

NATURALLY INDUCED AUTO-ANTI-IDIOTYPIC ANTIBODIES

Induction by Identical Idiotoxes in Some Members of an Outbred Rabbit Family*

BY STEVEN B. BINION‡ AND L. SCOTT RODKEY§

From the Division of Biology, Kansas State University, Manhattan, Kansas 66506

Immunoglobulin (Ig) idiotypes have been the subject of numerous investigations since their description by Kunkel et al. (1) and Oudin and Michel (2). The idiotypic epitopes (idiotopes), routinely found on Fab fragments, have been localized to H chains or to L chains in different experimental systems, but usually both chains are required for idioype expression. Hapten inhibition of idioype-anti-idioype reactions usually is interpreted as evidence that the idioype is located in, or very near, the antigen-binding site (paratope) of the antibody molecule. The property of hapten inhibitability of idioype-anti-idioype reactions is highly variable in different idioype systems. Some reactions are inhibitible, others are not. Thus, this property is not a consistent feature of these reactions. Originally, idiotypes were found to exhibit individual antigenic specificity, particularly in antibodies of the same specificity from outbred species such as humans and rabbits. Idiotypic cross-reactions have been reported in outbred species but are rare. An apparent exception to that finding seems to be anti-allotype antibodies (3-6).

Idiotopes are the structures on antibodies that govern interactions that seem to play a major role in regulating numerous immune processes. The conceptual basis for our current understanding of this system, originally conceived by Niels Jerne (7), has come to be known as the idioype network. The central feature of this concept is that the introduction of a "foreign" epitope upsets a dynamic balance of idioype-anti-idioype interactions, causing idioype concentrations to increase and thereby eliciting an increase in concentration of anti-idioype; a balance is then regained between the two reactant species.

A large body of accumulated evidence supports the hypothesis that idioype-anti-idioype interactions can modulate both humoral and cell-mediated interactions. These data have been reviewed (8). Suppressor T cells can bear idioype on their receptors, as shown by Lewis and Goodman (9) and can bear anti-idioype specificity, as shown by Bona and Paul (10). Cosenza et al. (11) showed that helper T cells can bear idioype on their receptors. Additionally, Hetzelberger and Eichmann (12), Eichmann et al. (13), and Bona (14) have presented evidence that helper T cells can

* Supported, in part, by grant PCM79-21110 from the National Science Foundation. Contribution 82-343-J from the Division of Biology, Kansas agricultural Experiment Station, Manhattan, KS.

‡ Present address is the Department of Pathology, University of Iowa, Iowa City, Iowa.

§ Recipient of a Public Health Service Research Career Development Award 1-K04-AI00217 from the National Institutes of Health. Present address is the Department of Pathology and Laboratory Medicine, University of Texas Medical School, Texas Medical Center, P. O. Box 20708, Houston, Texas 77025.

bear anti-idiotypic receptors. Cells mediating delayed-type hypersensitivity have been shown to bear idiotypes on their receptors (15). Thus, nearly all compartments of the immune system have been shown to bear idiotypic or anti-idiotypic receptors. Therefore, all compartments should be subject to idiootype network immunoregulatory interactions.

Despite the large number of model systems that have been studied to gain insight into network immunoregulation, reports of naturally induced anti-idiotypic mediated regulation of immune reactions are sparse. Tasiaux et al. (16) and Jackson and Mestecky (17) reported the appearance of cells bearing auto-anti-idiotypic (AAI) receptors after immunization. Naturally induced regulation has been shown in the T-15 system (18, 19), in an alloantigen system in rats (20, 21), in the levan (22) and trinitrophenyl systems (10, 23, 24) in BALB/c mice, the trinitrophenyl-Ficoll system (25-27), a delayed hypersensitivity response (15), in a dextran system (28), and in humans (29). Brown and Rodkey (30) described a rabbit that synthesized idiotypes and then synthesized naturally induced AAI antibodies in alternate rounds of immunization. The production of anti-idiotypic was related to the disappearance of specific antibody clonotypes.

This study was designed to determine the frequency with which natural AAI antibodies are found in rabbits after immunization. A further goal was to determine whether any similarities in antibody idiotypes could be detected in outbred, yet related, individuals.

Materials and Methods

Rabbits. Outbred New Zealand White rabbits obtained from commercial suppliers and our own rabbit colony were used.

Immunization Protocol. Rabbits were immunized according to Osterland et al. (31) and Strosberg et al. (32) with a vaccine prepared by suspending whole washed *Micrococcus lysodeikticus* cells (Worthington Biochemical Corp., Freehold, NJ) in normal saline. Each round of immunization lasted 3 wk and consisted of three intravenous injections of 3 mg (dry weight) micrococcus the 1st wk, three intravenous injections of 4 mg the 2nd wk, and three intravenous injections of 5 mg the 3rd wk. Successive rounds of immunization were alternated with rest periods.

Micrococcal Cell Wall Carbohydrate Immunoabsorbent. A micrococcal cell wall carbohydrate immunoabsorbent column was prepared by coupling soluble micrococcal cell wall polysaccharide to Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) as described previously (30). Briefly, cells were broken to make cell walls and were solubilized by a limited lysozyme treatment. The soluble fraction was coupled to Sepharose by cyanogen bromide treatment.

Purification of Antimicrococcal Carbohydrate Antibody. Antimicrococcal carbohydrate antibody was purified according to a modification of Wikler's procedure (33). Micrococcal antiserum was added to the Sepharose 4B micrococcal carbohydrate column, and unbound protein was washed through with 0.02 M phosphate-buffered saline, pH 7.5. Antibody was eluted with 0.2 M acetic acid.

