SUPPRESSION OF IDIOTYPE AND GENERATION
OF SUPPRESSOR T CELLS WITH
IDIOTYPE-CONJUGATED THYMOCYTES*

BY YOSHITANE DOHI†‡ AND ALFRED NISONOFF

From the Rosenstiel Research Center, Department of Biology, Brandeis University, Waltham, Massachusetts 02154

When most or all members of a strain of mice express a common idiotype on antibodies of a given specificity it is possible to suppress the appearance of the idiotype by prior inoculation of anti-idiotypic antibodies. This has been done in vivo (1–3) or in vitro (4). The suppressed state can be adoptively transferred by T cells (5, 6) or, in the case of a suppressed hyperimmunized donor, by B cells (7–9). After treatment with anti-idiotype, hyperimmunization, and a prolonged rest period, most of the suppressor T cells were found to possess receptors with anti-idiotypic specificity (6, 10). Such cells could be separated by rosette formation with syngeneic erythrocytes coated with Fab fragments possessing the idiotype.

Rowley et al. (11) have shown that challenge of BALB/c mice with protein T15, a myeloma protein with antiphosphorylcholine activity, reduces the subsequent humoral response to phosphorylcholine. The authors concluded that this effect was mediated by anti-idiotypic antibodies because it was brought about by injecting a protein, T15, possessing the idiotype. Because nearly all serum anti-PC antibodies carry the idiotype of protein T15 it was difficult to ascertain whether the antibodies with the idiotype were selectively suppressed.

Eichmann et al. have recently reported that some mice, when challenged with antistreptococcal antibody carrying the A5A idiotype, develop suppressor cells which can inhibit an antistreptococcal response. It was suggested that the inhibition may prove to be idiotype specific (12).

In the present investigation we have found that injection of molecules with the major idiotype characteristic of anti-p-azophenylarsonate (anti-Ar) antibodies in A/J mice selectively inhibits the idiotype response, without significantly diminishing the production of anti-Ar antibodies. The effect was markedly enhanced by injecting the idiotype conjugated to syngeneic or allogeneic thymocytes. This procedure also resulted in the formation of idiotype-specific suppressor T cells which could adoptively transfer the suppressed state; B cells were ineffective. There was no requirement for antigen in the induction of suppressor T cells. Suppression of the idiotype occurred in

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‡ Abbreviations used in this paper: Anti-Ar, anti-p-azophenylarsonate; BGG, bovine gamma globulin (fraction II); CFA, complete Freund’s adjuvant; CRI, cross-reactive idiotype common to anti-Ar antibodies of A/J mice; IFA, incomplete Freund’s adjuvant; KLH, keyhole limpet hemocyanin.
F₁(BALB/c × A/J) and the allotype-congeneic C.AL-20 strain, as well as in A/J mice.

Materials and Methods

Mice. Mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, except for the C.AL-20 strain, which was generously provided by Dr. Michael Potter and bred in our laboratory. This congenic strain has the heavy chain allotype of the AL/N strain on a BALB/c background and produces the anti-Ar idiotype characteristic of A/J mice. The mice used were males, 8–10 wk old at the start of each experiment.

Immunologic Reagents and Assays. The following methods have been described elsewhere (13–15): preparation of conjugates of proteins with Ar; preparation of anti-Ar in ascites fluids of mice; preparation of rabbit anti-idiotypic antibodies by inoculation of specifically purified A/J anti-Ar antibody and adsorption of anti-idiotypic antisera with A/J globulin conjugated to Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.); radioimmunoassay for the major cross-reactive idiotype. The latter assay utilizes 10 ng of ¹²⁵I-labeled purified A/J anti-Ar antibody and an antiglobulin reagent. The concentration of idiotype in unknown samples is estimated from their inhibitory capacity, per unit weight of anti-Ar antibody, in the radioimmunoassay. Anti-Ar antibodies were specifically purified by adsorption onto a column of Sepharose-4B conjugated with bovine gamma globulin (fraction II) (BGG)-Ar. The antibodies were adsorbed in the presence of 0.01 M EDTA and were eluted batchwise with 0.5 M p-aminophenylarsionate in 0.02 M Tris buffer, pH 8. The hapten was removed by exhaustive dialysis, and the antibody was passed through DEAE-cellulose in 0.04 M phosphate buffer, pH 8, for further purification. Concentrations of anti-Ar antibodies in sera were determined by the method of Klinman et al. (16), using specifically purified ¹²⁵I-labeled rabbit anti-mouse Fab as a developing reagent. Standards were run with sera containing known amounts of anti-Ar antibodies, as determined by precipitin analysis.

