In rabbits which are heterogeneous with respect to antigenic determinants on serum immunoglobulins (Ig), prenatal or neonatal exposure to antibodies specific for one allelic allotype leads to a long-lasting suppression of that type with compensatory production of the alternate allelic product. This phenomenon, called allotype suppression, was first described by Dray in 1962 (1). Although many studies since then have amply verified and further characterized Dray's original observations (reviewed by Mage in reference 2), the mechanism of suppression remains obscure. By using specific antiallotype sera it has been possible to identify b locus determinants on T2-neutralizing antibodies formed by spleen cells of heterozygous rabbits in vitro and to demonstrate that cells from b4b5 rabbits which were deficient in their ability to produce b4 Ig because of exposure to anti-b4 in utero formed anti-T2 antibodies which appeared to be entirely of the nonsuppressed type, namely, b5 (3). However, when such cells were cultured in the presence of anti-b5 serum made in b4 rabbits, b5 anti-T2 production was suppressed, while highly significant amounts of b4 anti-T2 were formed, indicating that the anti-b5 serum had caused release of the mechanism responsible for allotype suppression.

In the present study, the more general applicability of this phenomenon has been verified by culturing the spleen cells of b4-suppressed b4b6 rabbits with b4 anti-b6 serum. Results entirely analogous to those previously observed with the b4b5 system are reported. A further extension of the experimental conditions for bringing about the reversal of b4 suppression by using other rabbit antisera specific for the nonsuppressed type has revealed the participation of two factors in this process: antibody activity against the nonsuppressed allotype and normal b4 Ig.

Materials and Methods

Animals. Rabbits of known a and b locus allotypes were bred at this Institute.

Determination of Ig with Specific Allotypic Markers in Serum. The method for determining serum levels of Ig with specific allotypic markers using the technique of hemagglutination-inhibition (HI) has been described in a previous publication (3).
Preparation of Antiallotype Sera. Antisera specific for the b5 marker were prepared by immunizing a1a1/b4b4 or a2a2/b6b6 rabbits with a1a1/b5b5 or a2a2/b5b5 Ig, respectively. Anti-b6 serum was obtained by immunization of a3a3/b4b4 or a2a2/b5b5 rabbits with a3a3/b6b6 or a2a2/b6b6 Ig, respectively. Details of the immunization procedures have been given previously (3). The specificity of each serum was determined by hemagglutination and HI tests against a large number of sera from rabbits of known allotypes. The well known cross-reaction between b5 and b6 determinants was observed consistently when antisera were raised in b4 rabbits. However, no trace of cross-reaction between b4 and either b5 or b6 was ever seen. All sera were heated at 56°C for 30 min before use. Sera to be added to cultures were sterilized by passage through Millipore filters of 0.45μm pore diameter.

Culture Methods. Spleen cells from unprimed rabbits were cultured according to the technique of Mishell and Dutton (4) with the modifications described by Meiss and Fishman (5) and by Adler (3). Two lots of fetal calf serum (FCS) were used throughout these experiments (lots no. 81689 and no. 82082, Microbiological Associates, Bethesda, Md.) and were of equivalent efficacy in supporting the response to solubilized T2 phage (S-T2) preparations. Antiallotype serum was diluted in Eagle's minimum essential medium (MEM) and added at culture initiation in a vol of 0.03 ml to each culture consisting of 2 x 10^7 nucleated spleen cells. After 1 h of incubation under tissue culture conditions, S-T2 (0.2 ng phage protein nitrogen [PPN]) in a vol of 0.04 ml, and 0.05 ml of FCS were added to each 1 ml culture. Experiments were terminated at the end of 4 or 5 days, and the supernatant fluids, clarified by centrifugation, were stored at -20°C for assays of T2-neutralizing activity.

Neutralization Assays. The procedure used for determining the highest dilution of tissue culture fluid capable of neutralizing 30% of a test dose of T2 phage (NT, .), using amplification by specific antiallotype serum, was exactly as outlined in a previous report (3). Except when otherwise designated, each experimental group consisted of three identically treated cultures. The reproducibility of the response to S-T2 by replicate cultures and the significance of neutralizing activities obtained have been described in previous publications from this laboratory (3, 6).