Preparation of $F(ab')_2$ Fragments. $F(ab')_2$ fragments were isolated from peptic digests (34) of specifically purified antibodies or of chromatographically purified IgG.

Iodination of $F(ab')_2$ Fragments. $F(ab')_2$ fragments were iodinated by the ICl method of McFarlane (35), adjusted to yield an incorporation level of approximately two atoms of iodine per $F(ab')_2$ fragment.

Radioimmunoassay. A previously described, but modified, indirect radioimmunoassay (RIA) method (30) was used to detect AAI antibodies present in rabbit antisera. 10- μ l samples of antiserum were mixed with 5 ng of labeled anti-micrococcal $F(ab')_2$ fragments, incubated, and goat anti-rabbit Fc γ serum was added to precipitate complexes. Supernatants and precipitates

were assayed for radioactivity. Percentages of radioactivity precipitated were calculated after correcting for (a) background radioactivity, (b) trichloroacetic acid precipitability of the iodinated $F(ab')_2$ fragments, and (c) precipitate trapping of radioactivity. In inhibition experiments, antisera were mixed with aliquots of unlabeled $F(ab')_2$ fragments and incubated. After incubation, 5 ng of labeled anti-micrococcal $F(ab')_2$ fragments were added, followed by goat anti-rabbit Fc γ serum. Inhibition was calculated relative to counts precipitated in uninhibited controls.

Results

Naturally Induced Auto-Anti-Idiotypic Antibody Responses. The rabbits used in this study and their geneologic relationships to one another are shown in Fig. 1. Rabbit 102 was bred with four different females (7-7, 61-7, 63-7, and 64-7), and 10 offspring were raised. Father-daughter matings were set up by breeding 102 with daughters 89-7 and 94-7, and 8 offspring were raised. Rabbit 102 and each of his 18 offspring were immunized by injecting with 2-6 rounds of *M. lysodeikticus* vaccine. Specifically purified antimicrococcal carbohydrate antibodies from the peak of each round of response were isolated, pepsin digested, and radiolabeled. RIA assays were set up using individual bleedings taken after the peak of the antimicrococcal response to assay for the presence of AAI antibodies in each rabbit. Naturally induced AAI antibodies were detected in one rabbit during round two of immunization, but the AAI antibodies usually were found after three or four rounds of immunization. The natural AAI responses were detected 2-4 wk after the peak of the antimicrococcal response, and frequently they persisted even into later rounds of immunization, even though they were undetectable during the subsequent peak responses to *M. lysodeikticus*. As shown in Fig. 1, 42% (8/19) of the rabbits studied exhibited naturally induced AAI responses. The anti-idiotypic specificity of each AAI-positive serum was confirmed by using extensive controls consisting of RIA tests of these sera vs. (a) labeled $F(ab')_2$ from pooled normal rabbit IgG, (b) labeled $F(ab')_2$ prepared from normal serum of a rabbit with the same allotype as the mother of the responding rabbit, (c) labeled $F(ab')_2$ prepared from specifically purified antimicrococcal antibodies of rabbits unrelated to this family, and (d) labeled $F(ab')_2$ prepared from specifically purified antimicrococcal antibodies of rabbits in this family that showed no natural AAI responses (rabbits 142-7, 143-7, 88-7, 97-7, 90-7, 80-32, 80-33, 80-34, 80-35, 80-36, and 80-37). Rabbit 94-7 had both naturally induced AAI and pepsin agglutinator (36). Because of complications in interpreting data caused by the pepsin agglutinator activity, that rabbit was removed from the AAI study and used for breeding.

Characterization of Naturally Induced AAI Responses. Once we had clearly established the idiotypic nature of the AAI reactions (identified in Fig. 1), assays were designed to optimize each autologous idio-type-AAI reaction by titration of AAI to a plateau level and to test for cross-reactivity of each natural AAI serum with labeled $F(ab')_2$ fragments of antimicrococcal antibodies of the other offspring (from Fig. 1). Data are shown only for $F(ab')_2$ preparations yielding positive results. The results of these assays for cross-reactivity are summarized in Table I. The data show that each rabbit responded to only a fraction of the total autologous antimicrococcal antibody population that was elicited (11-41%), and the percentage of autologous idio-type that was recognized by naturally induced AAI antibodies varied substantially from individual to individual. Further, substantial cross-reactivity was observed when naturally induced AAI sera were assayed vs. labeled antimicrococcal $F(ab')_2$ from the other

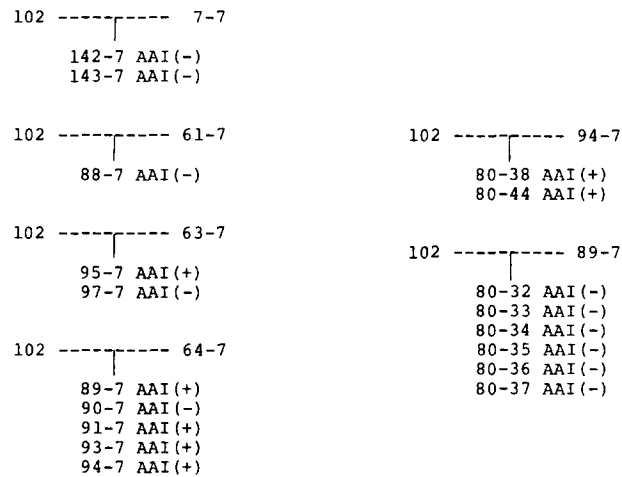


FIG. 1. Pedigree of the rabbits used in this study. AAI (+) and AAI (-) indicate that auto-anti-idiotypic antibodies were (+) or were not (-) detected in that individual after antimicrococcal antibodies had been elicited.

