SUPPRESSION OF IDIOTYPIC SPECIFICITIES IN ADULT MICE BY ADMINISTRATION OF ANTIIDIOTYPIC ANTIBODY*

By DAVID A. HART,‡ AI-LAN WANG, LAURA L. PAWLAK, AND ALFRED NISONOFF

(From the Department of Biological Chemistry, University of Illinois at the Medical Center, Chicago, Illinois 60612)

(Received for publication 31 January 1972)

There is a low frequency of idiotypic cross-reactions among antibodies of the same specificity from different outbred rabbits (1–4). When such cross-reactions do occur they are generally weak and involve a small proportion of the antiidiotypic antibody population (5, 6). This generalization applies also to rabbits belonging to the same family group (2, 7). Contrasting results were reported by Eichmann and Kindt (8), who investigated a family of rabbits consisting of a brother-sister mating pair and their F_1 and F_2 descendants. The mating pair was chosen on the basis of the capacity of both rabbits to produce high titers of antistreptococcal antibody of restricted heterogeneity. Antistreptococcal antibodies elicited in several members of the family group exhibited strong idiotypic cross-reactivity.

In mice of an inbred strain, idiotypic cross-reactions are more common. They have been observed among BALB/c myeloma proteins with antibody activity directed to the phosphoryl choline hapten group (9, 10). These same myeloma proteins also share idiotypic determinants with antiphosphoryl choline antibodies induced by immunization of some BALB/c mice with pneumococci (9). Strong intrastrain idiotypic cross-reactions have also been observed among antibenzoate antibodies of all BALB/c or A/J mice tested so far and among antiphenylarsonate antibodies of A/J mice (11). Examples of both strong and weak interstrain cross-reactivity were also noted (11). One cannot, however, make the generalization that the antibodies of all mice of the same strain exhibit idiotypic cross-reactions, since we have failed to detect strong cross-reactions, with two antiidiotypic antisera prepared so far, among antiphenylarsonate antibodies of C57/BL mice.

The existence of extensive cross-reactions within a strain makes it possible to test the effect in vivo of antiidiotypic antiserum on the immune response of isologous mice. We report here that the formation of antiphenylarsonate antibodies of a particular idiotype in adult A/J mice, in response to the administration of keyhole limpet hemocyanin (KLH)–azophenylarsonate,[1] is greatly sup-

* This work was supported by grants from the National Institutes of Health (AI-06281 and AI-10220).

‡ Supported by US Public Health Service Postdoctoral Fellowship AI-49223 FSG.

[1] *Abbreviations used in this paper:* D antibody, specifically purified anti-*p*-azophenylarsonate antibody from mouse 413 (this antibody was used to elicit antiidiotypic (anti-D) antibody in a recipient rabbit); KLH, keyhole limpet hemocyanin; RIgG, rabbit IgG.

pressed by prior administration of antiidiotypic antiserum directed toward the antihapten antibody. This suggests an alternative method for selectively inhibiting the formation of a particular antibody population without generalized suppression of the immune response.

Materials and Methods

Immunization of Mice.—Methods for the preparation of antigens used for immunization and testing have been described (11). Anti-*p*-azophenylarsonate antibodies used to elicit antiidiotypic antibody were produced in mouse 413 by immunization with KLH–*p*-azophenylarsonate. The protocol for immunization of the mice used in suppression experiments is given below.

Antiidiotypic Antiserum Directed to Anti-p-Azophenylarsonate Antibodies of an A/J Mouse.—The antiidiotypic antiserum used in these experiments was from the same pool as that employed in previous experiments (11); it was prepared in a rabbit against anti-*p*-azophenylarsonate antibody of A/J mouse 413. Evidence that the rabbit antiserum, after absorption with mouse immunoglobulin, was specific for idiotypic determinants was described in detail. The mouse immunoglobulin fraction that was used for absorption of the rabbit antiserum was prepared from normal A/J serum by two precipitations with sodium sulfate at final concentrations of 18% and 14%, respectively. For brevity, the immunogen (purified antihapten antibody from mouse 413) will be designated D and the antiidiotypic antiserum, anti-D. Antiphenylarsonate antibodies from other A/J mice cross-reacted extensively with the absorbed antiserum (11).

