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A Study of Idiotypic Suppression in Adult Rabbits Immunized with *Salmonella abortus-equi*

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Summary. It is possible to induce idiotypic suppression in adult rabbits immunized with *Salmonella abortus-equi* (*S.a.e.*). Ten months after priming we injected the rabbit with anti-idiotypic serum prepared against its own antibodies to *S.a.e.* and, 3 weeks later, gave it a booster injection of bacteria. A new anti-idiotypic serum was prepared with the serum to *S.a.e.* collected after this boost and was used for the following idiotypic suppression attempt made 10 months after the first one. Using this procedure we succeeded in two successive idiotypic suppression attempts in the same rabbit. In the three attempts we carried out, idiotypic suppression was totally effective, i.e. idiotypes detected by the serum used for the suppression totally disappeared after the suppression, and the suppression lasted during the life of the rabbits (maximum 10 months). This observation is consistent with a suppression resulting from an interaction of anti-idiotypic antibodies with the complementary receptors at the surface of memory cells. This suppression was without effect on antibody to *S.a.e.* titre and on IgG concentration.

Idiotypes detected by the anti-idiotypic serum prepared with the serum to *S.a.e.* collected after the suppression were already present in the serum to *S.a.e.* collected before the suppression. These idiotypes were different from those detected by the anti-idiotypic serum used for the suppression. This observation confirms that idiotypic recognition is confined to a limited number of clonal products, despite the fact that a very heterogeneous antibody population was used for the anti-idiotypic immunization. Thus we did not observe the appearance of new idiotypes produced by previously silent cell clones. All the different idiotypes we detected during the successive idiotypic suppression attempts carried determinants which remained peculiar to each individual rabbit.

INTRODUCTION

Suppression of specific immunoglobulin synthesis by antibodies directed against specific cell receptors is based on the assumption that the combining region of a cell receptor for antigen and the combining site of the antibodies produced against this antigen are very similar or identical. The presence of antigenic determinants such as allotypic determinants (Oudin, 1956a,b, 1960a,b,c) and idiotypic determinants (Oudin, 1966a,b; Oudin and Michel, 1963, 1969a,b,c) in this important region of antibody molecules, and consequently in specific cell receptors, renders this feasible.

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Allotypic suppression was first successfully executed in rabbits (Dray, 1962). Paternal allotypes were strongly suppressed in heterozygous F1 after exposure during foetal or neonatal life to antibodies directed against those allotypes. Since then, allotype suppression has been extensively studied in mice (review by Herzenberg, Jones and Herzenberg, 1974).

Two attempts to suppress idiotype synthesis were successfully carried out, almost simultaneously, by Hart, Wang, Pawlak and Nisonoff (1972) in A/J mice and by Cosenza and Köhler (1972) in BALB/c mice. A high degree of similarity in idiotypic determinants of antibodies against phenylarsonate is observed for almost the individuals of the A/J mouse strain. Hart *et al.* (1972) induced idiotypic suppression in adult A/J mice with an anti-idiotypic serum prepared against anti-phenylarsonate antibodies from other individuals of the same strain. Cosenza and Köhler (1972) used an anti-idiotypic serum directed against a BALB/c myeloma protein which binds phosphorylcholine to induce idiotypic suppression of antibodies to phosphorylcholine in BALB/c mice.

We were interested to know if idiotypic suppression would be possible in adult rabbits immunized with *Salmonella abortus-equi* (*S.a.e.*). Cross-reactivity between idiotypic determinants of antibodies to *S.a.e.* is a rare event in a rabbit population. We attempted to induce idiotypic suppression in the same rabbit which was itself the donor of anti-*S.a.e.* antibodies used to prepare the anti-idiotypic serum. We were also interested to know the effect upon immunoglobulin synthesis and production of antibody to *S.a.e.*, of successive idiotypic suppressions induced in the same rabbit with anti-idiotypic sera prepared with anti-*S.a.e.* sera collected after each new suppression.

MATERIALS AND METHODS

Animals

We used male and female 'Blanc de Bouscat' rabbits.

*Anti-*S.a.e. *and anti-idiotypic immunizations*

Rabbits primed with *S.a.e.* received a total of 7×10^9 killed bacteria in three intravenous injections made at 5-day intervals. They were bled three times at 2-day intervals, the first bleeding being made 6 days after the last injection. For each individual, we pooled these three serum samples. For each subsequent boost, primed rabbits received 4×10^9 bacteria in a single intravenous (i.v.) injection and, beginning 7 days later, were bled three times at 2-day intervals. Again, for each rabbit, we pooled the serum from these three bleedings. Antiserà to *S.a.e.* collected from individual rabbits were used to agglutinate bacteria. These agglutinates were injected into allotypically matched rabbits (Oudin and Michel, 1963) to prepare anti-idiotypic sera.

Schedule of idiotypic suppression

Rabbits subjected to the idiotypic suppression attempt received injections of either anti-idiotypic serum or anti-idiotypic serum IgG fraction. Each received 50 ml of anti-idiotypic serum, or IgG purified from 50 ml of anti-idiotypic serum, distributed equally in six daily injections alternately intraperitoneally (i.p.) and i.v. If we number the day of the first booster injection of bacteria as zero, the first i.p. injection of anti-idiotypic serum was made at day -21 and the last i.v. injection at day -16. The anti-idiotypic serum used for this idiotypic suppression attempt was prepared with the serum to *S.a.e.*

collected immediately after priming, i.e. at day −321. The first of the three consecutive bleedings following the boost occurred at day 7. Rabbits were allowed to rest until day 300 (about 10 months). They were then subjected to another idiotypic suppression attempt, following the same regimen as the previous one, except that the anti-idiotypic serum we used was prepared with their own serum to *S.a.e.* collected at day 7. At day 321 they received a second booster injection of bacteria.

