INDUCTION OF IDIOTYPE SUPPRESSION IN THE
ANTI-AZOPHENYLARSONATE RESPONSE OF
T-DEPLETED A/J MICE

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The serological response of strain A mice to the hapten p-azophenylarsonate
(Ar) is dominated by the presence of a cross-reactive idiotype (Id^CR) originally
defined by a heterologous rabbit antiserum. Administration of this antiserum
before Ar immunization resulted in an anti-Ar response that lacked idiotype-
positive (Id^+) antibodies (1). This suppression was shown to be associated with T
suppressor cells (Ts) capable of transferring the suppressed state to naive, lightly
irradiated recipients (2). An apparent suppressed state was also transferred by B
cells from mice suppressed with rabbit anti-Id antiserum, through a mechanism
of clonal dominance (3, 4).

It has been reported that homologous monoclonal antiidiotopes, as well as
heterologous anti-Id, suppress Id production in the Ar system (5, 6). In previous
work, we demonstrated that suppression induced by two monoclonal reagents
with overlapping specificities was idiotope specific, suggesting that homologous
antiidiotope exerts its effect through direct binding to diversified B cell surface
immunoglobulin (7). We have now further investigated the mechanism of sup-
pression induced by the homologous antiidiotope, MB. Suppression induced by
MB was remarkably stable, and long-lasting suppression could be transferred to
naive recipients by B cells from suppressed donors. The transfer experiments
indicated it was not necessary to invoke Ts to explain the perpetuation of idiotope
suppression, once established. We further sought to determine whether Ts must
be present initially to bring about suppression of idiotope expression, by studying
the effect of MB on T-depleted mice.

Materials and Methods

Animals. A/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

Suppression with Antiidiotope. MB is a homologous, murine, monoclonal antiidiotope
described previously (5). Naive, adult A/J mice were suppressed by intraperitoneal (i.p.)
inoculation of 0.1 cm³ of a 1:10 dilution of MB ascites fluid, containing 6 µg antiidiotope.

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One-tenth that amount was also suppressive, while lesser (to $10^{-5}$) amounts had no consistent effect on expression of the MB idiotope.

**Isolation of B Cells.** Two methods were used to isolate B cells from spleen cell suspensions. Cells adherent (Adh B) to rabbit anti-mouse immunoglobulin (RAMIg) coated petri dishes were obtained as described by Mage et al. (8). Alternatively, spleen cell suspensions were treated with mouse monoclonal anti-Thy-1.2 (Thy-1.2$^+$) plus guinea pig complement (9).

**$T$-depleted Mice.** A/J mice were suction thymectomized at 5 wk of age. 7 wk later they were given 600 rads total body irradiation, followed by intravenous administration of $3 \times 10^6$ syngeneic bone marrow cells that had been treated with anti-Thy-1.2 plus rabbit complement.

**$T$ Cell Supplementation of $T$-depleted Mice.** $T$ cells were prepared from splenic lymphocytes of A/J mice immunized with keyhole limpet hemocyanin (KLH) in complete Freund’s adjuvant (T$^\text{KLH}$) as described by Mage et al. (8). Lyt-2$^-T^\text{KLH}$ were prepared by treating T$^\text{KLH}$ with mouse monoclonal anti-Lyt-2,2 plus rabbit complement (10).

**Limiting Dilution Analyses.** The frequency of mitogen-responsive T cell precursors was determined on peripheral blood samples as described (11). In brief, responder cells were titrated at limiting dilutions into microtiter wells containing 3 ng/ml concanavalin A (Con A), 5 $\times 10^3$ irradiated, syngeneic splenic “feeder cells,” and conditioned medium containing IL-2; proliferation was measured by $[^3H]$thymidine incorporation after 7 d culture. Frequencies were calculated using the chi-square minimization method (11). For some tested mice, only a single group of wells contained both positive and negative wells. In these cases, the precursor frequencies were estimated from the zero-order term of the Poisson distribution.

**Radioimmunoassays.** Amounts of antibody expressing the MB idiotope (Id$^{MB}$) and anti-Ar antibodies were quantified as previously described (5), and are reported as microgram equivalents of HP 36-65 per milliliter.

