*Proc. Natl. Acad. Sci. USA*
Vol. 74, No. 11, pp. 5122–5125, November 1977
Immunology

# Idiotypic-anti-idiotypic regulation of antibody synthesis in rabbits

**(anti-ribonuclease antibodies/idiotypic repertoire/network hypothesis)**

P.-A. CAZENAVE

Service d'Immunochimie Analytique, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cédex 15, France

*Communicated by Jacques Oudin, July 18, 1977*

**ABSTRACT** **Anti-ribonuclease antibodies (Ab1) of one rabbit were used to prepare in two other rabbits anti-idiotypic antibodies (Ab2) against them. Anti-idiotypic antibodies (Ab3) were prepared against Ab2 of one rabbit in two other rabbits that had the same phenotype as the former three for the allotypic a and b systems. After a rest period of 4 months, the latter two rabbits were immunized against ribonuclease. The antibodies produced (Ab1′) crossreacted with the two anti-idiotypic sera (Ab2) against Ab1, showing an idiotypic similarity between anti-ribonuclease Ab1′ and anti-ribonuclease Ab1. No such crossreaction was observed between the anti-idiotypic sera (Ab2) against Ab1 and any of the 12 unrelated antisera against ribonuclease that were tested. These results suggest (*i*) that rabbits, at least with the same allotypes, possess a closely related idiotypic repertoire; and (*ii*) that the immune system is a network of variable (V)-domains.**

Idiotypy of antibodies has been defined as their property of possessing antigenic specificities that are different both among antibodies of one individual against distinct antigens and among antibodies of different individuals or groups of individuals against the same antigen (1, 2). The first observations of this property were made simultaneously in humans (3) and in rabbits (4).

It has been discussed "whether things seem to happen as though the choice of the idiotypic specificities of the antibodies against a given antigen were, in each individual, the result of lot-drawing among a large number of possibilities, all of which would be common to all individuals of the same species, or at least to all individuals of the same genotype in terms of immunoglobulin allotypy" (5). According to this model, if it were possible to cheat with the lot-drawing, idiotypic similarities that are not normally observed among antibodies of different individuals against the same antigen might eventually be elicited. A way of realizing the conditions favorable to the emergence of similar idiotypes in various rabbits has been suggested by the hypothesis that the immune system is a network of variable (V)-domains (6, 7).

Against anti-RNase antibodies (Ab1) of one rabbit, anti-idiotypic antibodies (Ab2) can be raised in a second rabbit. Theoretically, it is possible to prepare in a third rabbit anti-idiotypic antibodies (Ab3) against Ab2. This third rabbit can then be immunized against RNase and will produce anti-RNase antibodies (Ab1′). The aim of this work is to compare idiotypic specificities of Ab1 and Ab1′.

## MATERIALS AND METHODS

**Animals.** Rabbits were Bouscat Giant with a $(1^{-}2^{-}3^{+})$-$b(4^{+}5^{-}6^{-}9^{-})$ phenotype without known family relationship.

**Immunizations.** Several rabbits were immunized by injection in the hind footpads of 1 mg of ribonuclease emulsified in complete Freund's adjuvant. Two months later, they were boosted by intramuscular injection of the same quantity of antigen emulsified in complete Freund's adjuvant. Animals were bled weekly, and the sera of each rabbit were pooled.

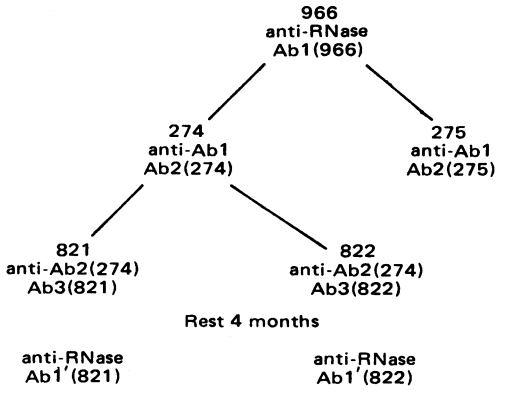


FIG. 1. Summary of the different immunizations.

