THE NATURE OF ANTIIDIOTYPE MOLECULES INDUCED BY ANTIALLOTYPE

Presence of Both Latent Allotype and Allotypic Internal Images

By DENNIS W. METZGER

From the Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

It has been shown that Ig idiotypes (Id)[1] and allotypes can serve as important recognition sites for immunoregulatory networks. Of particular interest are the findings that rabbit antiallotype antibodies display predominant Id detectable with homologous anti-Id antisera (1–4). However, the existence of these Id and their possible role in regulating allotype expression remains unclear. Recent reports have shown that immunization with antiallotype leads to the production, in some cases, of normally hidden, latent allotypes (5, 6), and in other cases, of allotype "internal images" (7, 8).

Work in my laboratory has focused on the allotypic markers present on rabbit variable heavy chain (V_H) regions. In the normal rabbit, 70–90% of Ig molecules express determinants that are encoded by the *a* subgroup locus (a1, a2, a3) and that appear to be inherited in a Mendelian fashion. My coworker and I have previously shown (9, 10) that virtually all anti-a1 antibody obtained from various species reacts with a rabbit anti-Id reagent. These results were taken to indicate the presence of a1-like epitopes within the anti-Id preparation; however, the exact nature of these a1 epitopes was unknown.

In this report, I present evidence that demonstrates the existence of two types of anti-Id molecules, each of which appears to express a1-like determinants. The serological evidence is consistent with the simultaneous induction of both latent a1 Ig and a1-like internal images by antiallotype immunization.

Materials and Methods

Animals. The rabbits used in this study were derived from the allotype-pedigreed colony maintained at St. Jude Children's Research Hospital.

Allotypic and Antiallotypic Reagents. The Ig fractions of normal rabbit sera were obtained by three sequential precipitations in 50% ammonium sulfate followed by extensive dialysis against physiologic saline. Conventional rabbit anti-V_Ha1 antiserum was prepared against a1 Ig as previously described (3). Production of the 3-2F1 monoclonal antibody (mAb) specific for a common a1 allotype has also been previously described (9). Goat anti-a1 antibody was obtained from an animal that was hyperimmunized with rabbit

This work was supported in part by grants AI 18880 and CA 21765 from the National Institutes of Health, Bethesda, MD, and by the American, Lebanese, Syrian Associated Charities.

[1] *Abbreviations used in this paper:* C, constant region of Ig; H, heavy chain of Ig; Id, idiotype; L, light chain of Ig; mAb, monoclonal antibody; RIA, radioimmunoassay; KLH, keyhole limpet hemocyanin; SRBC, sheep red blood cell; V, variable region of Ig.

a1 Fab fragments. This antiserum was exhaustively absorbed by passage over a Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) column containing pooled $a1^-$ rabbit Ig, and specificity for the a1 allotype was confirmed by radioimmunoassay (RIA) and by hemagglutination of Ig-coupled sheep red blood cells (SRBC).

For isolation of rabbit and goat anti-a1 antibody, antisera were applied to a1-coupled columns, and bound antibody was eluted with 0.2 M glycine sulfate buffer, pH 2.3. The 3-2F1 mAb was isolated from ascites fluid in a similar manner, except that 6.0 M guanidine-HCl buffer, pH 7.5, was used for elution. All antibodies were dialyzed against physiologic saline before use.

Preparation of Anti-Id. Anti-Id was obtained from nominal a2a3 rabbits that had been injected with allotype-matched anti-a1 conjugated to keyhole limpet hemocyanin (KLH) (Calbiochem-Behring, San Diego, CA), and was affinity-purified by elution from an anti-a1-coupled column as previously described (3). The population of anti-Id not expressing the a2 or a3 allotypes ($a2a3^-$ anti-Id) was prepared by passage over a column containing rabbit anti-a2 and anti-a3 antibody. A second anti-Id fraction expressing the a2 and a3 allotypes ($a2a3^+$ anti-Id) was obtained by elution of this column with 0.2 M glycine sulfate buffer, pH 2.3.