Keyhole limpet hemocyanin (KLH) was obtained from the Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. p-Aminophenylarsionic acid was purchased from Eastman Kodak Co., and was recrystallized twice from 50% ethanol. Complete Freund’s adjuvant (CFA) was obtained from Difco Laboratories, Detroit, Mich.

Preparation of T and B Cells. Single cell suspensions of spleen were exposed at 0°C for 5 min to a solution containing 0.155 M NH₄Cl, 0.01 M KHCO₃ and 0.1 mM EDTA, pH 7.4, to lyse the erythrocytes (17). The remaining cells were enriched for T cells by the method of Mage et al. (18). In brief, 6 × 10⁷ leukocytes in RPMI-1640 medium supplemented with 5% fetal calf serum were placed on a 100-mm Petri dish previously coated by sequential exposure to specifically purified Fab fragments of rabbit anti-mouse Fab (0.2 mg/ml), and to supplemented medium. After remaining in the dish for 1 h at 4°C with occasional agitation the cell suspension was removed and the cells were washed with RPMI-1640 medium. Approximately 95% of the remaining cells, as compared with 40–50% of the original leukocyte population, were killed by exposure to AKR anti-Thy-1.2 ascites (Litton Bionetics, Kensington, Md.) in the presence of guinea pig complement.

To enrich for B cells the splenic leukocytes were exposed to rabbit anti-A/J brain antiserum, prepared and absorbed by the method of Golub (19), in the presence of fresh guinea pig serum of low cytotoxicity. This procedure killed an average of 47% of the leukocytes (trypan blue exclusion). The cells remaining were not sensitive to significant further killing by AKR anti-Thy-1.2 ascites and complement.

Other Methods. Thymocytes were obtained from 4- to 5-wk-old mice and were washed three times with saline. Specifically purified anti-Ar antibodies were conjugated to thymocytes by using the reagent, N-ethyl-N’-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Fluka A. G., Buchs, Switzerland). 30 mg of the carbodiimide reagent was mixed with 4.8 mg of antibody in the presence of 1 × 10⁸ thymocytes in 0.16 M NaCl. The mixture was allowed to stand for ~2 h at room temperature. The purified antibodies were lightly labeled with ¹²⁵I to permit quantitation of the amount bound to the cells. The reaction time was adjusted so as to couple 12 ± 2 µg of antibody to 25 × 10⁶ cells.

Mice to be used as recipients in adoptive transfers were irradiated with 200 rad in a ¹³⁷Cs gamma irradiator (Shepherd, J. L., & Associates, Glendale, Calif.) over a 5-min period.
### Results

**Suppression of Idiotype Expression by Injection of Idiotype-conjugated Thymocytes.** Table I shows the results of two i.p. inoculations of idiotype-conjugated thymocytes, followed by immunization with KLH-Ar, and the results of a number of control experiments. The protocol is given in a footnote of the table. The data indicate that a suppressed state with respect to the formation of the common idiotype (CRI) was induced by two injections of A/J thymocytes, conjugated with anti-Ar antibodies carrying the idiotype, before inoculation of KLH-Ar (group 3). The suppressed mice produced normal or moderately reduced concentrations of anti-Ar antibodies, but the CRI was not detectable. In mice inoculated with thymocytes alone, or with thymocytes conjugated with normal A/J IgG, the idiotype was not suppressed (groups 2 and 4). Suppression induced by idiotype-conjugated thymocytes was essentially complete and persisted through the last bleeding, on day 38 after the first injection of antigen. When the anti-Ar antibodies were injected alone, or as a mixture with thymocytes (unconjugated) about half of the mice were suppressed with respect to the CRI (groups 5 and 7). Injection of a larger amount of anti-Ar antibody (total, 200 µg rather than 24
Adoptive Transfer of Suppression from A/J Mice Treated with Idiotype-conjugated A/J Thymocytes