TABLE I
Reactions of Naturally Induced AAI Antisera with ^{125}I -Antimicrococcal $\text{F}(\text{ab}')_2$ Fragments of All AAI-positive Individuals

Natural AAI	^{125}I -Antimicrococcal $\text{F}(\text{ab}')_2$						
	89-7	91-7	93-7	95-7	80-38	80-44	102
89-7	<i>16.1*</i>	15.4	4.1	16.4	9.6	15.1	24.2
91-7	12.2	<i>31.6</i>	9.5	16.4	13.7	19.2	29.0
93-7	17.5	33.3	<i>17.4</i>	30.8	23.3	24.4	38.4
95-7	9.8	15.3	3.6	25.7	6.3	13.3	35.1
80-38	7.7	11.9	0.7	11.2	<i>11.2</i>	15.7	19.2
80-44	4.9	8.9	1.0	10.0	15.4	<i>28.9</i>	33.1
102	4.5	6.7	0.5	11.4	5.2	15.6	<i>41.6</i>

* Numbers indicate percentage of each labeled antimicrococcal $\text{F}(\text{ab}')_2$ that was bound by each natural AAI antiserum. Autologous reactions are in italics.

rabbits in the family that made natural AAI. In several instances, the cross-reactions were quantitatively higher than were the autologous reactions. There was no cross-reaction of those sera when assayed by using labeled $\text{F}(\text{ab}')_2$ fragments of antimicrococcal antibodies from the AAI-negative rabbits (Fig. 1). Thus, the AAI-negative individuals in the family did not express the idiotypes that were expressed in all the AAI-positive offspring in the family. Those reactions were characterized for hapten inhibition properties by setting up each reaction in the presence of 5% glucose plus 0.5 M NaCl (33) or in the presence of the purified micrococcal carbohydrate antigen. Neither method successfully inhibited the reactions.

Cross-Reactivity of Naturally Induced AAI Antisera. With some exceptions, the idiotypes observed in individual outbred animals are individually unique. Further, because some of the cross-reactions in Table I gave quantitatively higher results than the autologous reactions, it was of interest to determine whether any of those reactions was heteroclitic. The reactions were examined by measuring the ability of unlabeled

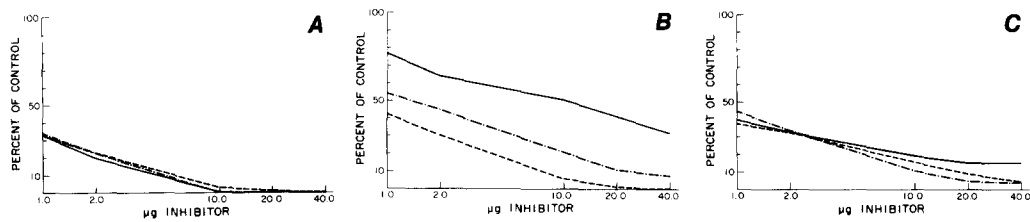


FIG. 2. RIA inhibition assays in which autologous AAI antiserum and labeled $F(ab')_2$ fragments of anti-micrococcal antibodies were used from each of three offspring. Each standard reaction (indicated in each headline) was inhibited with unlabeled antimicrococcal $F(ab')_2$ fragments of 89-7 (—), 91-7 (---), and 95-7 (-·-·-). A, 89-7 AAI vs. 89-7 [125 I]anti-CHO $F(ab')_2$; B, 91-7 [125 I]anti-CHO $F(ab')_2$; C, 95-7 AAI vs. 95-7 [125 I]anti-CHO $F(ab')_2$.

$F(ab')_2$ fragments to inhibit reactions between naturally induced AAI antiserum and autologous antimicrococcal $F(ab')_2$ fragments. The quantities of $F(ab')_2$ fragments used to inhibit a standard reaction between labeled idio type and anti-idio type were adjusted to contain the same amount of idio type. For example, if the labeled $F(ab')_2$ of rabbit A reacted with AAI from rabbit C after titration to the extent of 20% and the labeled $F(ab')_2$ of rabbit B reacted with AAI from rabbit C to the extent of 40% after titration, then the amount of unlabeled $F(ab')_2$ used from rabbit A would be twice the quantity used from rabbit B to inhibit the reaction of labeled $F(ab')_2$ from rabbit C with the AAI from rabbit C. The results of inhibition assays in which unlabeled $F(ab')_2$ fragments of antimicrococcal antibodies from rabbits 89-7, 91-7, and 95-7 were used to inhibit each autologous idio type-AAI reaction are shown in Fig. 2. Fig. 2A shows that the autologous 89-7 idio type-AAI reaction was inhibited equally well with fragments from 89-7, 91-7, and 95-7 and that the reaction was inhibited completely. As seen in Fig. 2B, the unlabeled fragments from 91-7 and 95-7 were nearly equivalent to unlabeled 91-7 fragments in inhibiting the 91-7 reaction by >90%; fragments of 89-7 inhibited by >60%. Fig. 2C shows that the autologous 95-7 idio type-AAI reaction was inhibited the same with unlabeled fragments from either 95-7 or 91-7, with both inhibiting the reaction totally. Inhibition by 89-7 fragments was nearly complete. These inhibition assays show that the naturally induced AAI antisera from 89-7, 91-7, and 95-7 recognized idio types that were present on the antimicrococcal antibody populations in each of these rabbits. The sets of idio types recognized by all three naturally induced AAI antisera overlapped substantially, as shown in Fig. 2A and 2B, or were identical, as shown in Fig. 2C.