Competitive RIA for Allotype Binding. Direct binding of a1 Ig to various anti-a1 preparations was assessed in a solid-phase RIA in which polyvinyl microtiter wells (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 50 μl/well of 100 μg/ml affinity-purified anti-a1 at 4°C overnight. Unbound antibody was recovered, and the plates were washed three times with 1% (wt/vol) bovine serum albumin (Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline. To each well, I then added 50 μl of inhibitor, together with 25 μl (32 ng) of ^{125}I-labeled a1 Ig (10^5 cpm). The plates were incubated at room temperature for 2 h, the wells washed 10 times with tap water, and the amount of bound ^{125}I-a1 Ig was determined using a Packard γ-counter.

Production of Anti-(anti-Id). Isolated $a2a3^-$ anti-Id and $a2a3^+$ anti-Id were each coupled to KLH using glutaraldehyde. Normal a2a3 rabbits were primed subcutaneously and intramuscularly with 0.5 mg of the anti-Id fractions emulsified in complete Freund's adjuvant, followed by monthly boosting with 0.5 mg in incomplete Freund's adjuvant. The resulting antisera were passed over immunoadsorbent columns containing a1 Ig, and the bound antibodies were eluted with 0.2 M glycine sulfate buffer, pH 2.3.

Hemagglutination Assays. Hemagglutination of Ig-coupled SRBC was performed as previously described (11). Inhibition of hemagglutination for the expression of *d* and *e* heavy chain constant region (C_H) allotypes was kindly performed by Dr. Rose G. Mage (National Institutes of Health, Bethesda, MD), essentially as described by Dubiski (12).

Isolation of Anti-Id H and L (Light) Chains. Anti-Id molecules were reduced with 0.02 M dithiothreitol (Sigma Chemical Co.) and alkylated with 0.05 M iodoacetamide (Sigma Chemical Co.) (13). The H and L chains were separated on a Sephacryl S-200 (Pharmacia Fine Chemicals) column equilibrated with 1.0 M acetic acid, and dialyzed into physiologic saline. The purity of the isolated H and L chain preparations was confirmed by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Binding of ^{125}I-labeled rabbit anti-a1 and of ^{125}I-goat anti–rabbit Ig was tested by solid-phase RIA as described above, using microtiter wells that were coated with 100 μl/well of 20 μg/ml isolated H and L chains at 4°C overnight.

Sequential Absorptions of a1 Ig. Anti-a1 antibody preparations bound to Sepharose 4B beads were incubated with ^{125}I-a1 Ig at room temperature overnight with continuous rocking. The mixtures were centrifuged, and the pellets were washed and counted. The supernatant fluids were transferred to tubes containing the second insolubilized antibody preparation, and mixed at room temperature for 5 h. The pellets were then counted for additional binding of radiolabel.

Results

Isolation of Two Anti-Id Populations. My coworker and I have previously shown (9, 10) that immunization of a3 rabbits with homologous anti-a1 antibody induces

the production of anti-Id molecules that appear to bear epitopes of the original antigen, i.e., the V_Ha1 allotype. To determine whether these a1 epitopes were present as internal images that resemble a1 or as actual latent a1 Ig, I tested for expression of the nominal V_H allotypes (a2 and a3) of the rabbit from which the anti-Id was obtained. The rationale for this experiment was that since internal images would be encoded by the antigen-combining site, molecules expressing such images could also bear a2 or a3; on the other hand, latent a1 Ig, encoded by the allotypic V_H framework regions, would lack these markers. Four consecutive rabbits of nominal a2a2, a3a3, or a2a3 phenotype were immunized with allotype-matched anti-a1. Each anti-Id preparation was affinity-purified; the purified molecules were then fractionated by passage over an immunoadsorbent column containing rabbit anti-a2 and anti-a3. This procedure thus allowed us to distinguish between $a2a3^-$ anti-Id (putative latent a1 Ig) and $a2a3^+$ anti-Id (putative a1-like internal images). In fact, as shown in Table I, each of the four immunized rabbits produced both $a2a3^-$ and $a2a3^+$ anti-Id molecules. The yield of anti-Id ranged from 73 to 533 μg/ml serum, the majority of which, in each case, did not express a2 or a3 allotypes.