^{125}I-labeled, specifically purified anti-*p*-azophenylarsonate antibodies from mouse 413 (^{125}I-labeled D) were prepared as described, and tested in an indirect assay system (11), which comprised 0.01 μg of ^{125}I-labeled D, 5 μl of a 10:1 dilution of the absorbed rabbit anti-D, and 30 μl of goat antiserum specific for the Fc fragment of rabbit IgG; the goat antiserum had been absorbed with A/J mouse serum. The anti-D antibody was present in slightly less than an optimal amount; the goat anti-Fc was present in a small excess over the amount needed for maximal precipitation, as shown by preliminary experiments. Immune precipitates were washed three times after standing overnight, and the percentage of radioactivity precipitated was determined. Unlabeled inhibitory sera, when present, were mixed with the antiidiotypic antiserum before addition of the ^{125}I-labeled D.

Immunosuppression Experiments.—An IgG fraction was prepared from the absorbed rabbit anti-D antiserum by two precipitations with sodium sulfate (18 and 14%) followed by chromatography on diethylaminoethyl (DEAE)-cellulose (12). The rabbit IgG was concentrated to 10 mg/ml, dialyzed against 0.1 M NaCl-phosphate buffer, pH 7.2, and sterilized by passage through a Millipore filter (Millipore Corp., Bedford, Mass.). Rabbit IgG from pooled normal serum was prepared and sterilized by the same procedure.

Normal 8-wk-old A/J mice (Jackson Laboratory, Bar Harbor, Maine) were injected intraperitoneally with 1 mg of the IgG fraction of anti-D antiserum (group 1) or with 1 mg of nonspecific rabbit IgG (group 2). There were nine mice in each group. 3 days later the injections were repeated. After 2 wk all animals were challenged intraperitoneally with 500 μg of KLH–*p*-azophenylarsonate in complete Freund's adjuvant. 2 wk later (wk 4) a second, identical injection was given; and the animals were bled retroorbitally 1 wk later (wk 5). Additional injections of 500 μg of antigen in incomplete Freund's adjuvant were given at wk 6 and 10; a bleeding was taken 1 wk after each inoculation. Each serum was tested by Ouchterlony analysis for its reactivity with KLH (the carrier protein) and with RIgG–*p*-azophenylarsonate. The serum of each bleeding was also analyzed for its capacity to inhibit the indirect precipitation of ^{125}I-labeled D from mouse 413 with its antiidiotypic antiserum.

Each serum was also tested by Ouchterlony analysis against rabbit IgG to determine

whether the 2 mg of IgG used for suppression had elicited antibodies. No precipitin lines were observed, presumably reflecting the fact that the two injections of rabbit IgG were given only 3 days apart and without adjuvant.

All of the 18 mice in the two groups survived the experiment, except for one mouse in the control group that died after the wk-7 bleeding.

RESULTS

All of the mice in both the control and suppressed groups produced precipitating antibodies against the carrier protein, KLH, and against the azophenylarsonate hapten group; the latter was shown by precipitation with RIgG-azophenylarsonate. These precipitating antibodies were present in each of the bleedings taken, i.e., at wk 5, 7, and 11 after the injection of normal or anti-idiotypic rabbit IgG. Tests of precipitation were done by the Ouchterlony

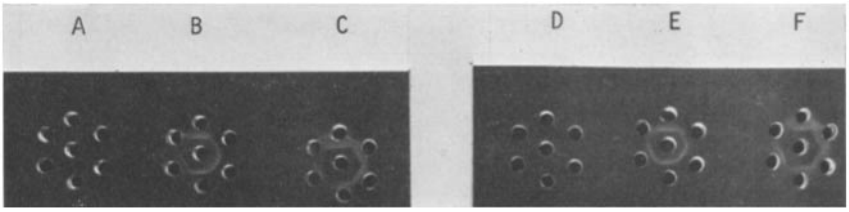

FIG. 1. Ouchterlony analysis of antisera to KLH–*p*-azophenylarsonate of a nonsuppressed mouse (C-2), patterns A, B, and C, and a suppressed mouse (S-1), patterns D, E, and F. Antiserum, taken 11 wk after injection of rabbit IgG (or 9 wk after the start of immunization), is in the center well; the test antigen is in the outer wells. In patterns A and D the antigen is RIgG; in B and E, RIgG–*p*-azophenylarsonate; in C and F, KLH (the carrier protein). Antigen concentrations, reading clockwise from the top well, are: patterns A, B, D, and E: 2, 1.5, 1, 0.5, 0.3, and 0.1 mg/ml; patterns C and F: 3, 2, 1.5, 1, 0.5, and 0.1 mg/ml.