These cross-reactions were examined further by determining the ability of unlabeled $F(ab')_2$ fragments to inhibit the cross-reactions between naturally induced AAI antiserum and labeled $F(ab')_2$ fragments from a different rabbit. Fig. 3A and 3B show the result of assays in which unlabeled $F(ab')_2$ fragments from rabbits 89-7, 91-7, and 95-7 were used to inhibit a reaction between 89-7 AAI antiserum and labeled $F(ab')_2$ fragments from 91-7 (Fig. 3A) and 95-7 (Fig. 3B). In both assays, inhibition curves for each of the three inhibitors were identical. Fig. 3C and 3D show inhibition of the cross-reaction between the naturally induced AAI antiserum from 91-7 and labeled $F(ab')_2$ fragments from 89-7 (Fig. 3C) and 95-7 (Fig. 3D). Once again, identical inhibition curves were generated, and each $F(ab')_2$ preparation inhibited the cross-reaction completely. Fig. 3E and 3F show the results of assays to measure the inhibition of the cross-reaction between naturally induced AAI from 95-7 and

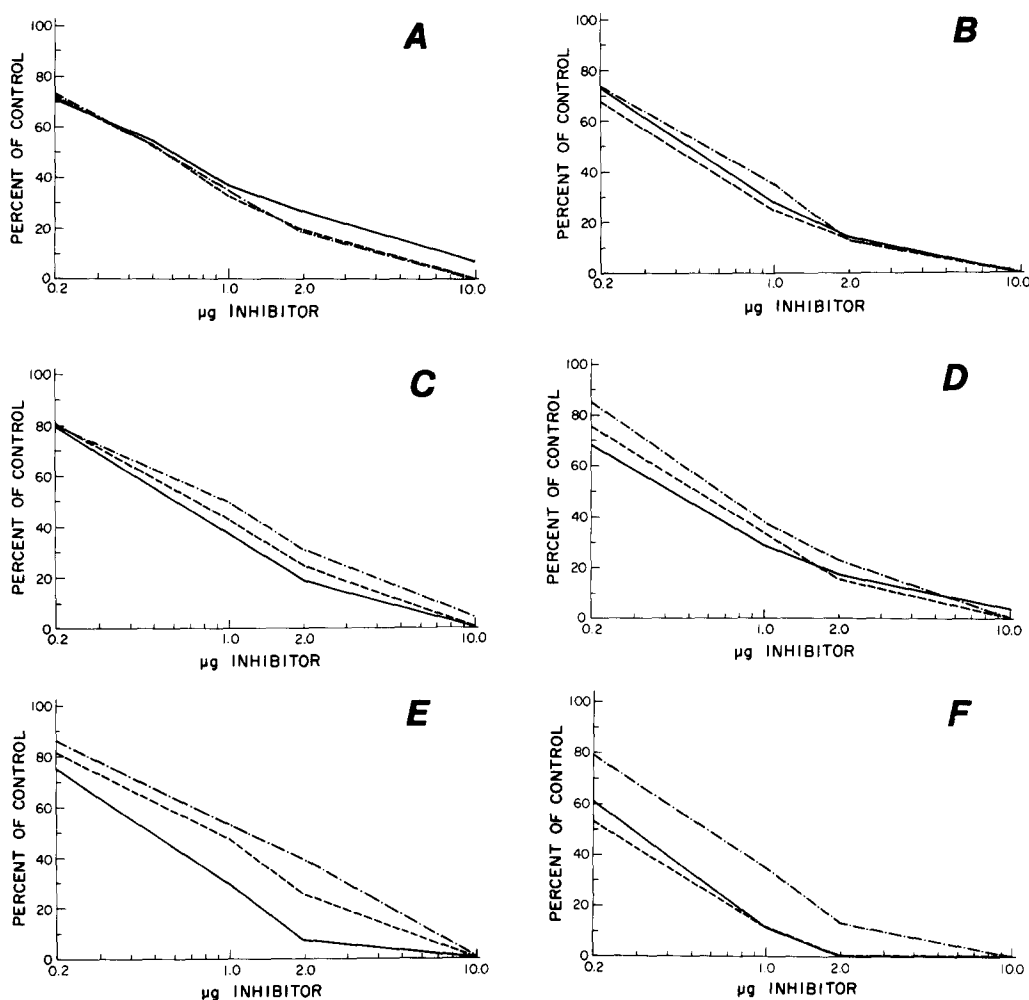


FIG. 3. RIA inhibition assays in which isologous AAI antiserum and labeled $F(ab')_2$ fragments of antimicrococcal antibodies were used from each of three offspring. Each standard reaction (indicated in each headline) was inhibited with unlabeled antimicrococcal $F(ab')_2$ fragments of 89-7 (—), 91-7 (---), and 95-7 (-·-·). A, 89-7 AAI vs. 91-7 [^{125}I]anti-CHO $F(ab')_2$; B, 89-7 AAI vs. 95-7 [^{125}I]anti-CHO $F(ab')_2$; C, 91-7 AAI vs. 89-7 [^{125}I]anti-CHO $F(ab')_2$; D, 91-7 AAI vs. 95-7 [^{125}I]anti-CHO $F(ab')_2$; E, 95-7 AAI vs. 89-7 [^{125}I]anti-CHO $F(ab')_2$; F, 95-7 AAI vs. 91-7 [^{125}I]anti-CHO $F(ab')_2$.