Binding Properties of the $a2a3^-$ and $a2a3^+$ Anti-Id Fractions. To assess the activities of the $a2a3^-$ and $a2a3^+$ anti-Id fractions, and to test for the presence of a1 epitopes within each fraction, a solid-phase RIA was performed. In this assay, the binding of ^{125}I-a1 Ig to microtiter plates coated with anti-a1 antibody obtained from various species was inhibited by varying amounts of $a2a3^-$ anti-Id or $a2a3^+$ anti-Id. As shown in Fig. 1, each anti-Id population completely inhibited the binding of a1 Ig to rabbit, goat, or mouse mAb anti-a1. These results demonstrate not only that both fractions possess anti-Id activity, but also, based upon the binding to heterologous anti-a1, that each fraction appears to express a1 epitopes.

Immunization of Rabbits with $a2a3^-$ Anti-Id and $a2a3^+$ Anti-Id. To further confirm the presence of a1 epitopes within the anti-Id fractions, each anti-Id population was tested for the ability to induce anti-a1 in normal a2a3 rabbits. Rabbit 867 was injected with $a2a3^-$ anti-Id, and rabbit 3103 was injected with $a2a3^+$ anti-Id. As shown in Table II, both rabbits showed specific hemagglutinating activity in their sera for a1 Ig-coupled SRBC after three injections of anti-Id.

The reactivity of these anti-a1 preparations with each of the anti-Id fractions

TABLE I

Frequency of $a2a3^+$ and $a2a3^-$ Molecules in Anti-Id Preparations

Rabbit	Allotypic phenotype	$a2a3^+$ anti-Id (nominal allotype)	$a2a3^-$ anti-Id (unknown allotype)
		%	
1096	a3a3	27	73
1182	a2a3	48	52
1184	a2a3	32	68
4015	a2a2	20	80

The yield of affinity-purified anti-Id was 253 μg/ml from rabbit 1096, 103 μg/ml from 1182, 73 μg/ml from 1184, and 533 μg/ml from 4015.

TABLE II

Induction of Anti-a1 Antibody by Immunization with Anti-Id

Allotype of Ig-conjugated SRBC	Hemagglutination titer of serum obtained from rabbit:	
	867 [anti-(a2a3$^-$ anti-Id)]	3103 [anti-(a2a3$^+$ anti-Id)]
a1	16	64
a2	0	0
a3	0	0

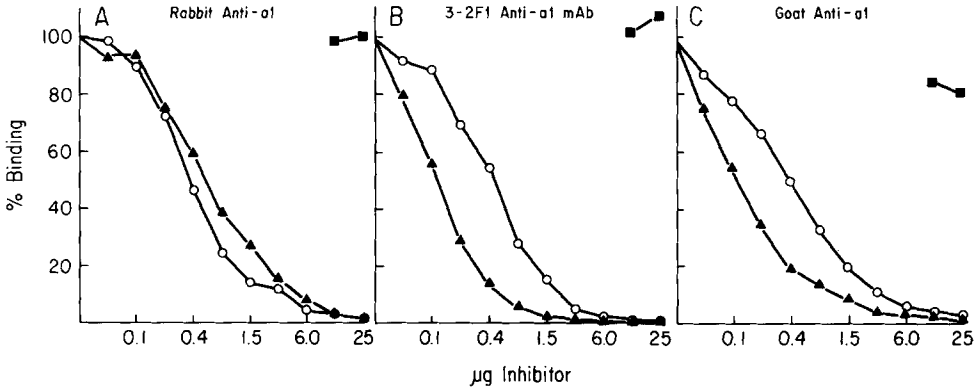

FIGURE 1. Ability of the a2a3$^-$ and a2a3$^+$ anti-Id fractions to inhibit binding of ^{125}I-a1 Ig to: (*A*), rabbit anti-a1; (*B*), 3-2F1 mAb; and (*C*), goat anti-a1. Inhibitors were: (▲), a2a3$^-$ 1096 anti-Id; (●), a2a3$^+$ 1096 anti-Id; and (■), normal a2a3 rabbit Ig. Binding in the absence of inhibitor was 41% of input cpm in (*A*), 38% in (*B*), and 37% in (*C*).