method. A typical pattern is shown in Fig. 1. Although there were differences in the strength of the reactions among individual mice, the average antibody titer did not differ markedly among the two groups of mice. Photographs were taken of all Ouchterlony plates, and the distances of precipitin lines from the antigen and antibody wells were correlated with antigen concentration. By this rough criterion there was no significant difference between the antihapten and anti-KLH titers in the two groups of mice. Thus, there was no generalized suppression of the immune response.

The results in Table I demonstrate that the group of mice preinoculated with the antiidiotypic (anti-D) antibodies produced far less antiphenylarsonate antibody capable of displacing the ^{125}I-labeled antibody of mouse 413 from its antiidiotypic antibodies. 10 μl of antiserum from the wk-11 bleeding of each suppressed mouse caused less inhibition than 0.3 μl of antiserum from any non-suppressed mouse. Thus the degree of idiotypic suppression exceeded 97% in each mouse. It appears, however, that suppression was not complete, since a few sera from suppressed mice had a small but significant inhibitory capacity.

This did not appear to increase as a function of time of immunization. The investigations are continuing with the surviving mice.

In previous work (11) all immune sera were shown to lose their inhibitory capacity entirely upon adsorption with Sepharose (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) coupled to RIgG–*p*-azophenylarsonate, but not with

TABLE I

*Inhibition of Binding of ^{125}I-Labeled Antiphenylarsonate Antibody from Mouse 413 to Its Rabbit Antiidiotypic Antiserum**

Mouse No.	Pre-immune serum (10 μl)	^{125}I-labeled purified D antibody bound (% of control)											
		Wk-5 bleeding‡				Wk-7 bleeding‡				Wk-11 bleeding‡			
		μl of serum tested as inhibitor											
		0.03	0.3	3	10	0.03	0.3	3	10	0.03	0.3	3	10
Controls§													
C-1	95 (2)	87 (2)	26 (0)	23 (3)		75 (1)	13 (2)	6 (4)	8 (1)				
C-2	100 (2)	95 (2)	76 (7)	37 (0)		93 (6)	38 (0)	26 (7)	10 (1)	92 (4)	56 (0)	23 (2)	
C-3	95 (2)	77 (3)	45 (3)	22 (0)		72 (2)	26 (3)	14 (3)	10 (2)	85 (6)	25 (2)	7 (3)	
C-4	95 (2)	84 (2)	41 (0)	28 (0)		73 (10)	29 (1)	8 (3)	14 (1)	92 (1)	41 (0)	10 (3)	
C-5	102 (1)	98 (5)	79 (2)	28 (1)		86 (3)	41 (7)	20 (4)	10 (0)	95 (6)	34 (1)	17 (1)	
C-6	95 (1)	88 (0)	59 (0)	25 (0)		82 (5)	44 (2)	21 (2)	10 (2)	92 (0)	36 (4)	12 (1)	
C-7	102 (2)	84 (3)	52 (1)	28 (1)		83 (0)	38 (1)	17 (1)	14 (4)	67 (7)	22 (1)	8 (1)	
C-8	98 (1)	78 (5)	50 (5)	30 (3)		72 (5)	36 (1)	17 (1)	11 (2)	80 (3)	22 (3)	11 (3)	
C-9	98 (1)	80 (1)	48 (0)	29 (3)		75 (8)	53 (2)	28 (2)	13 (5)	99 (5)	49 (4)	12 (1)	
Suppressed‖													
S-1	91 (6)			95 (0)	87 (5)			74 (2)	75 (2)			82 (2)	70 (4)
S-2	97 (1)			95 (1)	88 (1)			89 (3)	86 (2)			100 (1)	90 (2)
S-3	102 (3)			90 (0)	80 (3)			82 (1)	75 (2)			90 (1)	84 (1)
S-4	96 (2)			101 (2)	N.D.			84 (8)	88 (2)			91 (2)	92 (1)
S-5	100 (0)			92 (2)	81 (3)			77 (2)	75 (1)			77 (1)	75 (2)
S-6	100 (0)			93 (1)	82 (3)			83 (4)	88 (3)			93 (3)	94 (6)
S-7	100 (0)			90 (1)	88 (2)			86 (6)	74 (1)			85 (0)	84 (1)
S-8	98 (1)			96 (0)	86 (2)			89 (2)	87 (2)			84 (6)	78 (1)
S-9	109 (3)			87 (0)	77 (3)			83 (2)	80 (2)			93 (2)	82 (1)