labeled $F(ab')_2$ fragments of 89-7 (Fig. 3 E) and 91-7 (Fig. 3 F) by unlabeled $F(ab')_2$ fragments from 89-7, 91-7, and 95-7. Here again, the cross-reactions were inhibited in a quantitatively identical way, and the cross-reactions were inhibited completely. Both autologous and cross-reaction standard assays were used for inhibition experiments with several other combinations of reagents listed in Table I (data not shown), and the results were always the same: identical inhibition curves and reactions that were inhibited completely.

The specificities of the naturally induced AAI responses of rabbits 80-38 and 80-44 were examined closely because they were AAI-positive responders from a father-

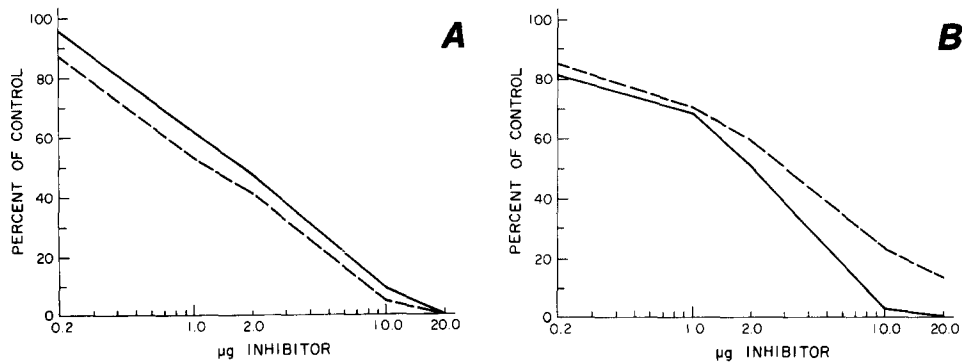


FIG. 4. RIA inhibition assays in which autologous AAI antiserum with labeled $F(ab')_2$ fragments of antimicrococcal antibodies were used from offspring 80-38 and 80-44. Each standard reaction (indicated in each headline) was inhibited with unlabeled antimicrococcal $F(ab')_2$ fragments of 80-44 (—) and 80-38 (---). A, 80-38 AAI vs. 80-38 [125 I]anti-CHO $F(ab')_2$; B, 80-44 AAI vs. 80-44 [125 I]anti-CHO $F(ab')_2$.

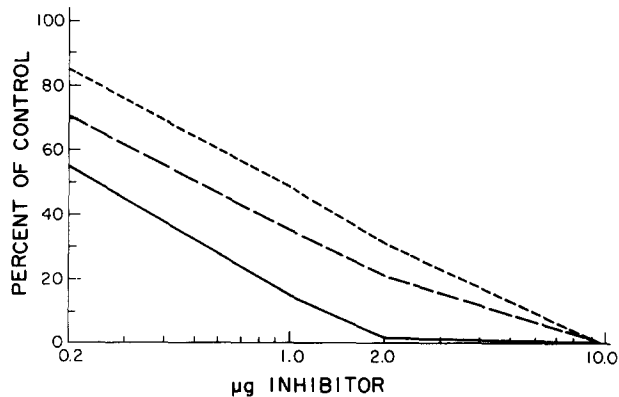


FIG. 5. RIA inhibition assay of an autologous reaction of 91-7 AAI with labeled $F(ab')_2$ fragments of 91-7 antimicrococcal antibodies (headline) inhibited with unlabeled $F(ab')_2$ fragments of antimicrococcal antibodies from 80-44 (—), 80-38 (---), and 91-7 (- - -).

daughter mating, and it was conceivable that the previous AAI response of the mother could have modified or suppressed the expression of some, or all, of the cross-reactive idiotype in the offspring. The naturally induced AAI antibodies of 80-38 and 80-44 were examined for mutual cross-reactivity in reciprocal inhibition experiments similar to those described above. In Fig. 4A, the autologous reaction of 80-38 naturally induced AAI antiserum and labeled 80-38 antimicrococcal $F(ab')_2$ was equally and totally inhibited with either 80-38 or 80-44 unlabeled $F(ab')_2$. (Fig. 4B shows equal and total inhibition of the autologous 80-44 idiotype-AAI reaction with unlabeled 80-38 and 80-44 fragments.)

Assays were done to determine whether the idiotypes recognized by naturally induced AAI antisera from the first group of offspring (89-7, 91-7, 93-7, 94-7, and 95-7) were similar to the idiotypes recognized by the AAI antisera from the offspring of 240the father-daughter mating (80-38 and 80-44). An autologous reaction between 91-7 naturally induced AAI antibodies and labeled 91-7 $F(ab')_2$ was inhibited with unlabeled 91-7, 80-38, and 80-44 $F(ab')_2$ fragments. The results are shown in Fig. 5.