Results

Effect of Anti-b6 Serum made in b4 Rabbits on the Responses of Spleen Cells from b4-Suppressed b4b6 Rabbits to S-T2. A series of experiments was undertaken in an effort to demonstrate that the release from allotype suppression in vitro reported previously (3) could be observed using a different combination of b loci specificities. For this purpose homozygous b6 female rabbits were immunized against b4 Ig and then bred with b4 males. The resulting b4b6 offspring were first bled at 8 wk of age and their sera assessed for Ig with the b4 and b6 markers. At that age, b4 was usually below the level of detectability (less than 2 μg per ml), and b6 Ig levels were elevated in comparison to normal b4b6 rabbits of the same age. At ages varying from 2½ to 8 mo the spleens of these b4-suppressed b4b6 rabbits were used to establish cultures to which decreasing amounts of b4 anti-b6 serum, diluted in MEM, were added. An optimal immunogenic dose of S-T2 (0.2 ng PPN) and FCS (5% of the culture volume) were added after 1 h of incubation. Each experiment was terminated after 4 or 5 days, and the tissue culture fluids were assayed for anti-T2 activity with the b4 and b6 specificities by using anti-b4 or anti-b6 serum as an amplifying reagent in the neutralization assay. Table I illustrates the results obtained in three such experiments. The ratio of b6 to b4 Ig in the serum at the time of sacrifice indicates the relative degree of b4 suppression. Normal b4b6 rabbits have serum b6/b4 ratios of 0.5–1.0. In each instance, no anti-T2 activity attributable to b4 was formed in the control cultures without anti-b6 serum. However, in the presence of anti-b6 serum there was a proportional inhibition of b6 anti-T2 expression with the concomitant generation of b4 anti-T2 activity. Similar results were obtained in experiments with the spleen cells of four other
**Table I**

**Effect of b4 Anti-b6 Serum on the Responses of b4-Suppressed b4b6 Spleen Cells to S-T2**

| Spleen donor (age in months) | b6/b4 Ratio in serum* | b4 Anti-b6 serum added to culture | 1/NT<sub>b2</sub> b4 Anti-T2: | b6 Anti-T2: |
|-----------------------------|-----------------------|---------------------------------|-----------------------------|-------------|
| D-232 (3 mo)                |                       |                                 |                             |             |
| > 360                       | 30                    | <4§                             | <4                         |             |
|                             | 12                    | <4                              | <4                         |             |
|                             | 6                     | <4                              | <4                         |             |
|                             | 3                     | 16-32                           | 16-32                      |             |
|                             | 0                     | <4                              | 128-256                    |             |
| D-239 (4.5 mo)              |                       |                                 |                             |             |
| 144                         | 30                    | 32-64                           | <4                         |             |
|                             | 12                    | 32-64                           | <4                         |             |
|                             | 6                     | 32-64                           | <4                         |             |
|                             | 3                     | 16-32                           | 8-16                       |             |
|                             | 1.5                   | 8                               | 32-64                      |             |
|                             | 0                     | <4                              | 64-128                     |             |
| D-285 (4 mo)                |                       |                                 |                             |             |
| 15                          | 30                    | <4                              | <4                         |             |
|                             | 12                    | 64-128                          | <4                         |             |
|                             | 6                     | 32-64                           | 4-8                        |             |
|                             | 3                     | <4                              | 8                          |             |
|                             | 0                     | <4                              | 16-32                      |             |

* Serum obtained on the day of sacrifice.
‡ Each determination represents the mean of triplicate cultures.
§ The lowest dilution tested was 1:4, and <4 indicate no significant neutralization at that dilution.

b4-suppressed b4b6 rabbits not shown here. In some cases, an optimal dose effect in the suppression-releasing activity of anti-b6 serum was observed, as is seen here with the cells of rabbit D-232 and to a lesser extent D-285. This seemed to be a property of the cells rather than of the releasing antiserum. With spleen cells from highly suppressed rabbits (b6/b4 > 300) it was usually found that the amount of b4 anti-b6 serum that would bring about release was rather critical, as seen with rabbit D-232. However, reversal of b4 suppression also did not occur in the presence of higher concentrations of anti-b6 serum on some occasions when less well-suppressed donors were used, e.g., D-285 in Table I.