Anti-RNase antibodies were isolated from the serum of one rabbit (no. 966) by using RNase bound to aminohexyl-Sepharose by means of glutaraldehyde (8). These antibodies will be designated as Ab1(966). Anti-idiotypic sera against Ab1(966) were prepared in rabbits 274 and 275 (9). Immunoglobulin fraction of anti-RNase serum 966, obtained by precipitation with $Na_2SO_4$ (10), was bound to aminohexyl-Sepharose. This immunoadsorbent was used to isolate from sera 274 and 275 anti-idiotypic antibodies against Ab1(966). These antibodies will be designated as Ab2(274) and Ab2(275).

Rabbits 821 and 822 were immunized against Ab2(274) and produced anti-idiotypic antibodies designated Ab3(821) and Ab3(822). After a rest period of 4 months, rabbits 821 and 822 were immunized against RNase following the schedule described above. The anti-RNase antibodies, designated Ab1′(821) and Ab1′(822), were isolated on RNase–aminohexyl-Sepharose. These immunizations are summarized in Fig. 1.

**Antigen-Antibody Reactions and Their Inhibition.** These reactions were studied using $^{125}I$-labeled isolated antibodies and insoluble polymers of antisera (11). The various preparations of antibodies—Ab1(966), Ab2(274), Ab2(275), Ab1′(821), and Ab1′(822)—were labeled with $^{125}I$ by the chloramin T method (12). The antisera were insolubilized by polymerization with ethyl chloroformate (13) with some modifications (14).

**IgG and $(Fab')_2$ Fragment.** IgG were isolated by chromatography on DEAE-cellulose from anti-RNase sera 966, 821, and 822 and anti-idiotypic sera 274 and 275. $(Fab')_2$ fragments were prepared by pepsin hydrolysis and fractionated on Sephadex G-100 (15). $(Fab')_2$ fragments of Ab1(966), Ab2(274), Ab2(275), Ab′(821), and Ab1′(822) were then specifically isolated by immunoadsorption, as described for entire antibodies.

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Abbreviation: Ab, antibody.

Table 1. Binding of labeled Ab2 on crosslinked Ab3(821) and Ab3(822) sera

| $^{125}$I-Labeled antibody | Binding, % | |
|---|---|---|
| | Ab3(821) | Ab3(822) |
| Ab2(274) | 42 | 45 |
| Ab2(275) | 25 | 21 |

## RESULTS

**Properties of Antisera 821 and 822 Directed against Anti-Idiotypic Ab2(274) Antibodies.** Rabbits 821 and 822 immunized against Ab2(274) synthesize Ab3(821) and Ab3(822) which faintly precipitate antiserum 274 but do not precipitate the serum taken in rabbit 274 before immunization. Binding values of $^{125}$I-labeled Ab2(274) on crosslinked antisera 821 and 822 are given in Table 1. The binding is inhibited by antisera 274, by isolated Ab2(274), and by $(Fab')_2$ fragment of Ab2(274), but not by the serum taken in rabbit 274 before immunization. Therefore, Ab3(821) and Ab3(822) have the properties of anti-idiotypic antibodies directed against Ab2(274).

Part of the labeled Ab2(275) binds to crosslinked antisera Ab3(821) and Ab3(822) (Table 1). Ab2(275) partially inhibits the binding of labeled Ab2(274) on crosslinked antisera Ab3(821) and Ab3(822) (Fig. 2). These results suggest that Ab2(275) carries idiotypic determinants with crossreactivity with those recognized on Ab2(274) by antisera Ab3(821) and Ab3(822). However, another possible interpretation will be considered below (*Discussion*).

**Idiotypy of Anti-RNase Antibodies Ab1'(821) and Ab1'-(822).** In Table 2 are given binding values of $^{125}$I-labeled anti-RNase Ab1(966), Ab1'(821), and Ab1'(822) on crosslinked anti-allotypic (anti-a3 and anti-b4) antisera and anti-idiotypic sera Ab2(274) and Ab2(275). There are, in anti-RNase Ab1'-(821) and Ab1'(822), idiotypic determinants* that are recognized by anti-idiotypic sera against Ab1(966). Anti-RNase antibodies isolated from the antisera of five other rabbits do not bind to Ab2(274) and Ab2(275). The proportion of anti-RNase Ab1'(821) that reacts with Ab2(274) and Ab2(275) is larger than among the Ab1(966) antibodies themselves (Table 2).