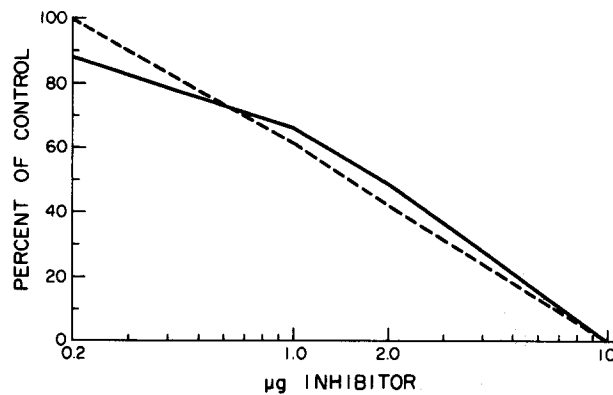


FIG. 6. RIA inhibition assay of an autologous reaction of 91-7 AAI with labeled $F(ab')_2$ fragments of 91-7 antimicrococcal antibodies (headline) inhibited with unlabeled $F(ab')_2$ fragments of antimicrococcal antibodies from 91-7 (----) and 102 (—).

It is evident that all three inhibitors inhibited the reaction in a nearly equivalent way and that the reaction was inhibited totally, showing that both groups of offspring shared the same set of antimicrococcal idiotopes that elicited AAI antibodies in each individual.

The Cross-Reactive Idiotypes Are of Paternal Origin. The apparent identity of the idiotopes recognized by the natural AAI antisera in each of several offspring of rabbit 102, when bred with unrelated females and with one daughter, led us to postulate that these idiotopes could have been present in the antimicrococcal antibodies of 102. Anti-micrococcal antibodies were purified from third-round antimicrococcal antiserum from rabbit 102, and $F(ab')_2$ fragments were made. Some of these fragments were labeled and were assayed with fourth-round AAI serum from 102 and with AAI serum from all AAI-positive offspring (Table I). Equivalent quantities of unlabeled $F(ab')_2$ from 102 and from 91-7 were used to inhibit the reaction between 91-7 AAI and labeled 91-7 $F(ab')_2$. The results (Fig. 6) clearly showed that the autologous 91-7 reaction was inhibited equally by 91-7 and 102 $F(ab')_2$, and the inhibition was complete in both cases.

Discussion

A central feature of the idiootype network theories proposed by Jerne (7) and Hoffman (37) is that immune responses may be regulated within the animal that mounts an immune response by another immune response specific for idiotopes on the antibodies elicited originally and that this system must function naturally. In numerous papers (10, 15, 18-30), it is suggested that AAI antibodies can appear spontaneously after an immune response and can act to regulate the original response. However, the question of the frequency of that type of regulatory response in the normal outbred animal has been largely ignored. In several attempts to study that problem in our laboratory, we consistently failed to detect naturally induced AAI antibodies. We were only successful when we used a labeling method for the antimicrococcal $F(ab')_2$ that did not have reducing agents in the protocol. The idiotopes that were recognized by the natural AAI antibodies in this study were destroyed by ultra-low concentrations of reducing agents. This is documented and

discussed in a separate paper.¹

The experiments in this paper originally were designed to determine what fraction of outbred individuals would show a naturally induced AAI response during routine immunization. The data in Fig. 1 show that 42% of the rabbits we surveyed mounted an AAI response that was detectable with the assay method we used. The percentage of responders could have been >42% because our assay method would detect only IgG AAI antibodies. Possibly some of the animals could have mounted an IgM AAI response that would have gone undetected by our assay, and possibly the animals that did respond inherited an appropriate set of Ir genes, whereas the non-AAI responders were unable to mount a response with the AAI-inducing idiotypic because of a lack of proper Ir genes (6). That could be so because inhibition assays showed that the antimicrococcal antibodies from the rabbits lacking AAI responses did not contain the AAI-inducing idiotypes. Additionally, we could argue that regulatory responses in the AAI-negative animals might have been accomplished by suppressor T cells specific for idiotypic (10, 38). Thus, we conclude from this study that the percentage of normal outbred animals that can mount AAI responses is significant and that idiotypic network regulation may be a common feature of immune responses in outbred animals.

The data presented here show that the set of idiotopes that induced the AAI responses appeared to be identical in each individual. Moreover, the idiotopes or the genetic ability to express the idiotopes appeared to be inherited from the father. Quantitation by titration of the idiotypic content of the unlabeled inhibitors used ensured that fair comparisons of inhibitory capacity were made in each assay. Thus, the idiotypes that were expressed in the AAI-positive animals were the same idiotypes and were expressed at different quantitative levels. No evidence for heteroclicity was found.

One aspect of network theories that has not been discussed in detail is whether all antibody idiotopes are equally capable of eliciting AAI responses as well as anti-idiotypic responses in isologous, heterologous, or xenogeneic individuals. That is a difficult problem experimentally because the triggering threshold concentrations of idiotypic may be different for different idiotopes. This study revealed a direct relationship between the presence of the cross-reactive idiotopes and the presence of a subsequent AAI response, with variations in the levels of the cross-reactive idiotypes from 11.2–41.6% of the total antimicrococcal antibodies in the individuals. That possibly means only that 11.2% concentration of this group of idiotopes was sufficient to trigger AAI synthesis. Alternatively, one might argue that the inheritance of the idiotypic genes also ensured inheritance of the proper AAI genes.

