc.) Appropriate background, ^{125}I-^{131}I overlap, and isotope half-life corrections were made to obtain normalized values for ^{125}I and ^{131}I from which rates of clearance could be determined.

All clearance curves showed an initial rapid fall in radioactivity, caused largely by equilibration of the IgG between intravascular and extravascular fluid (15). From day 6 (day 4 in many rabbits) through day 14, clearance data could be fit to a first-order exponential decay curve with a high correlation coefficient. The half-life of this decay, which is the value reported in Table I, was determined by least squares analysis of the linear portion of the plot (Fig. 1) of log percent radioiodine remaining vs. time.

Immunization with Antiallotype Antibodies. Antiallotype antibodies were isolated by immunoadsorbent chromatography on IgG columns of the appropriate allotype. Bound antibodies were eluted with 3 M NH_4SCN in 0.01 M phosphate-buffered saline, pH 7.0, and desalted on columns of Sephadex G-10. Isolated antibodies were lightly cross-linked with glutaraldehyde, and 1 mg of cross-linked antibody was injected subscapularly each month for 3 consecutive mo.

Streptococcal Immunization after Immunization with Antiallotype Antibodies. Rabbits were rested 3

[1] *Abbreviations used in this paper:* PFC, plaque-forming cell; RIA, radioimmunoassay.

TABLE I

Clearance of IgG of Different Allotypes

	Rabbit	Allotype	$t_{1/2}$				Accelerated clearance*
				d			
Experiment 1‡			a1	a3			
	5	1, 4	9.05	5.17			×
	B261	1, 4	8.42	9.09			
	2567	1, 4	7.75	8.19			
	5471	1, 4	7.81	7.81			
	5446	1, 3, 4	10.23	10.70			
	5480	1, 3, 4	6.53	6.79			
	5481	1, 3, 4	10.73	10.98			
	5486	1, 3, 4	9.81	10.28			
	23	3, 4	3.16	11.39			×
	25	3, 4	7.86	7.69			
	5436	3, 4	7.27	9.28			×
	5483	3, 4	8.20	8.42			
	B279	3, 4	9.85	10.24			
Experiment 2§			a2	a3			
	5451	2, 4, 5	5.02	5.22			
	5453	2, 5	5.49	3.93			×
	5454	2, 5	5.39	5.36			
	5455	2, 4, 5	6.79	7.09			
	5456	2, 4, 5	5.57	5.60			
	5432	3, 4	4.56	7.00			×
	5437	3, 4	6.03	7.35			
	5440	3, 4	6.99	7.35			
	5441	3, 4	4.82	8.11			×
Experiment 3‖			a1	a2	b4	b5	
	5	1, 4	6.62	3.79			×
	23	3, 4	2.64	7.88			×
	5432	3, 4			7.30	4.66	×
	5436	3, 4			6.06	6.21	
	5441	3, 4			7.90	5.03	×
	5453	2, 5			4.21	6.69	×
	5454	2, 5			5.57	5.60	

* Criteria for accelerated clearance: >20% difference in paired isotope clearance rates with faster clearance rate outside of the range of control rates measured in the experiment.

‡ IgG donors: (1, 4)-B261; (3, 4)-B279.

§ IgG donors: (2, 4)-5343; (3, 4)-5440; (2, 5)-5454; (3, 5)-21976.

‖ IgG donors: (1, 4)-5; (3, 4)-5432; (2, 4)-5343; (2, 5)-5454; (3, 5)-21976.

mo after the last antiallotype antibody injection, then immunized with group C streptococcal vaccine. Rabbits 4805, 4806, and 4809 received normal injections (three per week) whereas rabbits 5455 and 5456 received 1 ml of vaccine once a week for 3 wk.

Antistreptococcal antibodies were isolated from immune sera on an immunoabsorbent containing *p*-aminophenyl-*N*-acetylgalactosamine coupled to Sepharose 2B. Bound antibodies were eluted with 0.5% *N*-acetylgalactosamine in 2 M NaCl, desalted, and passed through a column of IgG containing all allotypes except the one to be determined in the latent allotype assay.