* Tests were carried out as described under Materials and Methods. In the control for a given set of experiments, the same amount of pooled normal A/J serum was substituted for the sera tested as inhibitors. In the absence of inhibitor, an average of 54.8% of the labeled D was precipitated (corrected for 5.5% precipitation, which occurred when rabbit antiovalbumin was substituted for anti-D). Experiments were done in duplicate; the average deviations are shown in parentheses.

‡ Number of weeks after the injection of normal or antiidiotypic rabbit IgG.

§ Preinjected with 2 × 1 mg of normal rabbit IgG.

‖ Preinjected with 2 × 1 mg of rabbit antiidiotypic IgG.

unconjugated Sepharose. This finding was confirmed in the present study by testing sera of the wk-11 bleeding of three mice in the control group. In each case all inhibitory capacity was lost upon treatment with the immunoadsorbent.

Sera from both the control and suppressed groups reacted strongly with monospecific goat antisera to mouse IgG1 and IgG2.

DISCUSSION

It is evident that antibodies having idiotypic specificities shared with the antiphenylarsonate antibodies of mouse 413 (D antibody) were almost com-

pletely suppressed in the sera of the group of nine A/J mice that had been treated with 2 mg of an IgG fraction of rabbit antiidiotypic antiserum (anti-D) 2 wk before the 9-wk course of immunization with KLH–*p*-azophenylarsonate. In contrast, hyperimmune sera of each of nine control mice of the same strain, preinoculated with nonspecific rabbit IgG, contained antibodies that reacted with nearly all of the antiidiotypic antibody population, as evidenced by inhibition of the indirect precipitation of ^{125}I-labeled D. These inhibitory antibodies were present in bleedings taken 3, 5, and 9 wk after the start of immunization (or 5, 7, and 11 wk after the injection of rabbit IgG). Immune adsorption of three of these control sera showed that, as in previous studies (11), the inhibition was caused by antiphenylarsonate antibodies. All preimmune sera were noninhibitory. In these and other experiments we have tested antiphenylarsonate antisera from a total of more than 20 hyperimmunized A/J mice; each antiserum displaced at least 75% of ^{125}I-labeled D of mouse 413 from its antiidiotypic antiserum.

Treatment of the experimental group with anti-D did not prevent the appearance of antibodies to the carrier protein (KLH) or to the hapten (*p*-azophenylarsonate). Ouchterlony patterns suggested that the titers of these antibodies were roughly comparable in the experimental and control groups, but the possibility of some suppression cannot be ruled out. The antiphenylarsonate antibodies that did appear thus possessed idiotypic specificities not recognized by the anti-D antibodies. The results are not attributable to generalized suppression of a major subgroup of IgG since sera of the suppressed animals reacted strongly with monospecific antisera to mouse IgG1 and mouse IgG2. Also, one would not anticipate suppression of a subgroup of IgG since the antiidiotypic antiserum injected was absorbed with excess mouse immunoglobulin and must also have been exposed in the recipient mouse to a large excess of subgroup-specific antigenic determinants. The same arguments would indicate that the effects observed were not attributable to suppression of a class other than IgG.

Three major possibilities may be considered to account for the suppression of idiotypic antibodies: (*a*) There was no suppression of biosynthesis but the antiidiotypic antibodies injected before immunization with KLH–*p*-azophenylarsonate simply absorbed the idiotypic antibodies produced; (*b*) a small amount of idiotypic antibody was injected along with the anti-D, and the presence of this antibody suppressed the formation of identical antibody molecules (13); (*c*) there was central suppression, acting at the cellular level.