Antigen–antibody precipitation reactions

Precipitation reactions were studied in liquid medium in small glass tubes of 2-mm internal diameter and in gel medium according to the method described by Oudin (1946). Classical precipitation curves and inhibition of direct precipitation were performed according to Kabat and Mayer (1961). Antigen–antibody precipitates were analysed by the Folin's method as modified by Lowry, Rosebrough, Farr and Randall (1951). Nitrogen content of precipitates was determined by the optical density at 750 nm, using the conversion factors established by McDuffie and Kabat (1956). IgG concentration in the diverse rabbit serum samples was determined by the quantitative method devised by Oudin (1946, 1949, 1958), using specific precipitation in gel medium, simple diffusion technique, in glass tubes of 2-mm inner diameter.

Preparation and labelling of S.a.e. *polysaccharide*

Extraction of polysaccharide from bacterial cell walls was performed according to the technique of Freeman (1942). The polysaccharide was coupled with tyramine, according to the technique of Mitchell, Humphrey and Williamson (1972), prior to its iodination with ^{131}I, by the technique of Hunter and Greenwood (1962).

Isoelectric focusing (IEF) analysis

Idiotype–anti-idiotype precipitates were prepared by mixing, at the equivalence ratio, anti-idiotypic serum with the corresponding serum to *S.a.e.* These precipitates were dissolved in 8 M urea and analysed by IEF with Ampholine carrier ampholytes (pH 5–8) in thin layers of 5 per cent polyacrylamide gels (Awdeh, Williamson and Askonas, 1968) containing 8 M urea. The gels were overlaid with ^{131}I-labelled *S.a.e.* polysaccharide. Autoradiography of dried gels showed uptake of ^{131}I-labelled polysaccharide by antibodies to *S.a.e.* which had been precipitated by anti-idiotypic antibodies. This technique has been previously described in detail (Bordenave and Askonas, 1974).

Purification of IgG and idiotypes

IgG was prepared by serum chromatography through diethylaminoethyl–cellulose (DEAE–cellulose Whatman DE-23) columns, according to the technique of Levy and Sober (1960). To prepare immunoadsorbents, crude IgG fractions, obtained by precipitating anti-idiotypic sera at 18 per cent sodium sulphate concentration, were coupled to Sepharose 4B by the cyanogen bromide procedure (Axen, Porath and Ernback, 1967). Idiotypes retained by antibodies to idiotypes in such an immunoadsorbent were released by means of 0·1 N HCl and immediately neutralized.

RESULTS

Four adult rabbits (numbers 702, 708, 709 and 713), primed with *S.a.e.*, received

successive boosts at approximately 10-month intervals. Two rabbits (numbers 702 and 708), used as controls, received only bacterial injections. The two remaining rabbits (709 and 713) were subjected to repeated idiotypic suppression attempts. Three weeks before each boost they received injections of anti-idiotypic serum prepared, in allotypically matched rabbits, with their own serum to *S.a.e.* collected after the previous bacterial boost. The idiotypic serum used for the first idiotypic suppression attempt was prepared with the serum to *S.a.e.* collected after priming.

The serum to *S.a.e.* collected after priming will be referred to as S1 (for serum 1). The corresponding anti-idiotypic serum will be referred to as anti-S1. The serum to *S.a.e.* collected after the first boost and consequently after the first idiotypic suppression attempt made with anti-S1 will be referred to as S2 (for serum 2). The corresponding anti-idiotypic serum will be referred to as anti-S2. In the same way, the serum to *S.a.e.* collected after the second boost and therefore after the second idiotypic suppression attempt made with anti-S2, will be referred to as S3 (for serum 3) and the corresponding anti-idiotypic serum will be referred to as anti-S3.

THE EFFECTIVENESS OF THE IDIOTYPIC SUPPRESSION

Rabbit 709 was subjected to only one idiotypic suppression attempt, and this suppression was effective. The anti-S1 serum which strongly precipitates the S1 serum, in gel medium, does not precipitate the S2 serum (Fig. 1a). We could not inhibit the reaction of S1 with anti-S1 by means of IgG from S2, despite the fact that S2 IgG was added at thirty times the amount of S1 IgG necessary for total inhibition. Thus, after injections of anti-S1, we cannot detect in S2 the idiotypes this anti-S1 recognized in S1.

With the control rabbit (number 702), receiving only injections of bacteria, the anti-S1 precipitates both S1 serum and S2 serum (Fig. 1b) and does not distinguish the S1 serum from the S2 serum in the reaction in gel medium (Fig. 1b).

In gel medium, the anti-709 S2 serum precipitates both 709 S1 and 709 S2 (Fig. 2). It does not distinguish 709 S1 from 709 S2 in this reaction in gel medium (Fig. 2).

We compared (Fig. 3a) the reaction in gel medium of anti-709 S1 and anti-709 S2 with 709 S1 which contains both the idiotypes recognized by anti-S1 and the idiotypes recognized by anti-S2. These idiotypes (Fig. 3a) behave as independent antigens reacting with their corresponding antisera. Contrarily, with the control, anti-702 S1 and anti-702 S2, which both precipitate 702 S1 and 702 S2, do not distinguish 702 S1 idiotypes in the reaction in gel medium (Fig. 3b).

709 S1 idiotypes specifically isolated on an anti-709 S1 immunoadsorbent react with anti-709 S1, in liquid medium, but not with anti-709 S2. 709 S1, totally absorbed by anti-709 S1 immunoadsorbent, does not react with anti-709 S1, but still reacts with anti-709 S2. 709 S2 idiotypes specifically isolated on an anti-709 S2 immunoadsorbent react with anti-709 S2, in liquid medium, but not with anti-709 S1 (Table 1, rabbit 709).