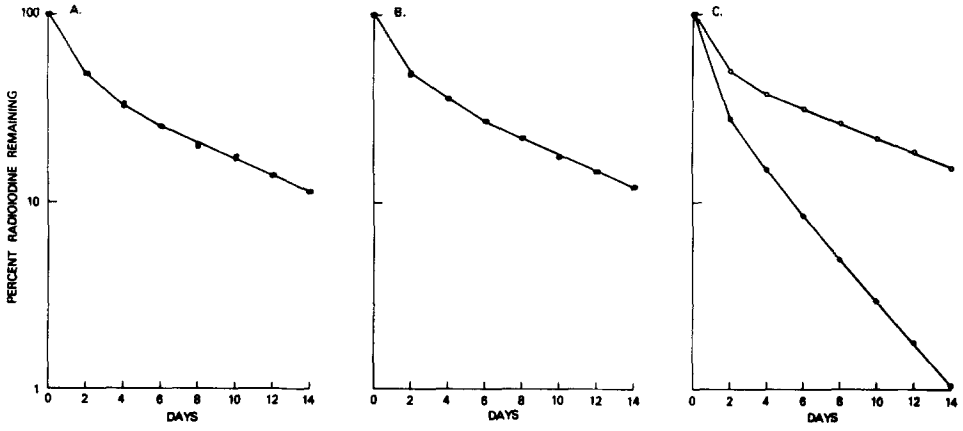

FIG. 1. Clearance of IgG by: A, 5480 (1, 3, 4) clearing 1, 4 (●) and 3, 4 (○) IgG; B, 5455 (2, 4, 5) clearing 2, 4 (●) and 3, 4 (○) IgG; C, 23 (3, 4) clearing 1, 4 (●) and 3, 4 (○) IgG.

Results

The rate of clearance of IgG was determined using freshly isolated IgG preparations lightly iodinated by the ICl method and freed from aggregated material by high-speed centrifugation. Preliminary experiments demonstrated considerable variation in rates of clearance among rabbits tested, so allotype-related clearance was examined by the paired label technique, using ^{125}I-labeled IgG of one allotype and ^{131}I-labeled IgG of another allotype. The three types of results obtained are shown in Fig. 1. When rabbits heterozygous for the allotypes in question were examined (panel A), the clearance rates of the two IgG preparations were invariably identical, within the limits of experimental error. Similarly, most homozygotes examined showed identical rates of clearance (panel B) for IgG of the self allotype and IgG of the foreign allotype. However, certain homozygous rabbits gave results that deviated from this pattern of equal clearance rates (panel C). In all cases of differential clearance, the foreign allotype was cleared more rapidly than the self allotype. Beyond 4 d, clearance curves (for normal or accelerated clearance) always followed first-order kinetics, with no indication of nonfirst-order clearance processes such as primary sensitization.

A summary of data from the three clearance experiments performed is shown in Table I. Mean half-lives of clearance, calculated on the basis of the self allotypes only, were 9.1, 6.5, and 6.9 d, for the three experiments respectively. The high value for experiment 1 was obtained under unfavorable environmental conditions (Materials and Methods). 3 of 10 rabbits in experiment 1 and 3 of 9 rabbits in experiment 2 cleared IgG of the foreign allotype at a rate significantly higher than that of the paired control sample. In these experiments the variable marker was the group a allotype. In experiment 3, two rabbits (5 and 23) from experiment 1 that cleared the foreign group a allotype rapidly, were retested with new preparations of the same allotypes. These rabbits again cleared the foreign allotype more rapidly. Four rabbits that had previously cleared the foreign group a allotype rapidly (5432, 5436, 5441, 5453) and one that did not (5454) were also tested in experiment 3 for clearance of a self and foreign group b allotype. Three of the five showed abnormally rapid clearance of the foreign group b allotype.