**Effect of b6 Anti-b5 Serum on the Responses of b4-Suppressed b4b5 Spleen Cells to S-T2.** In an earlier publication, spleen cells of b4-suppressed b4b5 rabbits were shown to produce anti-T2 neutralizing antibodies having the b4 marker as a result of treatment with anti-b5 serum made in b4 rabbits (3). In the previous section, analogous results were reported when cells from b4-suppressed b4b6 rabbits were cultured in the presence of anti-b6 serum taken from b4 rabbits. Experiments were now undertaken to determine if anti-b5 serum made in rabbits of a type other than b4 would be effective in bringing about release from b4 suppression in b4b5 cells. Table II presents data exemplifying the results...
obtained when spleen cells of such animals were incubated with anti-b5 serum made in rabbits of allotype b6. Although such sera significantly depressed the b5 anti-T2 responses of these cells, no release from b4 suppression was seen except with the cells of donor D-54. This rabbit appeared to be poorly suppressed with regard to b4 at the time of sacrifice, as indicated by the low serum b5/b4 ratio of 15. Of a total of nine b4-suppressed b4b5 rabbits tested in this manner with three lots of b6 anti-b5 serum, only two (including D-54) exhibited low titers of b4 anti-T2 activity. The second rabbit of this group had a b5/b4 ratio of 5.

Effect of b5 Anti-b6 Serum on the Responses of b4-Suppressed b4b6 Spleen Cells to S-T2. In a parallel series of experiments, cells of b4-suppressed b4b6 rabbits were treated with anti-b6 serum made in b5 rabbits. The results obtained in four out of a total of six such experiments are given in Table III. It can be seen that with cells from two well-suppressed rabbits (D-234 and D-239), no escape from b4 suppression occurred with any concentration of b5 anti-b6 serum tested. However, D-240 and D-250 (with b6/b4 ratios of 36 and 30, respectively) formed low but significant amounts of b4 anti-T2 in the presence of the higher concentrations of anti-b6 serum used. The two remaining donors not represented in this table had b6/b4 ratios of 51 and 60 at time of sacrifice and their spleen cells exhibited no escape from allotype suppression when cultured with b5 anti-b6 serum.

Reversal of b4 Suppression by a Mixture of Normal b4 Serum with b5 Anti-b6 or b6 Anti-b5 Serum. The observations made up to this point, i.e., that (a) b4
RELEASE FROM ALLOTYPE SUPPRESSION

TABLE III

Effect of b5 Anti-b6 Serum on the Responses of b4-Suppressed b4b6 Spleen Cells to S-T2

| Spleen donor (age in months) | b6/b4 Ratio in serum | b5 Anti-b6 serum added to culture | 1/NTb6 | b4 Anti-T2: b6 |
|-----------------------------|----------------------|----------------------------------|--------|---------------|
|                             | µl                   |                                  |        |               |
| D-234 (5.5 mo)              | 360                  | 30                               | <4     | <4            |
|                             |                      | 12                               | <4     | <4            |
|                             |                      | 6                                | <4     | 4             |
|                             |                      | 3                                | <4     | 8             |
|                             |                      | 1.5                              | <4     | 8-16          |
|                             |                      | 0                                | <4     | 64-128        |
| D-239 (4.5 mo)              | 144                  | 30                               | <4     | <4            |
|                             |                      | 12                               | <4     | <4            |
|                             |                      | 6                                | <4     | 16-32         |
|                             |                      | 3                                | <4     | 32-64         |
|                             |                      | 0                                | <4     | 64-128        |
| D-240 (5 mo)                | 36                   | 6                                | 4      | 8-16          |
|                             |                      | 3                                | <4     | 16-32         |
|                             |                      | 1.5                              | <4     | 32-64         |
|                             |                      | 0                                | <4     | 32-64         |
| D-250 (6 mo)                | 30                   | 30                               | 8      | <4            |
|                             |                      | 0                                | <4     | 32-64         |

* Serum obtained on day of sacrifice.

§ Each determination represents the mean titer of three identical cultures.