We used the method of isoelectric focusing (IEF) in polyacrylamide gel (Awdeh *et al.*, 1968) which permits analysis of clonal antibodies to compare the isoelectric (pI) spectra of 709 S1 and 709 S2 antibodies to *S.a.e.* specifically precipitated by anti-709 S1 or by anti-709 S2 (Fig. 4a). We also compared the pI spectra of 702 S1 and 702 S2 antibodies to *S.a.e.* specifically precipitated by anti-702 S1 or by anti-702 S2 (Fig. 4b). The pI spectra of antibodies to *S.a.e.* were visualized with iodinated polysaccharide extracted from bacterial cell walls. Anti-idiotypic antibody recognition is still confined (Fig. 4a and

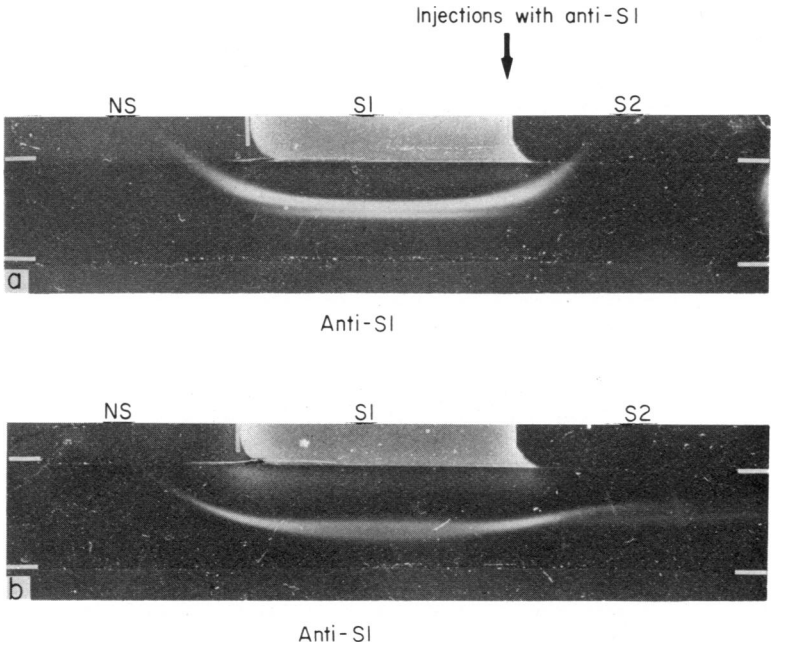

FIG. 1. Reactions in agar (double diffusion in cells with parallel walls). (a) Rabbit number 709. Reaction of the anti-idiotypic serum anti-709 S1 (lower gel layer) with (upper gel layers from left to right) the serum collected from rabbit 709 before any immunization (NS for normal serum) and two different anti-*S.a.e.* serum samples (S1 and S2) respectively collected from rabbit number 709 before and after the idiotypic suppression attempt made with anti-709 S1. (b) Control rabbit number 702. Reaction of the anti-idiotypic serum anti-702 S1 (lower gel layer) with (upper gel layers from left to right) the normal serum (NS) collected from rabbit 702 and two different anti-*S.a.e.* serum samples (S1 and S2) collected from rabbit 702 which was used as control and received only injections of bacteria, following the same regimen as the rabbits subjected to the idiotypic suppression attempt, i.e. 702 S1 was collected after priming and 702 S2 was collected after the first booster injection of bacteria. The white dashes indicate the levels of the interfaces between the various gel layers.

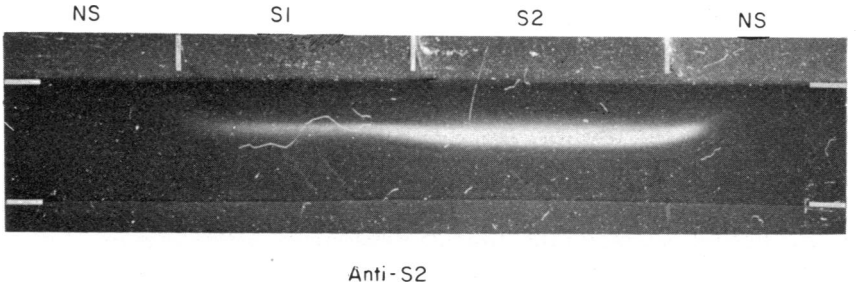

FIG. 2. Reactions in agar (double diffusion in cells with parallel walls). Reaction of the anti-idiotypic serum anti-709 S2 (lower gel layer) with (upper gel layers from left to right) the normal serum (NS) of rabbit number 709 and two different serum samples (S1 and S2) collected from rabbit number 709 before and after the idiotypic suppression attempt made with anti-709 S1. The white dashes indicate the levels of the interfaces between the various gel layers.

b), as it was previously described (Bordenave and Askonas, 1974), to a very limited number of cell clonal products despite the fact that a heterogeneous antibody population was used for the anti-idiotypic immunization. The pI spectrum of 709 S1 idiotypes precipitated by anti-709 S2 is totally different from the pI spectrum of 709 S1 idiotypes precipitated by anti-709 S1 (Fig. 4a, 1 compared with 3). The pI spectra of idiotypes precipitated by the same anti-709 S2 in 709 S1 and in 709 S2 are identical (Fig. 4a, 1 compared with 2). Thus, 709 S1 idiotypes precipitated by anti-709 S1 and those precipitated by anti-709 S2 are different cell clonal products, while 709 S1 idiotypes and 709 S2 idiotypes precipitated by anti-709 S2 are the same cell clonal products. If we compare the heterogeneity of antibodies to *S.a.e.* in 709 S1 and in 709 S2 (Fig. 4a, 4 compared