All rabbits that showed evidence of allotype-associated rapid clearance were tested

in order to determine whether the rapidly cleared foreign allotype were present as a latent allotype in serum. Three rabbits that did not show allotype-related rapid clearance were tested as controls. The results of these assays are shown in Table II. Four random bleedings, taken within a 6-mo period before the clearance experiments, and six weekly bleedings, taken starting 1 mo after the end of the clearance experiments, were tested. A high percentage of bleedings (24/36 for preclearance bleedings and 36/54 for postclearance bleedings) from rabbits showing rapid clearance of a foreign allotype had readily measurable levels of that (latent) allotype in the serum. From three control rabbits similarly monitored, only one of 24 bleedings contained any latent allotype.

Latent Allotype Induction by Immunization with Antiallotype Antibodies. In view of the striking correlation between latent allotype expression and abnormal clearance of the allotype, the effect of immunization with antiallotype antibodies on latent allotype expression was investigated in five rabbits. Three received anti-a2 antibody and two received anti-b6 antibody. The antiallotype antibodies were allotype matched to the recipients. Each rabbit was immunized three times at monthly intervals with 1-mg doses of affinity-purified antiallotype antibody. After a 3-mo rest period, the rabbits were immunized with streptococcal vaccine. Latent allotype levels were measured in bleedings taken before and after immunization as well as in affinity-purified antistreptococcal antibody fractions from immune bleedings. The allotype against which the immunizing antiallotype antibodies were directed was present in high levels in the immunized animals, particularly in the antistreptococcal antibody fractions (Tables III and IV).

Because anti-antiallotype antibodies would mimic allotype in the latent allotype assay, the contribution of such antibodies to measured latent allotype levels was determined by preadsorption of serum with insolubilized antiallotype reagents di-

TABLE II

Latent Allotypes in Bleedings Before and After the Clearance Experiment in Rabbits Showing Accelerated Clearance

Rabbit	Latent allotype	Preclearance*				Postclearance‡					
		1	2	3	4	1	2	3	4	5	6
						μg/ml					
5432	a2	2.9	—	2.6	2.6	—	3.1	1.7	2.7	—	5.3
	b5	—	—	3.7	3.1	3.0	6.2	6.1	4.0	—	2.3
5436	a1	—	—	4.0	4.0	1.3	—	—	2.4	1.7	—
5441	a2	4.0	—	—	3.0	3.4	1.8	2.8	—	1.5	1.9
	b5	2.1	1.5	1.7	4.2	—	4.0	4.7	3.0	—	—
5453	a3	1.3	4.1	—	2.2	—	3.8	3.4	6.6	8.2	—
	b4	—	11.2	4.8	1.2	5.4	—	3.9	5.7	—	5.1
5	a1	1.7	—	1.9	—	2.2	1.9	—	—	2.8	3.7
23	a3	3.3	3.8	2.0	—	2.4	—	—	2.0	4.0	3.1
5455§	a3	—	—	—	—	—	7.0	—	—	—	
5456§	a3	—	—	—	—	—	—	—	—	—	
6§	a3	—	—	—	—	—	—	—			

* Random bleedings taken within 6-mo period.

‡ Weekly bleedings starting 1 mo after clearance except for number 6 where bleeds were taken 2 wk after clearance.

§ Control rabbits not showing accelerated clearance.

TABLE III

Latent Allotype Induction by Antiallotype Antibody Immunization

Experimental stage	Bleeding number	Latent a2 in rabbits immunized against anti-a2 and numbered:			Latent b6 in rabbits immunized against anti-b6 and numbered:	
		4805	4806	4809	5455	5456
				μg/ml		
Preimmune	1	0	0	0	1.4	0
	2	ND	ND	ND	3.1	0
	3	ND	ND	ND	2.5	0
Primary antistreptococcal immunization	4	2.4	0	0	0	0
	5	0	2.1	0	0	0
	6	1.7	0	0	2.6	0
Postimmune	7	3.0	1.9	2.2	0	1.6
	8	2.0	2.8	0	0	0
Antiallotype immunization	9	3.1	4.1	2.3	2.3	2.0
	10	4.9	3.9	4.6	6.6	3.7
	11	7.0	6.8	5.9	5.7	5.7
	12	10.8	10.2	9.9	9.4	4.7
	13	12.1	8.9	7.1	8.1	7.1
Secondary antistreptococcal immunization	14	54.2	31.7	18.6	28.0	17.5
	15	59.0	29.4	ND	19.6	17.8
	16	32.8	ND	ND	23.2	9.3
Postimmune	17	19.0	ND	ND	9.0	7.1

ND, not done.