Possibility (*a*) can be ruled out by quantitative considerations. In each control animal, 11 wk after the start of the experiment, 0.3 μl of antiserum displaced more than 50% of the ^{125}I-labeled D antibodies from the antiidiotypic antibodies in the test system. Therefore, the number of anti-D molecules present in each test was not capable of binding more than the number of D molecules present in 0.6 μl of hyperimmune serum. Actually, the binding capacity of the anti-D molecules must have been smaller than this; because of competition with

labeled D, some unlabeled D molecules must have remained unbound. Thus the 0.5 μl of anti-D antiserum used in each test was capable of binding the D antibodies in less than 0.6 μl of control hyperimmune sera. The total quantity of rabbit anti-D IgG injected into suppressed animals was 2 mg, which is equivalent to about 200 μl of antiserum. The total binding capacity of the injected rabbit anti-D, therefore, could not exceed the amount of D antibodies present in 200 × 0.6/0.5 or 240 μl of a hyperimmune mouse serum. Thus at the time of the 11-wk bleeding, the serum of a hyperimmune control mouse contained far more D molecules than necessary to saturate all of the rabbit anti-D injected into a suppressed animal. In addition, idiotypic antibodies were present in comparable titer throughout a 6-wk period preceding this bleeding (Table I). Since the half-life of mouse IgG is about 5 days (14), the control mice must have synthesized many times as much idiotypic antibody as necessary to saturate the anti-D antibodies during the 6-wk interval. This calculation is based on the assumption that *none* of the rabbit anti-D IgG was eliminated from the mouse during the 10-wk interval; actually, most of it was probably cleared rapidly since it was injected without adjuvant. The differences between the control and the experimental groups therefore cannot be attributed to simple absorption of D antibodies by rabbit anti-D.

It is not possible to rule out theoretically suppression of D-antibody formation by traces of D antibodies that might have been present in the IgG fraction of rabbit anti-D. However, any such contamination must have been minute. The rabbit was challenged with a total of 3 mg of mouse antiphenylarsonate antibody. Even if none of this mouse antibody was cleared, the maximum degree of contamination by mouse antibody of the IgG in a 3-kg rabbit would have been less than 3 parts in 1000, or 6 μg in the 2 mg used for suppression. The actual contamination was undoubtedly much lower. Furthermore, the weight of KLH–*p*-azophenylarsonate injected into mice during the course of immunization was 2 mg. If suppression of antibody biosynthesis by antibody is due to sequestration of antigen (13), the effect of trace contamination of rabbit IgG by mouse antiphenylarsonate antibodies must have been completely negligible.

It seems very probable, then, that antiidiotypic antibodies act at the cellular level to suppress the formation of antibodies with the corresponding idiotype. This appears quite analogous to the suppression of allotypic specificities by anti-allotypic antisera (15–17). Allotype suppression, however, must be initiated in the newborn animal to be successful. Suppression of idiotype in the adult is probably feasible because only a very limited number of lymphocytes bear receptors with a given idiotypic specificity.

In all probability the antiidiotypic antibodies interact with immunoglobulin receptors bearing the idiotypic determinants which are present on the surfaces of lymphocytes. If the receptor corresponds in structure to the antibody to be produced by descendants of the lymphocyte after antigenic stimulation, inac-

tivation of the cell through interaction with antiidiotypic antiserum would account for the selective suppression observed. The cell type involved is probably the thymus-independent B lymphocyte.

Suppression of idiotype should provide a useful new approach for exploration of the mechanism of antibody biosynthesis. In addition, it represents another technique for specifically inhibiting the formation of a selected antibody population without generalized suppression of the immune response. Possible applications may eventually be found in the areas of transplantation and allergy.

SUMMARY

It has previously been shown that there are extensive idiotypic cross-reactions among antiphenylarsonate antibodies of A/J mice. The present work indicates that administration, into normal, adult A/J mice, of rabbit anti-idiotypic antibody directed to A/J antiphenylarsonate antibody suppresses almost completely the subsequent production of antibody of the corresponding idiotype. No effect was noted on the formation of antibodies to the protein carrier or of antiphenylarsonate antibody of a different idiotype. The data are consistent with central suppression of production of the idiotypic antibody mediated through interaction with immunoglobulin receptors on lymphocytes.