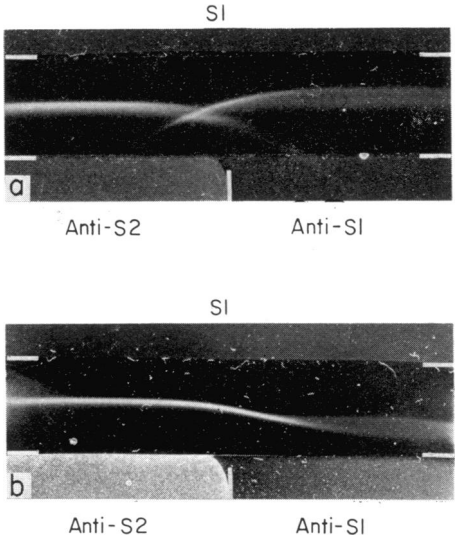

FIG. 3. Reactions in agar (double diffusion in cells with parallel walls). (a) Rabbit number 709. Reaction of the anti-idiotypic serum anti-709 S2 and of the anti-idiotypic serum anti-709 S1 (lower gel layers, from left to right) with (upper gel layer) the serum to *S.a.e.* (S1) collected from the rabbit 709 after priming and consequently before any idiotypic suppression attempt. (b) Control rabbit number 702. Reaction of the anti-idiotypic serum anti-702 S2 and of the anti-idiotypic serum anti-702 S1 (lower gel layers from left to right) with (upper gel layer) the serum to *S.a.e.* (S1) collected from the control rabbit 702 after priming. White dashes indicate the levels of the interfaces between the various gel layers.

with 5), it seems that the suppressed 709 S1 idiotypes are not totally eliminated in 709 S2 (Fig. 4a, 3 compared with 5). Very probably we have the superimposition of several different protein bands in a very close isoelectric region and therefore the disappearance of some bands cannot be detected after the suppression. With the controls (Fig. 4b) there is basically no difference between the pI spectrum of 702 S1 idiotypes precipitated by anti-702 S1 or by anti-702 S2 (Fig. 4b, 3 compared with 1). The intensity of the most anodic component remains high, while the relative amount of the most cathodic component is increased when 702 S1 idiotypes are precipitated by anti-702 S2. In addition, these two anti-idiotypic sera recognized the same clonal antibodies in 702 S1 and in 702 S2 (Fig. 4b, 1 compared with 2, 3 compared with 4).

TABLE 1

REACTION* OF THE SPECIFICALLY ISOLATED IDIOTYPES WITH THE DIFFERENT ANTI-IDIOTYPIC SERA

	S1	S1 idiotypes purified on anti-S1 immunoadsorbent	S1 absorbed out with anti-S1	S2	S2 idiotypes purified on anti-S2 immunoadsorbent	S2 absorbed out with anti-S2	S3	S3 idiotypes purified on anti-S3 immunoabsorbent	S3 absorbed out with anti-S3
Rabbit 709									
Anti-S1	+	+	0	0	0	0			
Anti-S2	+	0	+	+	+	0			
Rabbit 713									
Anti-S1	+	+	0	0	0	0	0	0	0
Anti-S2	+	0	+	+	+	0	0	0	0
Anti-S3	+	0	+	+	0	+	+	+	0

* Presence (+) or absence (0) of idiotypes was tested by allowing sera to *S.a.e.* or purified idiotypes to react, in liquid medium, with the different anti-idiotypic sera.

Consequently, suppressed idiotypes are idiotypically different from idiotypes escaping the suppression. Idiotypes escaping the suppression are not idiotypes produced by previously silent cell clones. They were already present in the serum to *S.a.e.* taken before the suppression but we had not the appropriate antiserum to reveal them.