anti-b5 or b4 anti-b6 serum readily brought about a reversal of b4 suppression in spleen cells from b4-suppressed b4b5 or b4b6 animals, respectively, and that (b) b6 anti-b5 or b5 anti-b6 serum was ineffective with the same respective cell types unless the rabbits were at the time of sacrifice well advanced in their spontaneous escape from suppression, suggested a possible role for normal serum b4 Ig in this phenomenon. Therefore, experiments were undertaken to determine if the cells of b4-suppressed b4b5 or b4b6 rabbits would form b4 anti-T2 antibodies when treated with a mixture of normal b4 serum and b6 anti-b5 or b5 anti-b6 serum, respectively. Representative experiments illustrating these two sets of conditions are shown in Tables IV and V. It can be seen from Table IV that the addition of normal b4 serum together with anti-b5 serum from a b6 rabbit to cultures of spleen cells from a b4-suppressed b4b6 rabbit resulted in the formation of T2 neutralizing antibodies with b4 specificity. No b4 anti-T2 activity was demonstrable in fluids obtained from cultures incubated with the b6 anti-b5 serum alone. Normal b4 serum had no effect on the responses of b4-suppressed cells, as has been observed repeatedly in the course of these studies (3). The data presented in Table V demonstrate that a mixture of normal b4 serum and b5 anti-b6 serum could bring about a reversal of b4 suppression in b4-suppressed b4b6 cells, while the anti-b5 serum alone was completely ineffective.
Table IV

Effect of a Mixture of b6 Anti-b5 Serum and Normal b4 Serum on the Responses of b4-Suppressed b4b5 Spleen Cells to S-T2

| b6 Anti-b5 serum/culture | b4 Normal serum/culture | 1/NT₄₅* |
|--------------------------|-------------------------|---------|
| µl                       | µl                      | b4      | b5 Anti-T2: |
| 30                       | 0                       | <4      | <4         |
| 12                       | 0                       | <4      | ND         |
| 6                        | 0                       | <4      | 8–16       |
| 3                        | 0                       | <4      | 16         |
| 30                       | 30                      | <8      | <4         |
| 12                       | 30                      | 16      | 8          |
| 6                        | 30                      | 8–16    | 8–16       |
| 3                        | 30                      | <4      | 16         |
| 0                        | 30                      | <4      | 64         |

Spleen cells used were from a b4-suppressed b4b5 rabbit (age 4 mo) whose serum b6/b4 ratio of 25 on the day of sacrifice.
* Mean of triplicate cultures.

Table V

Effect of a Mixture of b5 Anti-b6 Serum and Normal b4 Serum on the Responses of b4-Suppressed b4b6 Spleen Cells to S-T2

| b5 Anti-b6 serum/culture | b4 Normal serum/culture | 1/NT₄₅* |
|--------------------------|-------------------------|---------|
| µl                       | µl                      | b4      | b6 Anti-T2: |
| 30                       | 0                       | <4      | <4         |
| 12                       | 0                       | <4      | <4         |
| 6                        | 0                       | <4      | <4         |
| 30                       | 30                      | 32      | <4         |
| 12                       | 30                      | 64      | <4         |
| 6                        | 30                      | 32–64   | <4         |
| 0                        | 30                      | <4      | 128        |

Spleen cells used were from a 5-mo old b4-suppressed b4b6 rabbit whose serum b6/b4 ratio at the time of sacrifice was 51.
* Mean of triplicate cultures.

Table VI presents additional data which indicate a dose effect of the normal b4 serum. No b4 anti-T2 production occurred in the presence of any concentration of the b5 anti-b6 serum tested, although there was almost complete suppression of the b6 anti-T2 response. The admixture of at least 3 µl of normal b4 serum in this experiment was required to bring about detectable reversal of b4 suppression, and significantly more b4 anti-T2 activity was formed when 30 µl of normal b4...
serum was present. In this experiment release from b4 suppression occurred as a result of treatment with 12 µl of anti-b6 serum per culture, but not with 30 µl. The occurrence of such optimal zones has been noted in an earlier section. Findings such as those just cited suggested that in assaying the potency of b4 anti-b5 or b4 anti-b6 sera in releasing b4 suppression in b4b5 or b4b6 rabbits suppressed with regard to b4 production (ref. 3 and Table I), normal b4 Ig in the antiallotype serum might be a limiting factor. This possibility was tested using protocols such as that presented in Table VII. In this experiment, incorporation into the culture medium of as little as 1.5 µl of the b4 anti-b6 serum resulted in the production of significant anti-T2 activity with b4 specificity (1/NT30 of 4–8). When the same range of b4 anti-b6 serum concentrations was tested together with 6 or 12 µl of normal b4 serum, as little as 0.4 µl of the antiallotype serum was sufficient to provoke a significant b4 anti-T2 response. Moreover, the b4 anti-T2 titers are seen to be significantly higher with 12 µl than with 6 µl of normal b4 serum. The degree of depression of the b5 anti-T2 response is independent of the presence of normal b4 serum.