TABLE IV

Maximum Latent Allotype Concentration Observed During Different Stages of the Experiment

Experimental stage	Latent a2 in rabbits immunized against anti-a2 and numbered:			Latent b6 in rabbits immunized against anti-b6 and numbered:	
	4805	4806	4809	5455	5456
			μg/ml		
Before antiallotype immunization					
Bleedings lacking antistreptococcal antibody	3.0	2.8	2.2	3.1	1.6
Antistreptococcal antibody	2.4	2.1	0	2.6	0
After antiallotype immunization					
Bleedings lacking antistreptococcal antibody	12.1	10.2	9.9	9.4	7.1
Antistreptococcal antibody	59.0	31.7	18.6	28.0	17.8

rected against the nominal allotypes. These would remove anti-antiallotype antibodies and other Ig with nominal allotypes without affecting latent allotypes. In this way, it could be determined that anti-antiallotype antibodies were present neither in bleedings taken before immunization with antiallotype antibodies nor in purified antistrep-

tococcal antibody fractions, but were present in variable amounts in normal bleedings taken after antiallotype immunization.

Discussion

The present report documents a phenomenon of allotype-associated rapid clearance of radiolabeled IgG from serum. It was shown that although the majority of rabbits tested cleared lightly iodinated IgG bearing a foreign allotype at the same rate as they clear labeled IgG bearing a self allotype, certain rabbits showed enhanced clearance of the foreign allotype. No cases were observed in which the self allotype was cleared at an accelerated rate. In each case in which accelerated clearance was documented, the rapidly cleared allotype appeared as a latent allotype in the majority of serum samples obtained both before and after the clearance experiment.

In an extension of these experiments, the effect on latent allotype expression of immunization with purified antiallotype antibodies directed against the latent allotype was examined. It was shown that such immunization in each case greatly enhanced the serum levels of the (latent) allotype against which the antiallotype antibodies were directed.

Before discussing possible interpretations for these results certain details of the experimental procedures will be reviewed. The validity of the results depends strongly on proper experimental design, particularly in the determination of clearance rates for IgG and in the measurement of latent allotypes. The serologic detection of latent allotypes requires careful attention to details in the preparation of antisera and samples to be tested. The protocol used has been described thoroughly in another publication (7), and all of the experimental considerations have been reviewed recently (6). Clearance experiments are subject to a number of potential artifacts (15), and considerable attention was devoted to eliminating all such problems. First, the use of paired labels eliminates the problem of variability in the animal population because each animal is simultaneously tested for clearance of allotype-matched and allotype-unmatched IgG. Second, the iodinated IgG samples injected were prepared and given in a manner appropriate to eliminate artifacts caused by denaturation and aggregation. Iodinations were done so as to introduce an average of less than one atom of iodine per molecule of IgG. All iodinated IgG preparations were ultracentrifuged twice immediately before administration to eliminate any molecules greater than 7S in size. Sephadex G-200 chromatography verified the absence of macroglobulins or aggregated material. The dose injected was kept low to minimize the possibility of sensitization and to simulate observed levels of latent allotypes. Rabbits were given NaI in the drinking water to prevent iodine scavenging and reutilization.

Under these conditions, the average clearance rate for iodinated IgG with self allotypes was 6.6 d in experiments 2 and 3, with a range of 5.0–8.1 d. This number compares well with published values for IgG clearance in the rabbit, which range from 5.7 to about 8 d (16–20). The clearance rate observed in experiment 1 (mean 9.1, range 7.7–11.4 d) is clearly unusual and appeared to be caused by temporary, unfavorable environmental conditions in the animal room, as discussed in Materials and Methods. The unusual environmental conditions, however, altered only the absolute clearance rates, without distorting the relative clearance rates of the paired labels.

Analysis of the results of the clearance experiments suggests first that the increased

rates of clearance observed in some rabbits is causally related to recognition of foreign allotypic determinants and, second, that the recognition reflects previous sensitization of either the cellular or humoral immune system by autologous Ig bearing latent allotypes. A number of points of evidence support each of these conclusions.