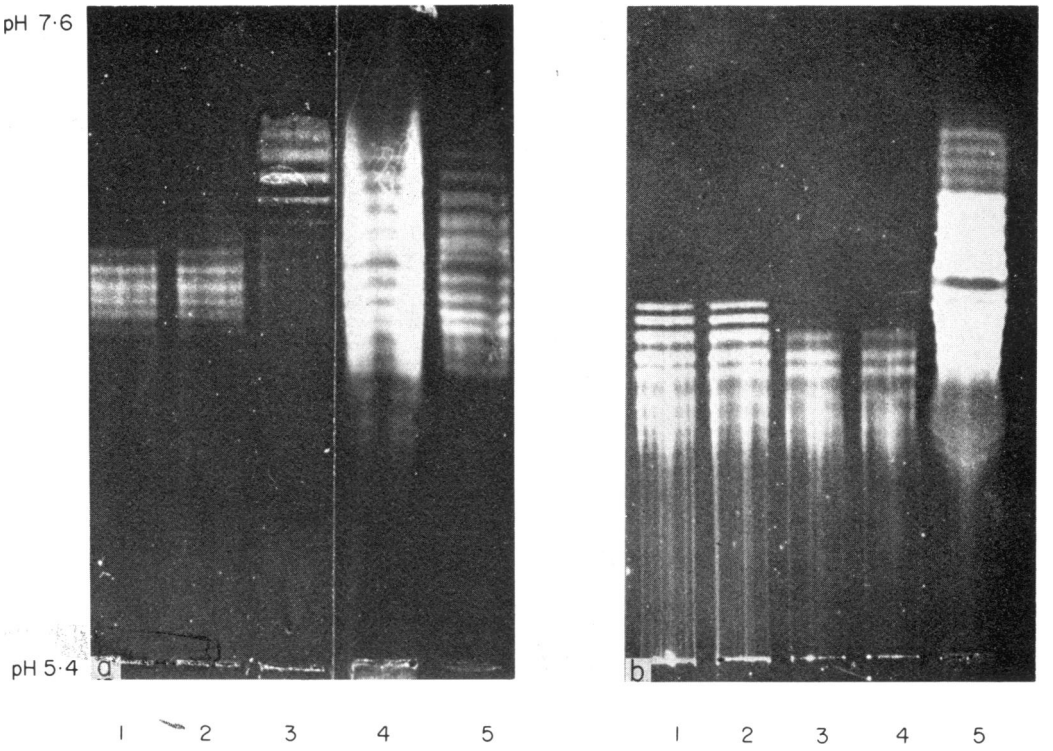

FIG. 4. Isoelectric pI spectra of antibodies to *S.a.e.* in the serum of (a) rabbit number 709 subjected to the idiotypic suppression, in the serum of (b) rabbit number 702 used as control and after precipitation with the corresponding anti-idiotypic sera. IEF was carried out in a thin layer of 5 per cent acrylamide gel using 8 M urea and pH 5–8 Ampholine; the gel was then coated with ^{131}I-labelled polysaccharide, dried and autoradiographed. (a) a1 and a2 = Antibodies to *S.a.e.* precipitated by the same anti-709 S2 in 709 S1 (a1) and in 709 S2 (a2). a3 = Antibodies to *S.a.e.* precipitated by the anti-709 S1 in 709 S1. a4 and a5 = Antibodies to *S.a.e.* in 709 S1 serum (a4) and in 709 S2 serum (a5). (b) b1 and b2 = Antibodies to *S.a.e.* precipitated by the same anti-702 S2 in 702 S1 (b1) and in 702 S2 (b2). b3 and b4 = Antibodies to *S.a.e.* precipitated by the same anti-702 S1 in 702 S1 (b3) and in 702 S2 (b4). b5 = Antibodies to *S.a.e.* in 702 S1 serum.

EFFECT OF TWO SUCCESSIVE IDIOTYPIC SUPPRESSIONS IN THE SAME RABBIT

The same holds true for another rabbit (number 713) which survived long enough for two successive idiotypic suppression attempts. It allowed us to study the reaction of three anti-*S.a.e.* sera (S1 collected after priming, S2 collected after the first idiotypic suppression made with anti-S1 and S3 collected after the second idiotypic suppression for which we used anti-S2) with the corresponding anti-idiotypic sera (anti-S1, anti-S2 and anti-S3). In gel medium, S1 is strongly precipitated by anti-S1, while S2 and S3 are not (Fig. 5a). We could not inhibit the reaction of S1 with anti-S1 by S2 IgG despite the fact that S2

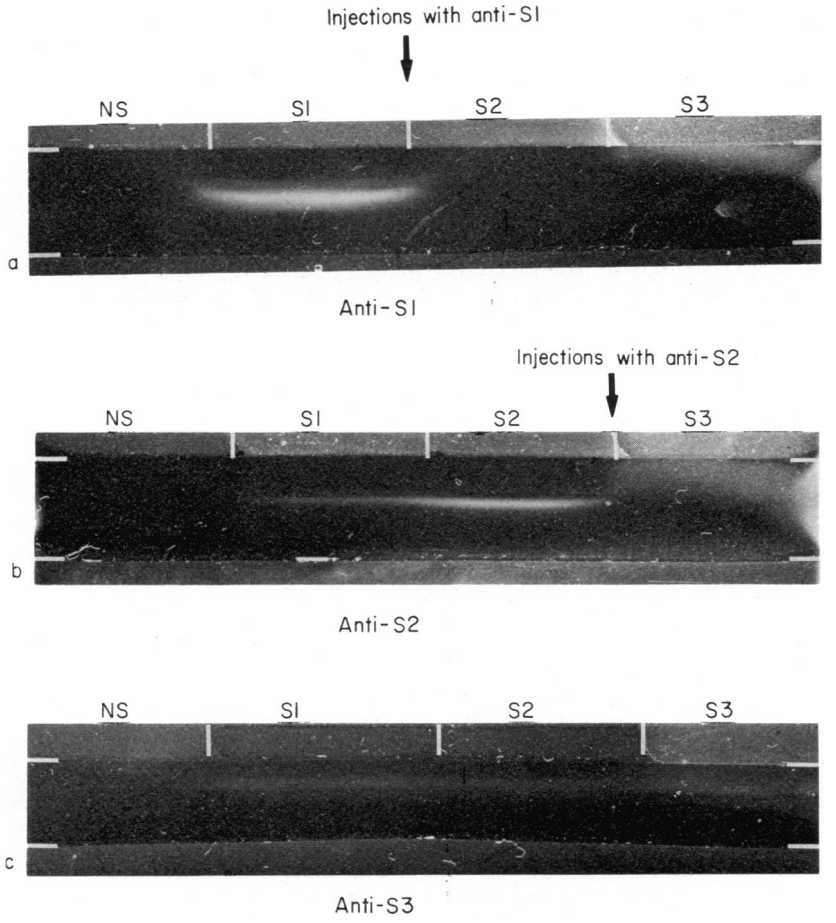

FIG. 5. Reactions in agar (double diffusion in cells with parallel walls). Rabbit number 713. Reaction of the anti-idiotypic sera anti-713 S1, anti-713 S2 and anti-713 S3, respectively (lower gel layer of cell a, cell b and cell c), with (from left to right in upper gel layers) the normal serum (NS) collected from rabbit 713 and three different serum samples collected from rabbit 713 after priming (S1), after the first boost (S2) following the first idiotypic suppression attempt made with anti-713 S1 serum and after the second boost (S3) following the second idiotypic suppression attempt made with anti-713 S2 serum. The white dashes indicate the levels of the interfaces between the various gel layers.

IgG was added at twenty-five times the amount of S1 IgG necessary for total inhibition. In the same manner, we inhibited by only 3 per cent the reaction of S1 with anti-S1 when we added S3 IgG at twenty-five times the amount of S1 IgG necessary for total inhibition. This percentage of inhibition is of the same order as fluctuations observed with controls, i.e. when we used IgG from a non-immunized rabbit. It appears that the suppression produced by anti-S1 persisted for 10 months, i.e. the time between anti-S1 injections and S3 collection. Anti-S2 which precipitates S2 also precipitates S1, but does not precipitate S3 (Fig. 5b). Here again, idiotypes recognized in S2 by the anti-S2 were already present in S1 but the anti-S1 we had was not able to detect them. The idiotypic suppression produced by anti-S2 was efficient: anti-S2 does not precipitate S3 and we could not inhibit the reaction of S2 with anti-S2 by S3 IgG at twenty-four times the amount of S2

IgG necessary for total inhibition. Finally, anti-S3 weakly precipitates the three serum samples S1, S2 and S3 and does not distinguish between them in the reaction in gel medium (Fig. 5c). Thus, S3 idiotypes recognized by this anti-S3 were already present in S1 and in S2.