Discussion

The demonstration of release from b4 suppression in b4b6 spleen cells through their exposure to antibodies specific for b6 strengthens the significance of the previously reported release in b4b5 cells that was mediated by anti-b5 (3).
observation that antiallotype serum which itself has the same allotypic specificity as the suppressed type, namely b4, is far more efficient in releasing allotype suppression than antisera made in rabbits of other allotypes (b5 or b6) appears to reveal a significant aspect of the mechanism of allotype suppression. Treatment of cells from b4-suppressed b4b5 or b4b6 rabbits with anti-b5 or anti-b6 sera made in b6 or b5 animals, respectively was generally seen not to result in a release from suppression. These observations suggested that b4 Ig plays an important role in release from b4 suppression, but they did not show whether it was some peculiarity in the b4 anti-b5 or b4 anti-b6 that was directly related to its antibody function or whether it was the allotypic specificity of such an antiserum that accounted for its effectiveness. Experiments such as those shown in Tables IV, V, and VI demonstrated that the addition of normal b4 serum to b6 anti-b5 or b5 anti-b6 serum rendered it as effective as b4 antisera in causing release from allotype suppression. Such experiments indicate therefore that nonantibody b4 Ig molecules in b4 antiallotype sera are one of the two factors involved in overcoming allotype suppression in our in vitro system, the other being antibody activity against the nonsuppressed type. Inspection of data shown in Tables II and III shows that anti-b5 or anti-b6 sera other than those made in b4 rabbits

Spleen cells used were from a b4-suppressed b4b6 rabbit (age 8 mo) with a serum b6/b4 ratio of 25 on the day of sacrifice.

* Mean of duplicate cultures.
were occasionally effective in bringing about release from b4 suppression, but only in cultures of spleen cells from b4-suppressed rabbits that were well on their way to recovery. Cultured cells from poorly suppressed rabbits, such as D-54 in Table II, and D-240 and D-250 in Table III form small amounts of b4 Ig which can be detected by hemagglutination-inhibition, although the anti-T2 fraction of Ig with the b4 marker is usually too small to be measurable in the neutralization assay. For example, 4-day culture fluids from cells of rabbit D-250 in Table III contained approximately 5 μg of b4 Ig per ml. The presence of small quantities of spontaneously formed b4 Ig might be enough to implement the action of b6 anti-b5 or b5 anti-b6 serum and thus facilitate the formation of low levels of b4 anti-T2 activity by cells of b4-suppressed rabbits already advanced in escape from suppression.

The essential participation of nonantibody Ig having the same b locus determinant as the suppressed type was further confirmed in studies such as that presented in Table VII, which clearly showed that b4 Ig is a limiting factor when the suppression-releasing activity of b4 anti-b6 serum on b4-suppressed b4b6 cells is assessed. Normal b4 serum alone has been shown repeatedly throughout the course of these investigations not to cause reversal of b4 suppression in spleen cells of rabbits that were well suppressed with respect to b4. Thus, the event reported here differs from the neutralization of allotype suppression in vivo which can be brought about only early in the life of the suppressed rabbit, either by injections of the suppressed Ig type or by putting the baby rabbits to nurse on foster mothers having that Ig type (7-10). This phenomenon has been attributed to neutralization of the antiallotypic antibodies which are the initiators of the chronically suppressed state and are still present in the young rabbit. Interestingly, however, Catty et al. (9) have noted that in b4b6 rabbits suppressed for b6 by intrauterine exposure to maternal b4 anti-b6 antibodies, “neutralization” of suppression could be achieved more readily by injecting b6 Ig which also had antibody activity against b4 than by using b6 Ig without antibody activity against the maternal allotype. They have attributed this superior neutralizing capacity to antiallotypic precipitation “in two directions.” The neutralization of suppression in b6-suppressed b4b6 neonates by b6 anti-b4 Ig and the reversal of suppression in spleen cells of b4-suppressed b4b6 rabbits by b4 anti-b6 serum seem on superficial inspection to have elements in common. In both sets of conditions Ig of the suppressed type is being furnished, as well as antibody activity against the nonsuppressed type, and in both cases the suppression-neutralizing reagent would bind the maternal type antibody both as an antigen and as an antibody. However, it should be kept firmly in mind that the spleen cells used in the experiments described in this report were from suppressed rabbits up to 8 mo of age, and that passively acquired maternal Ig is not detectable in the serum of the offspring after the age of about 8 wk (1).