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Cells transferred</th>
<th>Number of cells transferred</th>
<th>Antibody response (day 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-Ar titer (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>Normal spleen cells</td>
<td>10</td>
<td>260, 600, 1,000, 1,200, 1,300, 1,400</td>
</tr>
<tr>
<td></td>
<td>&quot;Suppressed&quot; spleen cells</td>
<td>10</td>
<td>190, 270, 840, 1,300, 1,500, 1,400</td>
</tr>
<tr>
<td>2</td>
<td>&quot;Suppressed&quot; B cells</td>
<td>5</td>
<td>190, 640, 1,400, 3,200, 4,300, 7,600</td>
</tr>
<tr>
<td></td>
<td>&quot;Suppressed&quot; T cells</td>
<td>2.5</td>
<td>120, 290, 400, 650, 1,300, 1,300, 2,500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>14, 260, 500, 1,200, 2,400, 2,500, 2,400, 2,500</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2.5</td>
<td>330, 700, 790, 1,500, 1,800, 2,000, 2,400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>220, 420, 430, 1,000, 1,200</td>
</tr>
</tbody>
</table>

* Donors were normal A/J mice or A/J mice that had been inoculated twice, on days -42 and -21, with idiotype-conjugated thymocytes, as described in a footnote of Table I. Cells were transferred on day 0. The recipients were challenged i.p. with 0.25 mg KLH-Ar in CFA on days 3 and 31 and were bled on day 41. Each value in the table represents an individual mouse.

† Cells from mice treated with idiotype-conjugated thymocytes.

μg; group 6) was not more effective in inducing the suppressed state. Preinoculation of idiotype-conjugated thymocytes from BALB/c or C57BL/10 mice similarly caused suppression of the idiotypic response in A/J recipients (groups 9 and 10), suggesting a lack of H-2 restriction with respect to the carrier cells. The degree of suppression appears to be somewhat less profound with BALB/c thymocytes.

The titers of anti-Ar antibody, rather than idiotype, produced by the various groups of mice showed considerable spread within a group but no very marked trend, although there was some evidence for a reduction in average antibody titer on day 21, but not day 38, in those groups whose idiotype concentration was decreased.

Mice of group 8, that received KLH-Ar before inoculation of idiotype-conjugated thymocytes, produced normal amounts of the idiotype per unit weight of anti-Ar antibody; i.e., an ongoing idiotypic response was not affected.

Induction of Idiotype-specific Suppressor T Cells. The data in Table II indicate that the inoculation of thymocytes conjugated with the idiotype results in the production of idiotype-specific suppressor T cells. 35 donor animals were challenged twice over a 3-wk period with 25 × 10⁶ thymocytes to which 12 µg of specifically purified A/J anti-Ar antibodies were conjugated. 3 wk later the mice were sacrificed. T and B cells were prepared from their pooled spleens and adoptively transferred into mildly irradiated (200 rad) recipients. The recipients were challenged with KLH-Ar 3 and 31 d after the adoptive transfer and were bled 10 d later.

Three of six mice that received 1 × 10⁸ splenic leukocytes from mice challenged with idiotype-conjugated thymocytes, and five of six mice that received 5 × 10⁷ T
Table III

Suppression of Synthesis of Idiotype in CAL-20 Mice by Inoculations of Idiotype-conjugated Thymocytes*

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Preinoculations</th>
<th>Antibody response (day 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-Ar titer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Conjugate, A/J thymocytes + anti-Ar</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>Conjugate, BALB/c thymocytes + anti-Ar</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>Mixture, A/J thymocytes + anti-Ar</td>
</tr>
</tbody>
</table>

* Preinoculations and immunization with KLH-Ar were as described in a footnote of Table I; bleedings were taken only on day 38.

Table IV

Suppression of Synthesis of Idiotype in F1 (A/J × BALB/c) Mice by Inoculation of Idiotype-conjugated Thymocytes*

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Preinoculations</th>
<th>Antibody response (day 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-Ar titer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Conjugate, F1 thymocytes + anti-Ar</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Mixture, A/J thymocytes + anti-Ar</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>Conjugate, A/J thymocytes + anti-Ar</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Conjugate, BALB/c thymocytes + anti-Ar</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>Conjugate, C57BL/10 thymocytes + anti-Ar</td>
</tr>
</tbody>
</table>

* Preinoculations and immunization with KLH-Ar were as described in a footnote of Table I; bleedings were taken only on day 38.

cells were strongly suppressed with respect to the CRI. Five of six mice receiving 5 × 10^7 B cells produced normal amounts of the CRI; the sixth mouse was partially suppressed with respect to idiotype production. Whereas 2.5 × 10^7 T cells or 1 × 10^7 T cells induced a state of suppression in some recipients, 2.5 × 10^7 B cells were not suppressive.