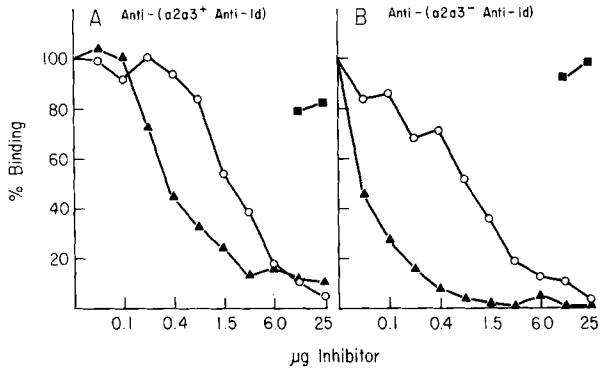

FIGURE 2. (*A*) Inhibition of ^{125}I-a2a3$^+$ anti-Id binding to 3103 anti-a1. (*B*) Inhibition of ^{125}I-a2a3$^-$ anti-Id binding to 867 anti-a1. Inhibitors were: (▲), a2a3$^-$ 1096 anti-Id; (●), a2a3$^+$ 1096 anti-Id; and (■) normal a2a3 rabbit Ig.

was next compared by competitive RIA. For this assay, microtiter wells were coated with affinity-purified 867 or 3103 anti-a1 antibody, and the binding of the homologous ^{125}I-anti-Id fraction was assessed in the presence of various inhibitors. As seen in Fig. 2, both anti-Id fractions completely inhibited the binding of ^{125}I-a2a3$^-$ anti-Id to 867 anti-a1-coated wells. However, the a2a3$^-$

anti-Id fraction was clearly more effective in inhibiting binding than the a2a3$^+$ anti-Id fraction. Similar results were obtained with ^{125}I–a2a3$^+$ anti-Id binding to 3103 anti-a1–coated wells. Again, despite the fact that the 3103 anti-a1 was prepared against a2a3$^+$ anti-Id, the a2a3$^-$ anti-Id population was the more efficient inhibitor.

I wanted to determine whether anti-a1 antibodies obtained after anti-Id immunization would recognize the same proportion of normal Ig molecules from an a1 rabbit as conventional anti-a1 antibody. For this purpose, I performed sequential absorptions by incubating ^{125}I–a1 Ig obtained from a nominal a1 rabbit with an excess of 867 or 3103 antibody bound to Sepharose beads. After this initial incubation, I tested the radiolabel remaining in the supernatant for additional binding to beads coupled with the same antibody or with rabbit 1112 antibody, which was prepared against nominal a1 Ig in a conventional manner. As shown in Fig. 3, absorption with 867 anti-a1 followed by absorption with 1112 anti-a1 precipitated approximately the same total amount of ^{125}I–a1 Ig as two sequential absorptions with 867 antibody. Although the overall amount of bound a1 Ig was lower, incubation with 3103-coupled beads showed essentially identical results. Taken together, these results show that both anti-Id populations were capable of inducing anti-a1 antibodies with specificities similar to those induced by normal a1 Ig; however, the a1 determinants present within the a2a3$^-$ fraction appear to bind anti-a1 antibody more efficiently than those in the a2a3$^+$ fraction.