The nonhapten inhibitability of the cross-reactive idiotypes reported here suggests strongly that the idiotypes of the antimicrococcal antibodies were true idiotypes recognized by the naturally induced AAI. Recently, Jerne et al. (39) proposed that two kinds of molecules could behave as anti-idiotypes. One molecule is the classic anti-idiotypic antibody that has a paratope that binds an idiotypic on the first antibody (Ab₁). This reaction may or may not be hapten-inhibitible, depending upon the proximity of the idiotypic to the paratope. The second reaction is due to Ab₁ inducing the production of antibodies by a large set of B cells that have receptors bearing an

¹ Binion, S. B., and L. S. Rodkey. Destruction of antibody idiotopes with ultra-low concentrations of reducing agents. Manuscript submitted for publication.

internal image of the original epitope that elicited Ab₁. This reaction should be hapten inhibitable. In that none of the cross-reactions reported here were hapten inhibitable, we seemed to be dealing with a true system of idiotype-natural AAI reactions. The idiotypic nature of these reactions was further documented here by using extensive controls. Fragments of antigen bound to free antibodies or to B cell receptors can interact with antibodies from other individuals specific for that antigen, and they appear to be anti-idiotypic. That was not the case in these experiments because antimicrococcal antibodies from only 42% of the offspring reacted with each naturally induced AAI. Natural anti-allotype antibodies specific for a noninherited maternal allotype (38, 40) could have bound purified antibodies with that allotype in antibodies from a second individual and appeared to be anti-idiotypic antibodies. That was controlled in these experiments by using controls of labeled F(ab')₂ fragments made for normal IgG isolated from maternal sera or of the same allotype as the mother. Another allotype-related problem that was controlled was the problem of latent allotypes. Antibodies of unexpected allotypes can be elicited by immunization (32), and these can elicit autologous anti-allotype antibodies that will bind to labeled antibodies from other individuals with that allotype. That was controlled in this study by assaying for allotype content of the F(ab')₂ fragments. Finally, the use of F(ab')₂ fragments in these experiments ruled out any complications that could be caused by the presence of rheumatoid factor of either the IgG or the IgM class in the antisera.

We conclude that the data are consistent with an interpretation that idiotype network-mediated regulation is a common feature of immune responses and that the idiotypes that naturally function to induce these immunoregulatory AAI antibodies may have a germ line basis or may be Ir gene controlled.

Summary

Naturally induced auto-anti-idiotypic (AAI) antibody responses specific for anti-micrococcal antibody idiotypes were detected in 42% of the rabbits in a family immunized with *Micrococcus lysodeikticus*. The natural AAI response of each rabbit recognized only a portion (11–41%) of that individual's total antimicrococcal antibody population. Cross-reactions of idiotypes were observed within the group of rabbits exhibiting natural AAI responses. Examination of the basis for the cross-reactions showed that the natural AAI antisera recognized identical idiotopes on the antimicrococcal F(ab')₂ fragments from each rabbit that made an AAI response. The cross-reactive idiotopes were shown to be of paternal origin and were found in the antimicrococcal antibodies of each offspring that synthesized AAI but not in antimicrococcal antibodies of AAI-negative offspring. The data strongly support the idiotypic network concept that naturally induced AAI responses may occur routinely in outbred normal individuals as a result of antigenic stimulation. Further, the data suggest that the induction of regulatory AAI antibody responses in outbred rabbits may depend on the expression of particular germ line idiotopes.

Received for publication 5 May 1982.

References

1. Kunkel, H. G., M. Mannik, and R. C. Williams. 1963. Individual antigenic specificity of isolated antibodies. *Science (Wash. D. C.)* **140**:1218.

2. Oudin, J., and M. Michel. 1963. Une nouvelle forme d'allotypie des globulines γ du s rum de lapin apparemment li e   la fonction et   la sp cificit  anticorps. *C. R. Acad. Sci.* **257**:805.
3. Roland, J., and P.-A. Cazenave. 1979. Mise en evidence d'idiotypes anti-b4 apparant s chez les lapins de ph notype a3+ immunis s contre l'allotype b4. *C. R. Acad. Sci.* **248**:571.
4. Gilman-Sachs, A., S. Dray, and W. Horng. 1980. A common idiotypic specificity of rabbit antibodies to the a2 allotype of the immunoglobulin heavy chain variable region. *J. Immunol.* **125**:96.
5. Rodkey, L. S. 1981. Public and private idiotypes on rabbit antibodies specific for rabbit a locus allotype epitopes. In *The Immune System*. C. M. Steinberg and I. Lefkovits, editors. S. Karger, Basel. **2**:315.
6. 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.
7. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. Inst. Pasteur.* **125c**:373.
8. Rodkey, L. S. 1980. Autoregulation of immune responses via idiootype network interactions. *Microbiol. Rev.* **44**:631.
9. Lewis, G. K., and J. W. Goodman. 1978. Purification of functional, determinant-specific, idiootype-bearing murine T cells. *J. Exp. Med.* **148**:915.
10. Bona, C., and W. E. Paul. 1979. Cellular basis of regulation of expression of idiootype. I. T suppressor cells specific for MOPC 460 idiootype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiootype. *J. Exp. Med.* **149**:592.
11. Cosenza, H., M. H. Julius, and A. A. Augustin. 1977. Idiotypes as variable region markers: analogies between receptors on phosphorylcholine-specific T and B lymphocytes. *Immunol. Rev.* **34**:3.
12. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotypic selectivity in the cooperation between T and B lymphocytes. *Eur. J. Immunol.* **8**:846.
13. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes of lymphocyte interactions. II. Antigen-dependent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. *Eur. J. Immunol.* **8**:853.
14. Bona, C. 1979. Regulation of lymphocyte function by antiidotype antibody. In *The Molecular Basis of Immune Cell Function*. J. G. Kaplan, editor. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands. 161.
15. Sy, M. S., J. W. Moorhead, and H. N. Claman. 1979. Regulation of cell mediated immunity by antibodies: possible role of anti-receptor antibodies in the regulation of contact sensitivity to DNFB in mice. *J. Immunol.* **123**:2593.
16. Tasiaux, N., R. Leuwenkroon, C. Bruyns, and J. Urbain. 1978. Possible occurrence and meaning of lymphocytes bearing auto-anti-idiotypic receptors during the immune response. *Eur. J. Immunol.* **8**:464.
17. Jackson, S., and J. Mestecky. 1979. Presence of plasma cells binding autologous antibody during an immune response. *J. Exp. Med.* **150**:1265.
18. Cosenza, H. 1976. Detection of anti-idiotype reactive cells in the response to phosphorylcholine. *Eur. J. Immunol.* **6**:114.
19. Kelsoe, G., and J. Cerny. 1979. Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation. *Nature (Lond.)*. **279**:333.
20. McKearn, T. J., Y. Hamada, F. P. Stuart, and F. W. Fitch. 1974. Anti-receptor antibody and resistance to graft-versus-host disease. *Nature (Lond.)*. **251**:648.
21. McKearn, T. J., F. P. Stuart, and F. W. Fitch. 1974. Anti-idiotypic antibody in rat