713 S1 is precipitable by the three anti-idiotypic sera: anti-S1, anti-S2 and anti-S3. The comparison of the reaction, in gel medium, of S1 with these three anti-idiotypic sera shows (Fig. 6) that the idiotypes recognized by each serum behave as independent antigens. The rabbits used as controls died before S3 was collected. Consequently, the reactions of the control serum samples have already been depicted in Fig. 1b and Fig. 3b. 713 S1 idiotypes specifically isolated on an immunoadsorbent prepared with anti-713 S1 react only with anti-S1. S1 serum, after absorption, is not precipitated by anti-S1 in liquid medium, but remains precipitable by anti-S2 and by anti-S3. Similarly, 713 S2 idiotypes specifically purified on an immunoadsorbent prepared with anti-713 S2 are only precipitable by anti-S2, in liquid medium. S2 after absorption is not precipitable by anti-S2, but is still precipitable by anti-S3. Finally, 713 S3 idiotypes specifically purified on an immunoadsorbent prepared with anti-713 S3 are precipitable only by anti-S3 (Table 1, rabbit 713). Thus, the idiotypes recognized by anti-S1, anti-S2 and anti-S3, respectively, are idiotypically different.

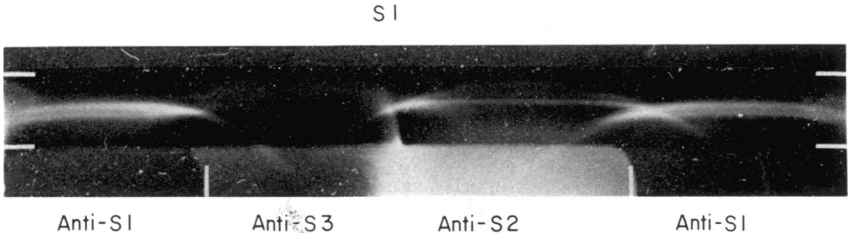

FIG. 6. Reactions in agar (double diffusion in cells with parallel walls). Rabbit number 713. Compared reaction of the anti-idiotypic sera anti-713 S1, anti-713 S3, anti-713 S2 and again anti-713 S1 (from left to right in the lower gel layers) with (upper gel layer) the serum to *S.a.e.* 713 S1 collected from rabbit number 713 after priming and consequently before any idiotypic suppression attempt. The white dashes indicate the levels of the interfaces between the various gel layers.

Consequently, idiotypic suppression in adult rabbits was efficient. Idiotypes recognized by the anti-idiotypic serum used for the suppression totally disappeared after suppression. We showed an example of idiotypic suppression which lasted until the rabbit died, i.e. approximately 10 months. We observed once with one rabbit and twice with another rabbit that idiotypes revealed after idiotypic suppression were not newly appearing idiotypes.

Because rabbits subjected to anti-idiotypic immunization received injections of bacteria agglutinated with anti-bacterial antibodies, they developed an antibody response against *S.a.e.* Antibodies to *S.a.e.* which were present in the anti-idiotypic serum used for the suppression could inhibit the formation of antibodies directed against the same antigen (Rowley and Fitch, 1964). We can rule out this possibility on several grounds. First, antibodies to *S.a.e.* contained in an anti-idiotypic serum represent a very heterogeneous antibody population. It would be surprising if the inhibitory effect of this population was restricted to such a limited number of clonal antibodies, i.e. the suppressed idiotypes. The same argument holds true for antibodies to *S.a.e.* used as immunizing material for

anti-idiotypic immunization and which could remain in the anti-idiotypic serum. Secondly, the serum collected from the rabbits a fortnight after the end of anti-idiotypic serum injections and just before the booster injection of bacteria was unable to agglutinate bacteria. Finally (see below), antibody titres to *S.a.e.* were of the same magnitude in rabbits just subjected to the suppression and in control rabbits.

DOES IDIOTYPIC SUPPRESSION AFFECT IgG CONCENTRATION AND/OR ANTIBODY CONCENTRATION?

IgG was titrated by Oudin's method (1946, 1949, 1958) of specific precipitation in gel medium, using a goat anti-rabbit IgG serum and simple diffusion in glass tubes. In rabbits subjected to idiotypic suppression, IgG concentration increased by about 1 mg/ml between the primary and the secondary response. Approximately the same increase was observed in control rabbits. For rabbit number 713, which was subjected to an additional idiotypic suppression and subsequently received two boosts, we observed an IgG concentration increase of about 0·5 mg/ml between the first and the second boost. The bacterial agglutinating titre of the principal serum samples (S1, S2, S3) was approximately 1: 10240 for rabbits subjected to the suppression and for control rabbits. Idiotypic suppression has no apparent effect on IgG concentration or on antibody titre to *S.a.e.*

For the successive serum samples collected from the two rabbits subjected to the idiotypic suppression, we compared the ratio of the concentration of idiotypes precipitated by the different anti-idiotypic sera to the concentration of antibodies to *S.a.e.* polysaccharide (Table 2). These ratios are approximate because they concerned only precipitating antibodies and they certainly are over-evaluated because antibodies to *S.a.e.* polysaccharide are only a fraction of anti-bacterial antibodies. Idiotypes which are precipitated by a given anti-idiotypic serum represent only a small proportion of the whole population of antibodies to polysaccharide (Table 2). The concentration of the nonsuppressed idiotypes is slightly increased after the suppression (Table 2). That disappearance of the suppressed idiotypes is apparently balanced by an increase in the remaining idiotypes, may explain why the final IgG concentration or the final antibody concentration is not affected, and even increases, after idiotypic suppression.

FREQUENCY OF CROSS-REACTIVITY BETWEEN IDIOTYPES SUCCESSIVELY REVEALED DURING REPEATED IDIOTYPIC SUPPRESSIONS AND ANTIBODIES TO *S.a.e.* OF A GIVEN RABBIT POPULATION

The reaction of an anti-idiotypic serum with the serum to *S.a.e.* used for its preparation is referred to as homologous reaction. Sometimes an anti-idiotypic serum can also precipitate serum to *S.a.e.* from other rabbits. These reactions are referred to as heterologous reactions (Oudin and Bordenave, 1971; Bordenave, 1973).