Localization of Anti-Id a1 Epitopes. As discussed above, it appeared likely that the a1 epitopes present on the anti-Id molecules represented either latent V_Ha1 Ig, and/or internal images that resemble a1. Indeed, Fab fragments of either the

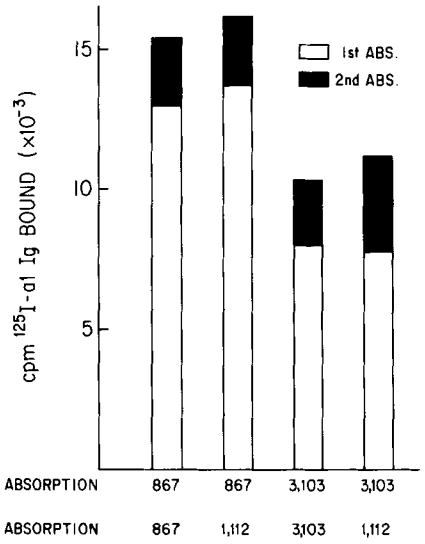

FIGURE 3. Sequential absorptions of ^{125}I–a1 Ig with insolubilized rabbit anti-a1. The 867 and 3103 antibodies were induced by a2a3$^-$ anti-Id and a2a3$^+$ anti-Id, respectively; the 1112 antibody was induced by a1 Ig. The amount of radiolabel bound in the first absorption is shown by the open bars; the amount of radiolabel additionally bound in the second absorption is shown by the solid bars.

$a2a3^-$ or $a2a3^+$ fraction showed anti-Id activity (data not shown). To further localize the a1 epitopes, each anti-Id population was separated into H and L chains, and the binding of ^{125}I–anti-a1 to the isolated chains was assessed. As a positive control, binding of ^{125}I–goat anti–rabbit Ig was also tested. The results in Table III show that, in the case of the $a2a3^-$ anti-Id fraction, isolated H chains were as effective as the unreduced molecules in binding anti-a1. With the $a2a3^+$ anti-Id fraction, on the other hand, neither H nor L chains alone were capable of binding anti-a1 to the same degree as unreduced anti-Id. Although the H chains did show some binding of anti-a1 antibody, this preparation also had greater reactivity with goat anti–rabbit Ig than any of the other preparations tested. These data are consistent with the presence of latent a1 Ig and of a1-like internal images, respectively, within each of the anti-Id populations.

Typing of Anti-Id for IgG C_H Allotypes. Wolf et al. (14) have reported that molecules that express latent V_Ha1 also bear unexpected IgG C region allotypes. I therefore wished to test for such a linkage in our anti-Id preparations. The results for rabbit 1096 are presented in Table IV. As detected by hemagglutination inhibition, this animal produced only d11e15 allotypes both before immunization, and at two time points, almost two years apart, after immunization with anti-a1 antibody. Identical results were obtained with affinity-purified $a2a3^-$ anti-Id molecules. Thus, the induction of anti-Id did not result in the concomitant expression of latent IgG C_H allotypes.

Discussion

This study shows that immunization of rabbits with homologous anti-V_Ha1 allotype antibody induces the production of two populations of anti-Id molecules.

TABLE III
Expression of Anti-Id Activity by Isolated H and L Chains

Anti-Id fraction		Binding of ^{125}I-labeled antibody	
Phenotype	Polypeptide chain	Rabbit anti-a1	Goat anti–rabbit Ig
$a2a3^-$	unreduced	21,440	47,391
	H chain	26,897	49,448
	L chain	5,236	29,240
$a2a3^+$	unreduced	18,920	34,548
	H chain	7,152	56,989
	L chain	1,540	24,601

TABLE IV
Expression of IgG C_H Allotypes on Anti-Id Preparations

Sample tested	Allotypic Phenotype			
	d11	d12	e14	e15
1096 preimmune serum	+	–	–	+
1096 anti-Id serum 8/82	+	–	–	+
1096 anti-Id serum 5/84	+	–	–	+
Purified 1096 $a2a3^-$ anti-Id	+	–	–	+

Both populations appear to express a1 allotypic determinants; they possess properties identical to those predicted for latent a1 Ig and a1-like internal images, respectively.