Anti-RNase sera Ab1'(821) and Ab1'(822) partially inhibit binding of $^{125}$I-labeled anti-RNase Ab1(966) on crosslinked anti-idiotypic Ab2(274) and Ab2(275) (Fig. 3). Similar results are obtained when isolated anti-RNase antibodies or their $(Fab')_2$ fragments are used as inhibitors. Sera taken in rabbits 966, 821, and 822 before immunization and anti-RNase sera of twelve other unrelated rabbits do not inhibit this binding. These results are in good agreement with the binding data presented in Table 2 and confirm a similarity between at least a part of the idiotypic determinants of Ab1(966) against which antisera Ab2(274) and Ab2(275) are directed and idiotypic determinants of Ab1'(821) and Ab1'(822).

To compare the idiotypic specificities of anti-RNase Ab1'-(821) and Ab1'(822), binding of $^{125}$I-labeled Ab1'(821) and Ab1'(822) on crosslinked anti-idiotypic antisera Ab2(274) and Ab2(275) by anti-RNase antisera Ab1(966), Ab1'(821) and Ab1'(822) have been studied (Figs. 4 and 5). Inhibition curves presented in Figs. 3, 4, and 5 show that there is a similarity between the idiotypy of Ab1(966), Ab1'(821), and Ab1'(822), but that this similarity, (*a*) between Ab1(966) and both Ab1'-(821) and Ab1'(822) and (*b*) between the latter two, is only partial.

* An idiotypic determinant is defined as a structure able to combine with a single anti-idiotypic antibody molecule (16). An idiotypic determinant is also called an idiotope (6).

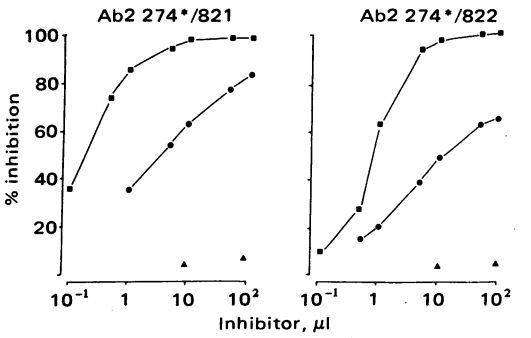


FIG. 2. Inhibition of binding of $^{125}$I-labeled Ab2(274) to crosslinked sera (*Left*) Ab3(821) and (*Right*) Ab3(822) by antisera Ab2(274) (■) and Ab2(275) (●) against Ab1(966) and by serum from rabbit 274 before immunization (▲).

## DISCUSSION

The results presented in this paper show that it is possible to prepare anti-idiotypic antibodies (Ab3) against anti-idiotypic antibodies (Ab2) directed against anti-RNase antibodies (Ab1).

When rabbits chosen at random were immunized against RNase in the usual manner, no crossreactivities were observed between the idiotypes of the anti-RNase antibodies produced. However, the experiments described here show that under special conditions anti-RNase antibodies with related idiotypes can be elicited in rabbits selected at random. Rabbits 821 and 822 were immunized against the isotypes of Ab2, which were themselves directed against the idiotypes of the anti-RNase Ab1(966). When these two rabbits were subsequently immunized with RNase, the anti-RNase (Ab1') produced shared part of the idiotypic determinants of Ab1 from the unrelated rabbit 966. On the basis of the evidence given here, we conclude that this preliminary immunization of 821 and 822 against Ab2 was both necessary and sufficient to bring about this result.