That accelerated clearance is allotype mediated is most immediately suggested by the fact that it was observed only for IgG of a foreign allotype, never a self allotype. For every rabbit that cleared an IgG preparation at an unusually rapid rate, there were several rabbits that cleared the identical preparation at a normal rate. This is sufficient proof to rule out degradation, improper radioiodination, or other sorts of denaturation as the cause of the abnormal clearance. Each rabbit cleared the self allotype at a normal rate, so a nonspecific process can be ruled out. The possibility that unknown idiotypic, subclass, or other nonallotypic differences might underlie the accelerated clearance is unlikely based on all that is known about rabbit IgG, but it is almost completely excluded by the repeat determination done with rabbits 5 and 23. These rabbits showed the same allotype-specific clearance in two experiments with two IgG preparations obtained from unrelated donors. It is of further note that those rabbits that rapidly cleared IgG of a given allotype invariably had an unusually high incidence of the cleared allotype as a latent allotype in serum samples obtained both before and after the experiment.

That the allotype-specific clearance observed is caused by a prior autosensitization rather than by primary sensitization during the experiment is established by several independent considerations. First, the relatively low dose, the lack of aggregates, and the route of administration make the test preparation an unlikely immunogen. Second, if sensitization were occurring, it would be expected to occur in a higher percentage of recipients. Third, primary sensitization leads to non-first-order kinetics, with a sharp increase in the rate of clearance at 7–10 d (21). Fourth, the repeat experiment with rabbits 5 and 23 showed no increase in relative clearance of self and foreign allotypes.

The mechanism of the accelerated clearance could not be determined; it may have been mediated by antiallotype antibodies or by allotype-specific cells. Hemagglutination assays for serum antiallotype antibodies were uniformly negative, but this does not exclude their involvement. In a preliminary experiment, an a2a3 rabbit was immunized once with a 1-mg dose of a1 IgG in complete Freund's adjuvant. At a time after the immunization when anti-a1 antibodies were barely detectable, a1 IgG was cleared so rapidly that the half-life could not be accurately measured. Thus, the modest rates of clearance observed in the present study would, if mediated by antiallotype antibodies, be compatible with undetectable levels of these antibodies.

In summary, the rabbits that showed accelerated clearance appear to have been previously sensitized to mount a cellular and/or humoral immune response to the allotype that was rapidly cleared. Because the rabbits were raised in the laboratory and had no previous experimental exposure to protein antigens, nor had any of them been bred, it seems most likely that autologous latent allotypes were the source of sensitization.

If latent allotypes are immunogenic, then serum latent allotypes may, in fact, constitute the "tip of an iceberg" (22), because active allotype-specific suppression may reduce the levels of many genetically possible latent allotypes to undetectable levels. The results obtained after immunization with antiallotype antibodies suggest

very strongly that this is true—that rabbits have the genetic information required to synthesize most, if not all, allotypic specificities.

The network concepts of Jerne (23) and experiments on network interactions in the control of idiotype expression in the rabbit provide a framework for interpreting the results obtained after immunizing rabbits with purified, allotype-matched antiallotype antibodies. It is clear that antiallotype antibodies are restricted in heterogeneity (24), and they have recently been shown to be idiotypically restricted as well (25, 26). Thus, the immunization would reasonably be expected to lead to antiidiotypic antibodies against the antiallotypic antibodies. The observed sharp increase in latent allotype levels attendant upon the immunization suggests that the antiidiotype response relieves an antiallotype-mediated suppression, which is important in suppressing latent allotype expression. Such a network of interactions has been shown by idiotypic analysis of antibodies of a variety of specificities by Urbain, et al. (27) and Yarmush and Kindt (28).