Table III shows the results of injecting BALB/c or A/J thymocytes, conjugated with antibody carrying the idiotype, into CAL-20 recipients. This congeneric strain possesses the AL/N heavy chain allotype on a BALB/c background and normally produces the CRI. It is evident that the formation of idiotype was almost completely inhibited by preinoculation with idiotype-conjugated thymocytes of either BALB/c or A/J origin. A mixture of A/J thymocytes with the idiotype-bearing antibodies (unconjugated) was relatively ineffective (group 4), although the concentration of
idiotype per unit weight of anti-Ar antibody was reduced in one recipient. Thus, the effect of conjugation is apparently more pronounced in the case of C.AL-20 than in A/J mice. The average idiotype content of anti-Ar antibodies in untreated C.AL-20 mice is somewhat lower than that of A/J mice.

The data in Table IV were obtained with F1 (A/J × BALB/c) mice. The results are similar to those observed with A/J or C.AL-20 mice. Three of four mice that were inoculated with a conjugate of F1 thymocytes and anti-Ar antibodies were idiotypically suppressed. A fifth mouse produced a very low titer of anti-Ar antibodies so that the degree of suppression is uncertain. A large majority of the recipients was suppressed when the anti-Ar antibodies were conjugated to A/J, BALB/c, or C57BL/10 thymocytes as well as to the F1 thymocytes. The results obtained with the F1 mice are similar to those obtained with C.AL-20 in that there was a very marked enhancement of suppression when the antibodies were conjugated to thymocytes. An unconjugated mixture of thymocytes and anti-Ar antibodies caused little if any suppression of idiotype production upon subsequent immunization with KLH-Ar.

Discussion

The experiments reported here demonstrate the feasibility of preventing the expression of idiotype and generating idiotype-specific suppressor T cells by inoculation of antibody molecules which bear the idiotype and are conjugated to thymocytes. There is no requirement for anti-idiotypic antibody or antigen to induce the formation of the suppressor cells. Thymocytes were used as the carrier because they represent a relatively homogeneous population of leukocytes; other cell types have not yet been tested as carriers in this system. Recipients of idiotype-conjugated thymocytes produced substantial amounts of anti-Ar antibodies upon subsequent immunization with KLH-Ar but the idiotype was undetectable by a sensitive assay in nearly all mice. The presence of suppressor T cells was shown by adoptive transfer experiments, using as donors mice that had been treated with idiotype-conjugated thymocytes but not with antigen. The adoptive transfer of 1 × 10⁸ splenic lymphocytes or 5 × 10⁷ T cells from such mice into mildly irradiated recipients suppressed the capacity to produce the CRI in three of six recipients of spleen cells and five of six recipients of T cells. Smaller numbers of cells were less effective.

Inoculation of antibody possessing the idiotype, without thymocytes, or mixed with thymocytes but unconjugated, induced a state of idioptopic suppression in ~45% of A/J recipients. The difference in induction of suppression by conjugated vs. unconjugated molecules bearing the idiotype was more pronounced in C.AL-20 and F1(A/J × BALB/c) mice in which idiotypic antibody was quite ineffective unless it was conjugated to thymocytes. Nearly all mice were suppressed after inoculation of conjugated cells.

The suppression of idiotype formation by preinoculation of idiotypic antibody has been reported by Rowley et al. (11) and more recently by Bona et al. (20). It was not ascertained in their experiments or in ours whether suppressor T cells were formed in response to the unconjugated antibody molecules. However, Bona and Paul (21) presented evidence that there are naturally occurring T suppressor cells specific for an idiotype present on the MOPC 460 protein, which binds the dinitrophenyl hapten group.

The enhancement of suppressive activity by conjugation to thymocytes was not
dependent upon the H-2 type of the thymocytes. As a carrier, C57BL/10 thymocytes were equally as effective as A/J thymocytes in A/J, C.AL-20, or F1(A/J × BALB/c) mice. BALB/c thymocytes were very effective in C.AL-20 or F1(A/J × BALB/c) mice. The degree of suppression, although very strong, was not complete when BALB/c thymocytes were used as the carrier in A/J mice.

A lack of H-2 restriction with respect to the carrier was also observed by Miller et al., who induced tolerance to delayed-type hypersensitivity against hapten or protein antigens conjugated to syngeneic or allogeneic spleen cells (22, 23). It seems possible that the thymocytes may simply act as an effective nonspecific carrier, although the cell membrane may be important.

Whether anti-idiotypic antibodies were elicited upon inoculation of idio-type-specific conjugated thymocytes was not determined. However, the idiotype-suppressed state could be adoptively transferred by enriched T cells but not by B cells (Table II).