The hypothetical mechanism of the action of the previous anti-idiotypic (anti-Ab2) immunization of rabbits 821 and 822 becomes fairly easy to imagine if the following assumptions are made: (*a*) According to the model cited above there is a large number of idiotypic patterns or rather of idiotypic determinants that can potentially be carried by antibodies synthesized by all allotypically similar rabbits (5). (*b*) There is some idiotypic similarity between anti-idiotypic Ab2 which is, or may be, synthesized, not only by rabbits 274 and 275, but also by all (or most) other rabbits, including 821 and 822. [The actual idiotypic similarity between Ab2(274) and Ab2(275) is separately discussed.] (*c*) According to the hypothesis put forward by Jerne (6, 7), anti-idiotypic antibodies (Ab2) against Ab1 are present in the organism before immunization in faint amounts, sufficient, however, to prevent the emergence of antibodies Ab1 able to react with these anti-idiotypic antibodies.

It follows from these three assumptions that the anti-idiotypic immunization against the idiotypic determinants of Ab2 prior

Table 2. Binding of labeled anti-RNase antibodies on crosslinked antisera

| Crosslinked sera | % binding of labeled antibodies | | |
|---|---|---|---|
| | Ab1(966) | Ab1'(821) | Ab1'(822) |
| Anti-a3 | 80 | 75 | 78 |
| Anti-b4 | 81 | 64 | 77 |
| Ab2(274) against Ab1(966) | 25 | 35 | 27 |
| Ab2(275) against Ab1(966) | 22 | 42 | 28 |

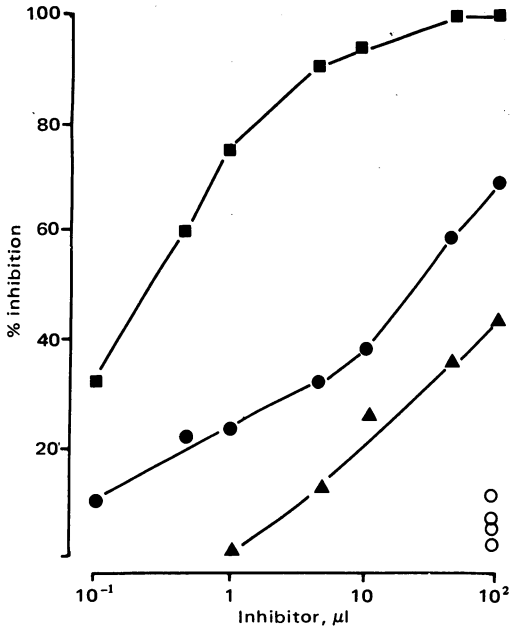


FIG. 3. Inhibition of the binding of $^{125}$I-labeled Ab1(966) to crosslinked Ab2(274) by anti-RNase sera Ab1(966) (■), Ab1'(821) (●), and Ab1'(822) (▲). Inhibition values obtained with anti-RNase sera from four unrelated rabbits are represented (○). Similar values were obtained with eight other anti-RNase sera.

to the anti-RNase immunizations will favor the expression of the Ab1 idiotypic determinants on the anti-RNase antibodies (Ab1') synthesized.

Urbain and his coworkers have independently obtained similar results using a quite distinct system, the *Micrococcus lysodeikticus* carbohydrate system. They have obtained anti-carbohydrate Ab1' antibodies bearing idiotypic specificities similar to those of original anti-carbohydrate Ab1 antibodies (J. Urbain, personal communication).

An example of an idiotype the synthesis of which was increased in mice by a previous injection of anti-idiotypic antibodies has been given by Eichmann and Rajewsky (17). However, (*a*) the anti-idiotypic antibodies used corresponded to the anti-Ab1 instead of the anti-Ab2 of the present experiments; and (*b*) the idiotype the synthesis of which was favored by the injection of anti-idiotypic serum was already known to exist as