Given the ever increasing mass of data supporting a functional network of idiotypes in the immune system, it is only a modest extension to suggest that network interactions control expression of latent allotypes. Latent allotypes have in common with idiotypes that they are immunologically recognizable self constituents, which are not routinely expressed and thus circumvent the usual mechanisms for induction of tolerance of self. What is surprising, if this speculative interpretation of our data is valid, is that it suggests a wider potential for latent allotype production than has been implied by analysis of allotypes in serum. All five rabbits immunized with antiallotype antibodies produced relatively large amounts of IgG bearing a randomly chosen latent allotype. It is particularly noteworthy that latent b6 appeared in both rabbits injected with anti-b6 antibodies, because latent b6 is the least frequent latent allotype in our colony.

The potentially widespread, if not universal, ability to make latent allotypes is also suggested by the work of McCartney-Francis and Mandy (29), who have reported induction of latent allotypes in vitro by treating spleen cell cultures with lipopolysaccharide and antiallotype serum directed against a nominal allotype. Under these conditions, expression of the nominal allotype was suppressed, and plaque-forming cells (PFC) were induced with the allotype of the antiallotype serum. Thus, treatment of a spleen culture from a b4 rabbit with b5 anti-b4 suppressed b4 PFC but led to the appearance of large numbers of b5 PFC.

Further work will be necessary to establish whether latent allotypes are under cellular or humoral antiallotype control and to what extent that control can be overcome. The immunization procedure reported here offers a means for the routine induction of latent allotypes. Such a method would permit a rapid resolution of questions concerning the distribution, control of expression, and genetic significance of latent allotypes.

Summary

The role of allotype recognition in the regulation of the expression of latent allotypes has been investigated in two series of experiments. The first experiments were designed to investigate the apparent instability of latent allotypes in circulation. In these experiments, clearance rates of IgG preparations bearing allotypes matched and unmatched to the recipient were examined. In all cases, iodinated IgG matched

in allotype to the recipient was cleared at a normal rate from the serum. However, in several cases, iodinated IgG of an unmatched allotype was cleared at a rate and in a manner suggesting prior sensitization of the recipient to IgG of that allotype. Such apparent sensitization correlated with the presence of the foreign allotypes as a latent allotype in several bleedings taken both before and after the clearance experiment.

In the second series of experiments, designed to test the ability of antiallotype antibodies to affect the expression of latent allotypes, five rabbits were immunized first with purified antiallotype antibodies and then after 3–4 mo, with streptococcal vaccine. Examination of the antistreptococcal antibodies for latent allotype revealed, in all cases, that the allotype against which the antiallotype antibodies were directed was present in levels 8- to 20-fold greater than were observed before the antiallotype injections.

These results indicate that recognition of allotypic determinants is an important element in the control of latent allotype expression and suggest the existence of a regulatory network involving antiallotype antibodies.

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References

1. Lobb, N., C. C. Curtain, and C. Kidson. 1967. Regulatory genes controlling the synthesis of H chains of human immunoglobulin-G. *Nature (Lond.).* **214:**783.
2. Bosma, M. J., and G. C. Bosma. 1974. Congenic mouse strains: the expression of a hidden immunoglobulin allotype in a congenic partner strain of BALB/c mice. *J. Exp. Med.* **139:** 512.
3. Strosberg, A. D., C. Hamers-Casterman, W. Van der Loo, and R. Hamers. 1974. A rabbit with the allotypic phenotype: a1a2a3 b4b5b6. *J. Immunol.* **113:**1313.
4. Foppoli, J. M., E. K. Wakeland, and A. A. Benedict. 1978. Structural and genetic studies on chicken 7S immunoglobulin allotypes. IV. The presence of an unexpected chicken immunoglobulin heavy chain allotype: subclass or pseudoallele? *J. Immunol.* **120:**812.
5. Hunt, L., and S. Duvall. 1976. Rat immunoglobulin allotypes: expression by thymus-independent cells. *Biochem. Soc. Trans.* **4:**39.
6. Kindt, T. J., and M. L. Yarmush. Expression of latent immunoglobulin allotypes and alien histocompatibility antigens: relevance to models of eukaryotic gene regulation. *CRC Crit. Rev. Immunol.* In press.
7. Mudgett, M., B. A. Fraser, and T. J. Kindt. 1975. Nonallelic behavior of rabbit variable-region allotypes. *J. Exp. Med.* **141:**1448.
8. Mudgett-Hunter, M., M. L. Yarmush, B. A. Fraser, and T. J. Kindt. 1978. Rabbit latent group a allotypes: characterization and relationship to nominal group a allotypic specificities. *J. Immunol.* **121:**1132.
9. McCartney-Francis, N., and W. J. Mandy. 1979. Serological and chemical studies of latent allotypes in the rabbit. *Ann. Immunol. (Paris).* **130C:**115.
10. Wolf, B., R. Urban, A. B. Miller, E. S. Kimball, M. Mudgett, D. Catty, and J. Daneman. 1979. Nonallelic inheritance of V region group *a* allotypes: cell surface and serum studies in double and triple expressing rabbits. *J. Immunol.* **123:**1858.
11. Yarmush, M. L., H. C. Krutzsch, and T. J. Kindt. 1980. Amino acid sequence analysis of