When the inoculation of idiotype-bearing thymocytes was preceded 5 d earlier by the antigen, KLH-Ar, there was no evidence of suppression of idiotype upon subsequent challenge with antigen. We have previously observed that idiotype-specific suppressor T cells are ineffective in animals that had already received antigen (24). A similar observation was reported by Rowley et al., who induced suppression with unconjugated idiotypic antibody (11).

The results may be related to those of Ramseier and Lindenmann (25), Binz and Wizell (26), and Krammer and Eichmann (27), who have shown that inoculation of T cells can elicit anti-idiotypic antibodies directed to receptors on the cells. In addition, Binz and Wizell (28) have observed the formation of suppressor T cells specific for idiotypic determinants of receptors on T cells. In such experiments one might consider the T cell as the carrier for idiotypic determinants of receptors. A state of suppression with respect to idiotypes on T-cell receptors, induced by inoculation of T cells, has also been demonstrated by Bellgrau and Wilson (29) and Andersson et al. (30). A number of studies have demonstrated that the lymphocyte is an effective carrier for hapten or protein antigens in the induction of suppression or suppressor T cells with specificity for antigens (22, 23, 31–34).

Immunization with idiotype-bearing molecules under appropriate conditions can elicit predominantly helper (5, 35, 36) rather than suppressor activity specific for idiotypic determinants. Conditions favoring one or the other response remain to be elucidated.

Our results do not establish the mechanism of induction of idiotype-specific suppressor cells. One possibility is that there is a direct stimulation of suppressor T cells with anti-idiotypic receptors by molecules with the idiotype. The existence of suppressor cells with anti-idiotypic receptors has been demonstrated in the anti-Ar system by rosette formation, using T cells from suppressed hyperimmunized A/J mice (6, 10). Also, Bona and Paul (21) have identified naturally occurring T suppressor cells with anti-idiotypic receptors specific for the MOPC 460 myeloma protein in nonimmunized BALB/c mice.

The presence of idiotype-positive, rather than anti-idiotypic, suppressor T cells has been observed by Lewis and Goodman after challenge of A/J mice with mouse IgG-Ar (37). Also, the presence of idiotypic determinants on idiotype-specific suppressor cells has been reported by Hetzelberger and Eichmann (38) and by Bottomly et al. (39). It thus appears that T cells with idiotypic or anti-idiotypic receptors may induce
suppression in vivo. It seems possible that the two cell types may be mutually stimulatory. One mechanism of suppression, demonstrated by Eichmann et al. (12), has as its target an idiotype-specific helper T cell (40). The presence of idiotypic determinants on suppressor factors derived from T cells has been demonstrated by Germain et al. (41) and Bach et al. (42).

It has recently been found that antibodies with CR1, when coupled to autologous lymphocytes and administered intravenously into A/J mice, stimulate the development of T cells which can adoptively transfer a state of suppression of delayed hypersensitivity to the Ar hapten group. A preliminary report of these observations has been made (43), and they will be described in detail elsewhere.

Summary

Inoculation of A/J mice with syngeneic thymocytes conjugated with specifically purified A/J anti-phenylarsonate (anti-Ar) antibodies, selectively suppressed the subsequent synthesis of those anti-Ar antibodies which carry the major cross-reactive idiotype. High titers of anti-Ar antibodies were produced upon subsequent immunization but in most mice the idiotype was undetectable. Suppression similarly occurred in F1(A/J × BALB/c) and in C.AL-20 mice. Although some mice were suppressed when unconjugated antibody was injected, the suppressive effect was much more pronounced, particularly in the F1 and C.AL-20 recipients, when the antibody was coupled to thymocytes. The state of suppression could be adoptively transferred with T cells to mildly irradiated syngeneic recipients. A population enriched for B cells had little if any suppressive effect. There was no requirement for antigen in the generation of suppressors. Thymocytes conjugated with antibody did not induce idiotype-specific suppression in mice that had been recently challenged with antigen. Thymocytes from BALB/c and C57BL/10 mice were effective carriers for the anti-Ar antibodies, i.e., there was no evidence for H-2 restriction. The experiments demonstrate the feasibility of suppressing idiotype production and generating idiotype-specific suppressor T cells without the use of anti-idiotypic antibody or antigen.

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References

cell populations having receptors with the same specificity but of different idiotype. J. Immunol. 114:610.


SUPPRESSION WITH IDIOTYPE-CONJUGATED THYMOCYTES