Latent V_H allotypes and allotype internal images can be easily distinguished by the presence or absence of nominal V_H allotype markers. The various properties of anti-Id molecules fractionated on this basis are summarized in Table V. As expected for molecules that express a1 epitopes, both anti-Id fractions were found to react with anti-a1 antibodies obtained from various species, and were capable of inducing anti-a1 when injected into normal animals. In accordance with the presence of internal images, the a1 epitopes within the $a2a3^+$ anti-Id population required both H and L chains for maximal expression. On the other hand, the a1 determinants in the $a2a3^-$ anti-Id population were fully displayed on H chains alone. In fact, using high-performance liquid chromatography, we have been able to identify an a1-specific peptide in tryptic digests of these H chains (M. Abolhassani, K. H. Roux, and D. W. Metzger, unpublished results). Recent immunoelectron microscopic studies (15) showing that there are at least two a1 epitopes on such anti-Id molecules, and the observation binding to anti-a1 antibody is in a manner essentially identical to that of a1 Ig, are fully consistent with the presence of latent a1 Ig in the $a2a3^-$ anti-Id population. However, it is clear that antiallotype immunization does not lead to activation of an entire latent allogroup, as reported by Wolf et al. (14), since no latent IgG C_H allotypes could be detected in the anti-Id reagent.

The finding of both latent a1 Ig and a1-like internal images in our anti-Id preparation may serve to reconcile the results obtained in various laboratories concerning the effects of antiallotype immunization. Previously, Yarmush et al. (5) observed that injection of antibody directed to a genetically unexpected *a* or *b* allotype, when followed by hyperimmunization with streptococcal vaccine, induced expression of the latent Ig. In addition, Kazdin et al. (6) recently reported that some anti-Id prepared against anti-a2 appeared to be similar to latent a2 Ig in that these molecules lacked nominal V_H allotype determinants and were able to induce anti-a2. The nature of the anti-Id fraction that expressed nominal V_H markers was not investigated in this study. In contrast to these reports, however,

TABLE V
Summary of Anti-Id Characteristics

Property	$a2a3^-$ anti-Id (latent a1 Ig)	$a2a3^+$ anti-Id (a1 internal image)
Nominal V_H allotype expression	−	+
Reactivity with heterologous anti-a1	+	+
Ability to induce anti-a1 antibody	+	+
Location of a1 epitopes	H chain	H and L combinatorial
Allotype configuration by electron microscopy*	+	ND‡

* Roux, et al. (15).
‡ Not determined.

Jerne et al. (7) found that only allotype internal images were induced by antiallotype immunization. This preferential induction, in individual laboratories, of either latent allotypes or internal images may be related, in part, to subtle differences in experimental design and/or genetic background of the particular rabbit colony. Nevertheless, it is evident from the data presented here that both types of Ig molecules can be present within one anti-Id reagent. In light of these results, it would be interesting to further characterize anti-Id molecules that have been induced with anti–mouse Ig (16–18) and anti–human Ig (19, 20) antibodies, since in at least one case, such molecules have also been shown to bear internal images (18).

The precise nature of the epitopes that mimic a1 within the internal image a2a3$^+$ anti-Id fraction remains unknown. The finding that both H and L chains are required for expression shows that these determinants are not identical to V_Ha1, and also makes it unlikely that the molecules are somatic mutants of a2 or a3 Ig, with portions of both a1 and a2/a3 allotypes coexpressed on the V_H framework regions of one molecule. In addition, a2a3$^-$ anti-Id (latent a1) was found to consistently inhibit the binding of a1 Ig to anti-a1 more efficiently than a2a3$^+$ anti-Id (internal images), even when the anti-a1 was induced by a2a3$^+$ anti-Id. Recently, we have prepared mouse mAb that appear to display rabbit a1 epitopes (21). These antibodies should allow a more detailed examination of the molecular basis for allotypic internal images.