We compared the frequency of heterologous reactions observed with the different anti-idiotypic sera prepared during this idiotypic suppression attempt and 137 anti-*S.a.e.* sera collected from 137 unrelated rabbits. We observed no heterologous reaction using two different anti-idiotypic anti-709 S1 sera, while we observed four heterologous reactions using two different anti-idiotypic anti-709 S2 sera. We did not observe any heterologous reaction with the single anti-idiotypic serum anti-713 S1. We observed only one heterologous reaction with the single anti-idiotypic serum anti-713 S2 and only one with the single

TABLE 2

COMPARISON, IN THE DIFFERENT SERUM SAMPLES COLLECTED DURING THE SUCCESSIVE IDIOTYPIC SUPPRESSION ATTEMPTS, OF THE CONCENTRATION OF ANTIBODIES TO *S.a.e.* WITH THE CONCENTRATION OF IDIOTYPES PRECIPITATED BY THE DIFFERENT ANTI-IDIOTYPIC SERA

		Idiotypes precipitated by anti-S1		Idiotypes precipitated by anti-S2		Idiotypes precipitated by anti-S3		
	Antibodies to *S.a.e.* polysaccharide (μg N/ml of serum to *S.a.e.*)	Micrograms of N in the precipitate (per ml of S1)	Ratio* (w/w) idiotypes versus antibodies to polysaccharide	Micrograms of N in the precipitate (per ml of S1 or S2)	Ratio* (w/w) idiotypes versus antibodies to polysaccharide	Micrograms of N in the precipitate (per ml of S1 or S2 or S3)	Ratio* (w/w) idiotypes versus antibodies to polysaccharide	Total of the ratios (for the same bleeding)
Rabbit 709								
S1	65	17	1/19	34	1/9·5			3/19
S2	67	0	0	50	1/7			1/7
Rabbit 713								
S1	53	16	1/17	23·5	1/11	8	1/33	6/33
S2	47·5	0	0	30·5	1/8	7·5	1/32	5/32
S3	50	0	0	0	0	9·6	1/25	1/25

* We assumed that we had four anti-idiotypic antibody molecules combined with one idiotype molecule.

anti-idiotypic serum anti-713 S3. Idiotypic determinants carried by the non-suppressed idiotypes do not seem more common in antibodies to *S.a.e.* of this rabbit population than those carried by the suppressed idiotypes.

The heterologous reactions observed with anti-713 S2 and with anti-713 S3 were in fact a reaction between anti-713 S2 and anti-713 S3. Rabbits subjected to the anti-idiotypic immunization are also subjected to an anti-bacterial immunization because they received injections of bacteria agglutinated with anti-bacterial antibodies. Thus, an anti-idiotypic serum is also an anti-bacterial serum and we can observe a heterologous reaction between two anti-idiotypic sera. Anti-713 S3 absorbed with 713 S3 does not precipitate anti-713 S2, while anti-713 S2 absorbed with 713 S2 is still precipitable by anti-713 S3. Consequently, the antibodies of this heterologous reaction are in anti-713 S3, while the antigen is in anti-713 S2. In the reaction in gel medium, anti-713 S3 does not distinguish 713 S3 from anti-713 S2 (Fig. 7). To totally inhibit the reaction of 713 S3 with anti-713 S3, we need the same amount of 713 S3 or of anti-713 S2. Thus, the same idiotypic determinants are shared by antibodies to *S.a.e.* in 713 S3 and by antibodies to *S.a.e.* in anti-713 S2. This observation can explain, in this precise case, why only a part of the antibodies to *S.a.e.* used for an anti-idiotypic immunization are immunogenic.

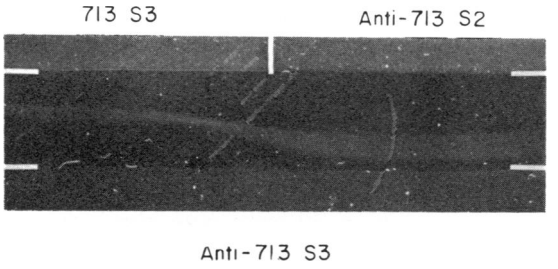

FIG. 7. Reactions in agar (double diffusion in cells with parallel walls). Rabbit number 713. Reaction of the anti-idiotypic serum anti-713 S3 (lower gel layer) with 713 S3 serum and anti-713 S2 serum (from left to right in the upper gel layers). The white dashes indicate the levels of the interfaces between the various gel layers.

DISCUSSION

It is possible to induce idiotypic suppression in adult rabbits immunized with *Salmonella abortus-equi* (*S.a.e.*). Three weeks before a booster injection of bacteria, rabbits received injections of anti-idiotypic serum prepared with their own serum to *S.a.e.* collected after priming. Idiotypes revealed by this anti-idiotypic serum in the serum to *S.a.e.* collected before the suppression, totally disappeared in the serum to *S.a.e.* collected after the suppression. The anti-idiotypic serum prepared with the serum to *S.a.e.* collected after the suppression allowed us to detect idiotypes present in the serum to *S.a.e.* collected before the suppression and in the serum to *S.a.e.* collected after the suppression. These idiotypes were different from those detected by the anti-idiotypic serum used for the suppression. Thus, idiotypes revealed after the suppression are not products of new cell clones previously silent. They are the products of clones already present before the suppression. Suppressed idiotypes and idiotypes escaping suppression showed different pI spectra upon IEF analysis and, in each case, the pI spectrum was restricted to a very limited number of cell clonal products.