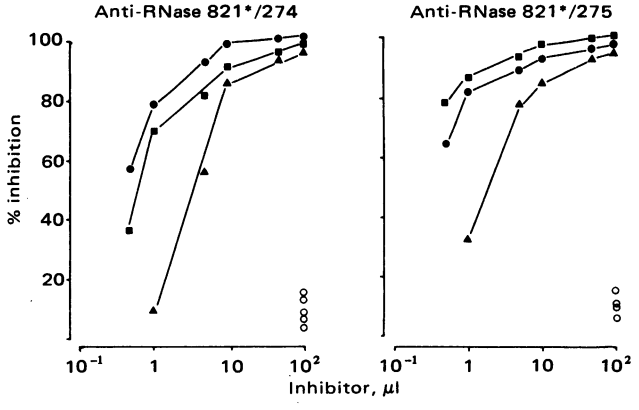


FIG. 4. Inhibition of the binding of $^{125}$I-labeled Ab1'(821) anti-RNase antibodies to crosslinked Ab2(274) (*Left*) and Ab2(275) (*Right*) by anti-RNase sera Ab1(966) (■), Ab1'(821) (●), and Ab1'-(822) (▲). Inhibition values obtained with anti-RNase sera from five unrelated rabbits are represented (○). Similar values were obtained with seven other anti-RNase sera.

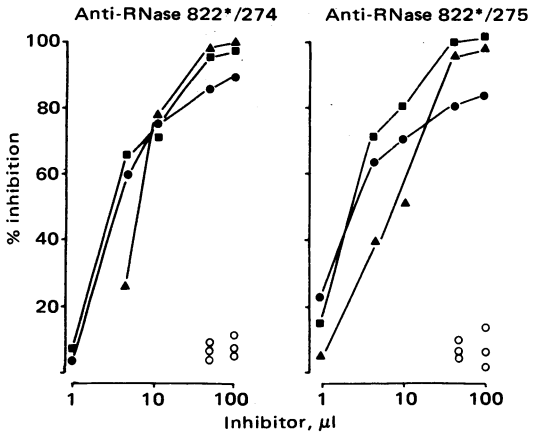


FIG. 5. Inhibition of the binding of $^{125}$I-labeled Ab1'(822) anti-RNase antibodies to crosslinked Ab2(274) (*Left*) and Ab2(275) (*Right*) by anti-RNase sera Ab1(966) (■), Ab1'(821) (●), and Ab1'-(822) (▲). Inhibition values obtained with anti-RNase sera from three unrelated rabbits are represented (○). Similar values were obtained with nine other anti-RNase sera.

a minor component of the anti-group A streptococcal carbohydrate of the mouse strain treated, so that its concentration was only increased.

The mechanism outlined above implies at least some similarity between anti-idiotypic antibodies against Ab1 synthesized by different rabbits. An indication of this similarity is given in this paper: part of Ab2(275) binds to antibodies against Ab2(274) (Table 1) and partially inhibits the binding of labeled Ab2(274) to insolubilized antisera against Ab2(274) (Fig. 2). However, these results could also be explained by a similarity between idiotypic specificities of Ab3(821), Ab3(822), and Ab1(966). In this case the inhibition observed could be the inhibition of the binding of labeled anti-idiotypic Ab2(274) antibodies on crosslinked idiotypes Ab3(821) and Ab3(822).†

It will be of interest to know whether the results presented here in this RNase system and those obtained by Urbain and his coworkers in the *Micrococcus* system are general in the rabbit and whether they can be found in other species. It will also be of interest to know the influence of allotypic background on these phenomena.

I am very grateful to Dr. Jacques Oudin for his continual encouragement and helpful discussion during this work. I thank Prof. N. Jerne for several discussions on idiotypy of antibodies, and Dr. J. S. Ingraham for discussion and help while writing the manuscript. This work was supported by a grant from the Centre National de la Recherche Scientifique to the Service d'Immunochemie Analytique of Institut Pasteur (ER 67, Chef de Service: Dr. J. Oudin) and by a grant from the Université Pierre et Marie Curie.

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† Ab3(821) and Ab3(822) inhibit the binding of Ab1 to crosslinked Ab2 (data not shown). Two interpretations of this result can be drawn: (*a*) Ab3 possesses idiotypic determinants similar to idiotypic determinants of Ab1; or (*b*) the combination of Ab3 with Ab2 causes a steric hindrance which does not allow the binding of Ab1 to Ab2.

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