The results presented here and elsewhere (5, 6) show that antiallotype immunization is a reproducible and efficient method for inducing unexpected allotypic markers. Unlike most earlier studies (reviewed in 22 and 23), the appearance of latent allotypes in this case does not appear to be transitory, nor is it dependent on antigenic stimulation. With regard to possible induction mechanisms, it may be that antiallotype directly stimulates allotype-bearing B cells which, for some reason, remain quiescent under normal conditions. However, at least for the results reported here, this would require that antibody conjugated to KLH and emulsified in Freund's adjuvant remains capable of activating B cells in this manner. Another possibility is that antiallotype immunization leads to indirect release of latent allotype-secreting cells from active suppression. One might reason that antiallotype induces antiidiotype which in turn inactivates allotype-specific suppressor cells, similar to the findings of Bona et al. (24) in the BALB/c 460 idiotype system. Further work should elucidate the mechanisms responsible for production of unexpected allotypic determinants, as well as the conditions leading to their expression in the form of latent allotypic Ig or internal images.

Summary

Previously (9), I found that immunization of rabbits with antibody directed against variable region heavy chain V_H polypeptides of a1 allotype induced the production of antiidiotype (anti-Id) molecules that appeared to bear images of the original a1 allotype. I now show that these anti-Id molecules can be fractionated into two populations: one population (a2a3$^-$ anti-Id) that lacks the nominal V_H a2 or a3 allotype of the rabbit from which it was derived, and another population (a2a3$^+$ anti-Id) that expresses these allotypes. Both anti-Id populations display epitopes that resemble a1 since: (*a*) they were capable of inhibiting ^{125}I–

a1 Ig binding to rabbit anti-a1, goat anti-a1, and mouse anti-a1 mAb; and (*b*) immunization of normal a2a3 rabbits with either anti-Id fraction led to the formation of specific anti-a1 antibody. Reductive cleavage of the anti-Id molecules showed that the a1 determinants in the $a2a3^-$ population were fully displayed on isolated H chains, consistent with the presence of latent a1 Ig. On the other hand, as expected for internal images encoded by the antigen-combining site, the $a2a3^+$ anti-Id population required intact H and L chains for maximal a1 expression. The a1-like images within the $a2a3^+$ anti-Id population do not appear to be identical to nominal or latent a1, however, since $a2a3^-$ anti-Id was invariably a more efficient inhibitor of a1 Ig–anti-a1 binding than $a2a3^+$ anti-Id. These results indicate that immunization with antiallotype can result in the simultaneous production of both latent allotypes and allotypic internal images.

My thanks to Dr. Rose G. Mage for testing the anti-Id reagents for expression of C_H allotypes, Dr. Kenneth H. Roux for helpful advice, Ms. Robin Reed and Nancy Moore for outstanding technical assistance, and Ms. Chris Winston for excellent secretarial help in the preparation of this manuscript.