Anti-idiotypic antibodies probably suppress idiotype production by combining with

memory cells bearing the corresponding determinants rather than by combining directly with idiotypes secreted by these cells (central block opposed to peripheral block) (Hart *et al.*, 1972; Rowley, Fitch, Stuart, Köhler and Cosenza, 1973). The cells may be either destroyed or diverted from idiotype production. In our experiment, once established, idiotypic suppression persisted for the life time of the rabbit. The half life of rabbit IgG in the rabbit is 6 days (Spiegelberg and Weigle, 1965). In one instance, the suppressed idiotypes did not reappear despite the fact that the rabbit lived for 10 months after the suppression and received two booster injections of bacteria. After 10 months, passively injected anti-idiotypic antibodies were certainly cleared out of the blood circulation of the rabbit and therefore could not act as in a peripheral block by neutralizing freshly produced idiotypes.

Two successive idiotypic suppression attempts were successful in the same rabbit. The same kind of results were repeatedly observed, i.e. idiotypes detected by the serum used for the suppression disappeared after the suppression and idiotypes detected after the successive suppressions were not new idiotypes. They were idiotypically different from idiotypes revealed by the serum used for the suppression, but they were already present in the rabbit before the suppression although, at that time, we did not have an antiserum to reveal them. Rabbits seemed to have a broad array of idiotypes for antibodies to *S.a.e.* During an anti-idiotypic immunization, only a very restricted number of these idiotypes were immunogenic despite the fact that a very heterogeneous idiotype population was used.

Idiotypes change infrequently during a rabbit's life time (Oudin and Michel, 1969a,c; MacDonald and Nisonoff, 1970; Oudin and Bordenave, 1971). We had hoped to increase the frequency of new idiotypes by suppressing the production of idiotypes detected by a given anti-idiotypic serum. Idiotypic suppression was confined to a very restricted number of cell clonal products compared to the heterogeneity of the antibodies to *S.a.e.* We did not observe the appearance of new idiotypes, but rather an increase in the concentration of idiotypes already produced that escaped suppression. The life span of a rabbit is, unfortunately, not long enough for one to expect to progressively suppress all the idiotypes expressed after priming and observe the effect, upon immunoglobulin synthesis and antibody synthesis, of such a suppression.

We can compare our results with the results of idiotypic suppression in mice. In A/J mice, idiotypic determinants of antibodies to the phenylarsonate group possess a high degree of cross reactivity. It is possible to induce idiotypic suppression in newborn or adult mice with an anti-idiotypic serum prepared against antibodies to phenylarsonate from other individuals of the same strain (Hart *et al.*, 1972; Pawlak, Hart and Nisonoff, 1973, 1974; Hart, Pawlak and Nisonoff, 1973). The idiotypes revealed after such suppression are peculiar to each individual mouse (Hart *et al.*, 1973). Unfortunately, it was not reported if idiotypes revealed after the suppression were already expressed in the immune serum collected before the suppression. It is therefore not known if the 'new' idiotypes were produced by clones previously silent or clones escaping from the suppression. Different results were obtained with antibodies to phosphorylcholine in BALB/c mice. Injections of anti-idiotypic antibodies to TEPC 15, a BALB/c myeloma protein which binds phosphorylcholine, totally abolished the antibody response to phosphorylcholine in normal BALB/c mice. Cell clones producing antibody to phosphorylcholine did not appear after suppression (Cosenza and Köhler, 1972; Lee, Cosenza and Köhler, 1974). The number of V genes coding for antibodies to phosphorylcholine in BALB/c mice is

very limited, while the number of V genes coding for antibodies to *S.a.e.* in rabbits is certainly very large.

In rabbits immunized with *S.a.e.*, we sometimes observed that an anti-idiotypic serum which precipitated the serum to *S.a.e.* used for its preparation (homologous reaction) can also precipitate serum to *S.a.e.* from other rabbits (heterologous reaction). We looked for heterologous reactions with anti-*S.a.e.* sera collected from a given population of unrelated rabbits. We observed no significant difference in the frequency of heterologous reactions when the sera to *S.a.e.* were reacted with anti-idiotypic sera prepared before the suppression or with anti-idiotypic sera prepared after each successive suppression. Compared with A/J mice, it would be possible that the initially detected rabbit idiotypes (using antisera prepared by isoimmunization) were those bearing the least common determinants in a given rabbit population. Likewise, idiotypes detected after the successive suppressions would bear determinants more and more frequently found in the rabbit population. For the rabbit, a significantly increased frequency of heterologous reactions is not observed; all the different idiotypes we detected had determinants which remained peculiar to one individual.

In the rabbits subjected to the anti-idiotypic immunization, the antibody response is restricted to a limited number of idiotypes, despite the fact that a very heterogeneous idiotype population was used for the immunization. Some of the injected idiotypes might bear determinants having some similarity with those of antibodies to *S.a.e.* of the rabbits subjected to the immunization. This resemblance could prevent the immunized rabbits from mounting an antibody response against these idiotypes. We were lucky enough to observe a case of identity between idiotypic determinants on some of the antibodies to *S.a.e.* used for the anti-idiotypic immunization and those on some antibodies to *S.a.e.* of the rabbit being immunized. This rabbit developed antibodies against other idiotypes in the whole population used for the immunization.

Another possibility is suggested by the clonal dominance phenomenon observed during successive clonal transfers into irradiated mice (Askonas and Williamson, 1972). A cell clone producing antibodies to the dinitrophenyl group was selected and propagated into several generations of irradiated CBA mice (Askonas, Williamson and Wright, 1970). During all the time that the cells of this selected clone produced a high level of antibodies with high affinity, they exerted a kind of dominance on the other cell clones which could not express their potentialities. When the strong cell clone degenerated progressively, its clonal dominance diminished and the response of the irradiated recipients became heterogeneous. This kind of phenomenon could be very pronounced during anti-idiotypic responses. Thus, the first cell clones producing anti-idiotypic antibodies would prevent the other cell clones from expressing their potentialities. The degeneration of the dominant clones could not happen during all the immunization period and the anti-idiotypic response could remain limited to a very small number of idiotypes.

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