Although in the examples documented here of reversal of b4 suppression by b4 anti-b6 (or b4 and anti-b6), there is an obvious double relationship between the

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2 In the interest of simplicity, in the remainder of the discussion the example of b4-suppressed b4b6 cells will be used, although the same principles also apply to the b4b5 system.

3 Dray's measurements are based on precipitation in agar gel. By the more sensitive HI test, traces of maternal Ig are still found in the serum at 10-11 wk (F. L. Adler, unpublished observations).
suppression-releasing combination and the maternal antibody which brought about suppression, the longlasting nature of allotype suppression makes it difficult to envision a role for maternal antibody in maintenance of the condition. If one discards the notion of a relevant and necessary role for maternal antibody in the maintenance of chronic suppression, it is possible to explain the observed results in the following manner: one would assume that most of the splenic lymphocytes in a b4-suppressed b4b6 rabbit display b6 on their membranes. Direct experimental evidence for this comes from the fluorescence studies of Harrison et al. (11) and also from Sell's observation on blast transformation in peripheral blood lymphocytes of allotype-suppressed rabbits (12). Binding of anti-b6 antibody by such cells could then initiate a series of events beginning with capping and endocytosis of the antibody (13, 14), or endocytosis without capping (15), leading to possible blast transformation (16) and depression of specific Ig synthesis (17-20). Cells not displaying b6 Ig and thus potentially producers of b4 Ig or their precursors would then proliferate. A variation of this theory would propose that the b4-suppressed animal possesses precursor cells that are potential producers of either b4 or b6 Ig, but which carry membrane b6. The anti-b6 serum might then bring about simultaneous repression of b6 and derepression of b4. Recent evidence obtained by Jones et al. (21), indicating that in normal heterozygous rabbits B lymphocytes marked with a given membrane Ig are committed to differentiate into plasma cells capable of producing that Ig type would speak against this idea, but one cannot rule out the possibility that the properties of lymphocytes from allotype-suppressed animals may differ from those of normal animals in this respect. Similar hypotheses were first offered by Dray (1) to explain the induction of allotype suppression in heterozygotes, which is always accompanied by a compensatory increase in the product of the alternate allele so that normal total Ig levels in the serum are maintained.

However, the results presented in Tables II and III clearly show that b4 suppression is not overcome by inhibition of b6 expression alone but also requires the presence of nonantibody b4 Ig, which in itself does not release suppression. A possible role for this component could be that of providing a "priming" effect for b4 production. Such a mechanism for regulation of Ig synthesis by feedback control has been proposed by Mage et al. (22) to account for the somewhat higher levels of a given Ig type found in adult heterozygous rabbits when that type is contributed by the maternal rather than by the paternal gene.

Alternatively, a completely different hypothesis to integrate the findings reported here and in a previous publication (3) suggests itself if they are viewed in terms of interference with a specific suppressive mechanism initiated by the presence of antiallotype antibodies in prenatal or neonatal life. Jacobson, Herzenberg et al. have presented evidence that chronic allotype suppression in the mouse is an active process dependent on the activity of suppressor T cells in the lymphoid system (23-25). Recently obtained evidence showing the transfer of b4 suppression from lymphoid cells of b4-suppressed rabbits to normal b4 cells indicates that an active suppression mechanism exists in allotype-suppressed rabbits as well.

The specificity of this suppression is currently under investigation.
present model would have b6 Ig on their membranes and would therefore be targets for inactivation by anti-b6. Although no direct cytotoxic effect of antiallotypic antibodies has been demonstrated (26–28), Mage (2) has recently discussed alternative mechanisms by which prolonged exposure to such antibodies might lead to eventual cell inactivation or death. A number of investigators have studied the ability of antibodies directed against membrane-bound antigens to interfere with the activity of such cells in the graft-vs.-host or the mixed lymphocyte reactions (29–34). Where tested, the antisera used were shown not to be cytotoxic. Such reports seem to be especially relevant to the current discussion of suppressor cells, since they deal with known activities of lymphoid cells against other lymphoid cells. Assuming, then, a restraining role of the antiallotypic antibody upon the suppressor cell, one could once again assign to the nonantibody b4 Ig the priming function that has been discussed earlier.