immunoglobulin light chains by gas chromatographic-mass spectrometric techniques: structural identity of nomal and latent b9 molecules. *Mol. Immunol.* **17:**319.

12. Krause, R. M. 1970. The search for antibodies with molecular uniformity. *Adv. Immunol.* **12:** 1.
13. Gottlieb, A. B., R. K. Seide, and T. J. Kindt. 1975. Quantitative determination of allotypic and idiotypic markers using antisera coupled to *N*-hydroxysuccinimide-activated Sepharose. *J. Immunol.* **114:**51.
14. McFarlane, A. S. 1958. Efficient trace labelling of proteins with iodine. *Nature (Lond.).* **182:** 53.
15. Waldmann, T., and S. Strober. 1969. Metabolism of immunoglobulins. *Prog. Allergy.* **13:**1.
16. Dixon, F. J., D. W. Talmage, P. H. Maurer, and M. Deichmiller. 1952. The half-life of homologous γ-globulin (antibody) in several species. *J. Exp. Med.* **96:**313.
17. Taliaferro, W. H., and D. W. Talmage. 1956. Antibodies in the rabbit with different rates of metabolic decay. *J. Infect. Dis.* **99:**21.
18. Svehag, S. E., and B. Mandel. 1964. The formation and properties of poliovirus neutralizing antibody. *J. Exp. Med.* **119:**1.
19. Spiegelberg, H. L., and W. O. Weigle. 1965. The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. Exp. Med.* **121:**323.
20. Spiegelberg, H. L., and W. O. Weigle. 1965. Studies on the catabolism of γ-G and subunits and chains. *J. Immunol.* **95:**1034.
21. Glenny, A. T., and B. E. Hopkins. 1922. Duration of passive immunity. *J. Hyg.* **21:**142.
22. Strosberg, A. D. 1977. Multiple expression of rabbit allotypes: the tip of the iceberg? *Immunogenetics.* **4:**499.
23. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris).* **125:**373.
24. Aasted, B., J. A. Sogn, and T. J. Kindt. 1976. Isolation and characterization of rabbit antibodies directed against group a allotypic determinants. *J. Immunol.* **116:**387.
25. Roland, T., and P. A. Cazenave. 1979. Mise en evidence d'idiotypes anti-b4 apparentes cez les lapins de phenotype $a3^+$ immunises contre l'allotype b4. *C. R. Acad. Sci. (Paris).* **288:** 571.
26. Gilman-Sachs, A., S. Dray, and W. J. Horng. 1980. A common idiotypic specificity of rabbit antibodies to the a2 allotypes of the immunoglobulin heavy chain variable region. *J. Immunol.* **125:**96.
27. Urbain, J., C. Wuilmant, and P. A. Cazenave. Idiotypic regulation in immune networks. *Contemp. Top. Mol. Immunol.* In press.
28. Yarmush, M. L., and T. J. Kindt. 1979. Idiotypes of rabbit antistreptococcal antibodies: probes for inheritance and immune regulation. *In* Immunology of Bacterial Polysaccharides. A. Rudbach and P. Baker, editors. Elsevier North-Holland, Inc., New York.
29. McCartney-Francis, N., and W. J. Mandy. 1980. Control of latent allotype expression by rabbit splenocytes. *Fed. Proc.* **38:**357. (Abstr. 460)