References

1. Roland, J., and P.-A. Cazenave. 1981. Rabbits immunized against b6 allotype express similar anti-b6 idiotypes. *Eur. J. Immunol.* 11:469.
2. Gilman-Sachs, A., S. Dray, and W. J. Horng. 1980. A common idiotypic specificity of rabbit antibodies to the a2 allotype of the immunoglobulin heavy chain variable region. *J. Immunol.* 125:96.
3. Metzger, D. W., and K. H. Roux. 1982. A predominant idiotype on rabbit anti-V_Ha1 allotype antibodies: sharing by both major and minor V_H subgroups. *J. Immunol.* 129:1138.
4. Gilman-Sachs, A., S. Dray, and W. J. Horng. 1982. Idiotypic specificities of rabbit antibodies to immunoglobulin allotypes. *J. Immunol.* 129:1194.
5. Yarmush, M. L., J. A. Sogn, P. D. Kern, and T. J. Kindt. 1981. Role of immune recognition in latent allotype induction and clearance. Evidence for an allotypic network. *J. Exp. Med.* 153:196.
6. Kazdin, D. S., A. Gilman-Sachs, S. Dray, and W. J. Horng. 1984. Induction of "latent a2" allotype in a^1a^1 homozygous rabbits as the major part of a bi-directional immune response to immunization with anti-a2 antibody. *J. Immunol.* 132:1909.
7. Jerne, N. K., J. Roland, and P.-A. Cazenave. 1982. Recurrent idiotypes and internal images. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:243.
8. Cazenave, P.-A., and J. Roland. 1984. Internal images of rabbit immunoglobulin allotypes. *Immunol. Rev.* 79:139.
9. Metzger, D. W. 1984. A mouse monoclonal antibody against rabbit V_H allotype shares the predominant idiotype with a rabbit antibody of the same specificity. *Eur. J. Immunol.* 14:304.
10. Roux, K. H., and D. W. Metzger. 1984. Crossreactive idiotypes on heterologous anti-allotype antibody. *J. Immunol.* 132:1341.
11. Adler, F. L., and L. T. Adler. 1980. Passive hemagglutination and hemolysis for estimation of antigens and antibodies. *In* Methods in Enzymology. H. Van Vunakis and J. J. Langone, editors. Academic Press, New York. 70:455.
12. Dubiski, S. 1969. Immunochemistry and genetics of a "new" allotypic specificity Ae^{14} of rabbit γG immunoglobulins: recombination in somatic cells. *J. Immunol.* 103:120.

13. Fleishman, J. B., R. Pain, and R. R. Porter. 1962. Reduction of γ-globulin. *Arch. Biochem. Biophys.* 1(Suppl.):174.
14. Wolf, B., J. Yee-Foon, and M. T. Ikeda. 1983. Three closely linked rabbit IgG allotype gene products: a1, d12, e14. *J. Immunol.* 131:1860.
15. Roux, K. H., D. W. Metzger, D. S. Kazdin, and W. J. Horng. 1984. Induced latent allotypes within rabbit anti–crossreactive idiotype reagents. Direct immunoelectron microscopic evidence. *Eur. J. Immunol.* 14:910.
16. Bona, C., P. K. A. Mongini, K. E. Stein, and W. E. Paul. 1980. Anti-immunoglobulin antibodies. I. Expression of cross-reactive idiotypes and *Ir* gene control of the response to IgG_{2a} of the b allotype. *J. Exp. Med.* 151:1334.
17. Bona, C., and J. Kearny. 1981. Anti-immunoglobulin antibodies. II. Expression of individual and cross-reactive idiotypes on syngeneic and homologous anti-idiotype antibodies. *J. Immunol.* 127:491.
18. Bona, C. A., S. Finley, S. Waters, and H. G. Kunkel. 1982. Anti-immunoglobulin antibodies. III. Properties of sequential anti-idiotypic antibodies to heterologous anti-γ globulins. Detection of reactivity of anti-idiotype antibodies with epitopes of Fc fragments (homobodies) and with epitopes and idiotopes (epibodies). *J. Exp. Med.* 156:986.
19. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-γ-globulin activity. *J. Exp. Med.* 137:331.
20. Førre, Ø., J. B. Natvig, and T. E. Michaelsen. 1977. Cross-idiotypic reactions among anti-Rh(D) antibodies. *Scand. J. Immunol.* 6:997.
21. Van Cleave, V. H., and D. W. Metzger. 1985. Characterization of monoclonal antibodies bearing rabbit V_Ha1 allotypic epitopes. *Fed. Proc.* 44:1693.
22. Kindt, T. J., and M. Yarmush. 1981. Expression of latent immunoglobulin allotypes and alien histocompatibility antigens: relevance to models of eukaryotic gene regulation. *CRC Crit. Rev. Immunol.* 2:297.
23. Roux, K. H. 1983. Rabbit latent allotypes: current status and future directions. *Surv. Immunol. Res.* 2:342.
24. Bona, C., R. Hooghe, P. A. Cazenave, C. Leguérn, and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. *J. Exp. Med.* 149:815.