However, another role for b4 Ig becomes apparent if one considers the likelihood that in a mechanism of allotype suppression involving the activity of suppressor cells, a precursor cell beginning to differentiate into a b4-producing cell would be the target for a suppressor cell with recognition for its identity as a b4 cell. It is conceivable that Ig marked with the b4 determinant might compete with target cells for specific sites on suppressor cells involved in b4 suppression. In support of such a concept, Schirrmacher et al. (35) have presented evidence for interference by free antigen with lymphoid cell activity against antigen-coated target cells. However, since the data show that both anti-b6 activity and the participation of normal b4 Ig are needed in order to bring about release from b4 suppression, it becomes necessary to postulate that the hypothetical suppressor cell must suffer “two hits” before becoming inoperative, one against its b6 identity and the other against its receptor activity for b4-labeled targets.

A different explanation for the failure of b5 anti-b6 to cause release from b4 suppression would require the assumption of cross-reactivity between antibodies in such sera and b4. Thus, these antibodies might bind emerging b4 Ig or inhibit cells that escape from b4 suppression, and the requirement for the addition of b4 Ig would be created by the need to neutralize these postulated cross-reactive antibodies. We do not believe this explanation to be correct for a number of reasons. Among these is our consistent failure to detect anti-b4 activity in b5 anti-b6 sera when using hemagglutination procedures that are not only highly sensitive tests for antibody activity but also readily detect the known cross-reactivity between b5 and b6 in antisera to b5 or b6 that were made in b4 rabbits. Also speaking against this explanation, and possibly the strongest evidence against it, is the observation that b6 anti-b5 serum effectively suppresses the b5 anti-T2 response of normal b4b5 spleen cells but totally lacks inhibitory activity toward the b4 anti-T2 response of such cells (20).

Continued investigation of the factors and conditions underlying the release from allotype suppression in vitro using purified antibodies and normal globulins may help to unravel the enigma of allotype suppression and to answer basic questions concerning the control of Ig synthesis in normal animals. The commonly observed “pecking order” among Ig allotypes in heterozygous normal rabbits (i.e., b4 > b6; a1 > a2) suggests the possible occurrence of a control
mechanism similar in basis to that observed in allotype-suppressed animals (36). Recent experimental evidence obtained by treating cultured spleen cells of certain normal b4b5 rabbits with anti-b4 or anti-b5 serum more directly supports this concept (20).

Summary

Spleen cells of b4b6 rabbits, shown to be deficient in their ability to produce b4 Ig due to prenatal exposure to anti-b4, formed anti-T2 antibodies marked with the b4 determinant in response to solubilized T2 phage (S-T2) only when cultured in the presence of antibodies specific for the nonsuppressed type (b6), thus confirming and extending the previously reported observation of release from b4 suppression in cultured cells of b4-suppressed b4b5 rabbits treated with anti-b5 serum. Only antiallotype sera made in b4 rabbits were active in reversing b4 suppression. Anti-b5 or anti-b6 sera from rabbits of allotypes b6 or b5, respectively, when used in concentrations which completely or partially inhibited the formation of anti-T2 antibodies marked with the corresponding nonsuppressed allotype of the spleen donor, proved to be almost completely ineffective in causing release of suppression. Exceptions were noted when spleen cells of rabbits advanced in spontaneous escape from suppression were tested with such sera. The addition of normal b4 serum to non-b4 antiallotypic sera rendered them as effective in releasing b4 suppression in vitro as were antisera from b4 rabbits. Furthermore, the capacity of a b4 antiallotype serum to cause reversal of b4 suppression could be potentiated by the addition of normal b4 serum, indicating that nonantibody b4 Ig is a limiting factor in such a serum.

Thus, the release from allotype suppression observed in cultures of spleen cells from b4-suppressed heterozygous rabbits is dependent upon the presence of two components: antibodies directed against the nonsuppressed allotype of the donor and normal b4 Ig. These findings are interpreted in terms of alternate hypotheses involving (a) a mechanism of b4 derepression and (b) inactivation of a suppressor cell with recognition for a b4-labeled target.

The authors wish to thank Mr. Raymond Harris, Mrs. Marsha Feldman, and Mr. Randolph Noelle for skillful technical assistance during the course of this work, and also gratefully acknowledge the excellent secretarial skills of Miss Katherine Kelly, who prepared the manuscript.

Received for publication 18 February 1975.

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