- transplantation immunity. I. Production of anti-idiotypic antibody in animals repeatedly immunized with alloantigens. *J. Immunol.* **113**:1876.
22. Bona, C., R. Lieberman, C. C. Chien, J. Mond, S. House, I. Green, and W. E. Paul. 1978. Immune response to levan. I. Kinetics and ontogeny of anti-levan and anti-inulin antibody response and of expression of cross-reactive idiotype. *J. Immunol.* **120**:1436.
 23. Bona, C., P.-A. Cazenave, and W. E. Paul. 1979. Regulation of anti-TNP response of antiidiotypic and anti-(antiidiotypic) antibodies. *Ann. Immunol. (Paris)*. **130C**:303.
 24. Bona, C., R. Hooghe, P.-A. Cazenave, C. Leguern, 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.
 25. Schrater, A. F., E. A. Goidl, G. J. Thorbecke, and G. W. Siskind. 1979. Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll. I. Occurrence in AKR/J and BALB/c mice of hapten-augmentable, anti-TNP plaque-forming cells and their accelerated appearance in recipients of immune spleen cells. *J. Exp. Med.* **150**:138.
 26. Goidl, E. A., A. F. Schrater, G. W. Siskind, and G. J. Thorbecke. 1979. Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll. II. Hapten-reversible inhibition of anti-TNP plaque-forming cells by immune serum as an assay for auto-anti-idiotypic antibody. *J. Exp. Med.* **150**:154.
 27. Schrater, A. F., E. A. Goidl, G. J. Thorbecke, and G. W. Siskind. 1979. Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll. III. Absence in nu/nu mice: evidence for T-cell dependence of the anti-idiotypic-antibody response. *J. Exp. Med.* **150**:808.
 28. Fernandez, C., and G. Möller. 1979. Antigen-induced strain-specific autoantiidiotypic antibodies modulate the immune response to dextran B 512. *Proc. Natl. Acad. Sci. U. S. A.* **76**:5944.
 29. Cunningham-Rundles, C. 1982. Naturally occurring autologous anti-idiotypic antibodies. Participation in immune complex formation in selective IgA deficiency. *J. Exp. Med.* **155**:711.
 30. Brown, J. C., and L. S. Rodkey. 1979. Autoregulation of an antibody response via network-induced auto-anti-idiotype. *J. Exp. Med.* **150**:67.
 31. Osterland, C. K., E. J. Miller, W. W. Karakawa, and R. M. Krause. 1966. Characterization of streptococcal group-specific antibody isolated from hyperimmune rabbits. *J. Exp. Med.* **123**:599.
 32. Strosberg, A. D., C. Hamers-Casterman, W. Van Der Loo, and R. Hamers. 1974. A rabbit of allotypic phenotype ala2a3b4b5b6. *J. Immunol.* **113**:1313.
 33. Wikler, M. 1975. Isolation and characterization of homogeneous rabbit antibodies to *Micrococcus lysodeikticus* with specificity to the peptidoglycan and to the glucose-N-acetylaminomannaronic acid polymer. *Z. Immunitaetsforsch.* **149**:193.
 34. Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley. 1960. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem.* **89**:230.
 35. McFarlane, A. S. 1958. Efficient trace labeling of proteins with iodine. *Nature (Lond.)*. **182**:53.
 36. Mandy, W. J. 1967. A new serum factor in normal rabbits. III. Specificity for antigenic determinants uncovered by papain or pepsin digestion. *J. Immunol.* **99**:815.
 37. Hoffman, G. W. 1975. A theory of regulation and self-nonsel self discrimination in an immune network. *Eur. J. Immunol.* **5**:638.
 38. Owen, F. L., S. T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T

- cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* **145**:1559.
39. Jerne, N. K., J. Roland, and P.-A. Cazenave. 1982. Recurrent idiotopes and internal images. *EMBO Journal.* **1**:243.
40. Adler, F. L., and R. J. Noelle. 1975. Enduring antibody responses in "normal" rabbits to maternal immunoglobulin allotypes. *J. Immunol.* **115**:620.
41. Hagen, K. L., L. E. Young, R. G. Tissot, and C. Cohen. 1978. Factors affecting natural antiallotype antibody production in rabbits. *Immunogenetics.* **6**:355.