Note Added in Proof.—Two control and three suppressed mice were tested by allowing them to rest for 10 wk after the wk-11 bleeding, then challenging with the KLH–*p*-azophenylarsonate antigen. A week later (wk 22) the animals were bled. It was found that 3 μl of each control serum caused nearly complete inhibition of indirect precipitation, whereas 10 μl of the serum from each suppressed mouse caused less than 12% inhibition.

REFERENCES

1. Oudin, J., and M. Michel. 1963. Une nouvelle forme d'allotypie des globulines γ du serum de lapin apparemment liée à la jonction et à la specificité anticorps. *C. R. Seances Acad. Agr. Fr.* **257**:805.
2. Kelus, A. S., and P. G. Gell. 1968. Immunological analysis of rabbit anti-antibody systems. *J. Exp. Med.* **127**:215.
3. Daugharty, H., J. E. Hopper, A. B. MacDonald, and A. Nisonoff. 1969. Quantitative investigations of idiotypic antibodies. I. Analysis of precipitating antibody populations. *J. Exp. Med.* **130**:1047.
4. Braun, D. G., and R. M. Krause. 1968. The individual antigenic specificity of antibodies to the streptococcal carbohydrate. *J. Exp. Med.* **128**:969.
5. Hopper, J. E., A. B. MacDonald, and A. Nisonoff. 1970. Quantitative investigations of idiotypic antibodies. II. Nonprecipitating antibodies. *J. Exp. Med.* **131**:41.
6. Oudin, J., and G. Bordenave. 1971. Observations on idiotypy of rabbit antibodies against *Salmonella abortus-equi*. *Nature (New Biol.) (London)*. **231**:86.
7. Oudin, J., and M. Michel. 1969. Idiotypy of rabbit antibodies. I. Comparison of

idiotypy of antibodies against *Salmonella typhi* with that of antibodies against other bacteria in the same rabbits, or of antibodies against *Salmonella typhi* in various rabbits. *J. Exp. Med.* **130:**595.

8. Eichmann, K., and T. J. Kindt. 1971. The inheritance of individual antigenic specificities of rabbit antibodies to streptococcal carbohydrates. *J. Exp. Med.* **134:**532.
9. Cohn, M., G. Notani, and S. A. Rice. 1969. Characterization of the antibody to the C-carbohydrate produced by a transplantable mouse plasmacytoma. *Immunochemistry.* **6:**111.
10. Potter, M., and R. Liebermann. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma protein that bind phosphoryl choline. *J. Exp. Med.* **132:**737.
11. Kuettner, M. K., A. L. Wang, and A. Nisonoff. 1972. Quantitative investigations of idiotypic antibodies. VI. Idiotypic specificity as a potential genetic marker for the variable regions of mouse immunoglobulin polypeptide chains. *J. Exp. Med.* **135:**579.
12. Levy, H. B., and H. A. Sober. 1960. A simple chromatographic method for preparation of gamma globulin. *Proc. Soc. Exp. Biol. Med.* **103:**250.
13. Uhr, J. W., and G. Möller. 1968. Regulatory effect of antibody on the immune response. *Advan. Immunol.* **8:**81.
14. Waldman, T. A., and W. Strober. 1969. Metabolism of immunoglobulins. *Progr. Allergy.* **13:**1.
15. Mage, R., and S. Dray. 1965. Persistent altered phenotypic expression of allelic γG-immunoglobulin allotypes in heterozygous rabbits exposed to isoantibodies in fetal and neonatal life. *J. Immunol.* **95:**525.
16. Dubiski, S., 1967. Suppression of synthesis of allotypically defined immunoglobulin and compensation by another sub-class of immunoglobulin. *Nature (London).* **214:**1365.
17. Herzenberg, L. A., L. A. Herzenberg, R. C. Goodlin, and E. C. Rivera. 1967. Immunoglobulin synthesis in mice. Suppression by anti-allotype antibody. *J. Exp. Med.* **126:**701.