**Results**

**Stability of Suppression Induced by MB.** Seven adult A/J mice were suppressed by administration of MB intraperitoneally on day 1, followed by 0.1 mg Ar-KLH in complete Freund’s adjuvant (CFA) on day 14 and in incomplete Freund’s adjuvant (IFA) on days 28 and 45. These mice were then rested for 24 wk. They were immunized again on day 216 with 0.1 mg Ar-KLH in CFA i.p. In bleedings just before (day 215) and after (day 228) the last immunization, all mice failed to express Id$^{MB}$ (<1 ng equivalent of HP 36-65 per ml). Mean anti-Ar titers were 534 and 4,200 ng/ml, respectively. In a separate, but similar, experiment, 18 adult A/J male mice were given MB on day 1, followed by 0.1 mg Ar-KLH in CFA on day 38 and in IFA on day 75. All 18 mice were bled on day 85 and failed to express Id$^{MB}$. The mean anti-Ar titer was 2,095 ng/ml. These mice were then immunized three more times with Ar-KLH and 14 surviving mice were bled on day 167. Again all mice failed to express Id$^{MB}$, the mean anti-Ar titer was 1,860 ng/ml.

**Adoptive Transfer of Id$^{MB}$ Suppression by B Cells.** Spleen cell donors were suppressed with MB and then hyperimmunized with five doses of 0.1 mg Ar-KLH in Freund’s adjuvant. 12 wk after the last immunization, suppressed B cells were adoptively transferred to lightly irradiated (100 rads) recipient mice. Light irradiation was used to overcome the barrier to syngeneic transplantation (12), while producing minimal effects on the immune response (13). Recipients were immunized with 0.2 mg Ar-bovine gamma globulin (BGG) in CFA 3 d later, followed by five doses of 0.1 mg Ar-BGG in IFA over 6 months. Mice were bled 10 d after each immunization and the sera assayed for titers of Id$^{MB}$ and anti-Ar.
antibodies. Results from the second (day 43) and sixth (day 198) bleedings are shown in Table I. All mice that received $10 \times 10^6$ B cells isolated by either method failed to express Id\textsuperscript{MB}, and the apparent suppressed state persisted for more than 6 months despite hyperimmunization. The number of anti-Thy-1.2 treated B cells adoptively transferred was titrated; as few as $1 \times 10^6$ B cells produced a long-lasting apparent suppressed state. Similar results were obtained in two additional experiments.

**Induction of Id\textsuperscript{MB} Suppression in T-depleted Mice.** A/J mice were depleted of mature T lymphocytes as described in Materials and Methods. Depletion of T cells was confirmed by determining the frequency of T lymphocyte precursors that proliferated in response to Con A (pPTL assay) through limiting dilution analysis of peripheral blood lymphocytes (see Materials and Methods). Results in Fig. 1 are reported as frequency of pPTL per $10^8$ nucleated cells and are displayed on a log scale. Three normal A/J mice had pPTL frequencies of 88–

### Table I

| Group: Cell no. | Adh B 10 × 10^6 | Thy-1.2^- 10 × 10^6 | Thy-1.2^- 1 × 10^5 | Control None |
|----------------|-----------------|---------------------|-------------------|--------------|
| Id\textsuperscript{Mb} | <1 | <1 | <1 | 25.5 |
| Titer | <1 | <1 | <1 | 319 |
| Day 43* | <1 | <1 | <1 | 2.3 |
| Anti-Ar | 343 | 469 | 161 | 47.9 |
| Titer | 446 | 492 | 198 | 306 |
| Day 43* | 851 | 662 | 352 | 46.2 |
| Id\textsuperscript{Mb} | <1 | <1 | <1 | 25.5 |
| Titer | <1 | <1 | <1 | 100 |
| Day 198 | <1 | <1 | <1 | <1 |
| Anti-Ar | 89.4 | 250 | 116 | 41.2 |
| Titer | 200 | 138 | 252 | 141 |
| Day 198 | 326 | 244 | 90.8 | 118 |

* Microgram equivalents of HP 36–65.

* Below the limit of reliable detection; 1 λ/well failed to produce any significant inhibition and thus contained <1 ng equivalents of HP 36–65.

![Figure 1](image_url)

**Figure 1.** Depletion of pPTL in experimental mice. The frequencies of pPTL per $10^8$ nucleated cells from peripheral blood specimens are shown for two groups of mice assayed concurrently. Results are plotted on a log scale. See Materials and Methods for details.
The mean pPTL frequency for 16 experimental mice was 2.5  
+/- 1.4, representing 98% depletion of the normal complement of circulating  
T cells. None of these mice had >5% of the normal frequency of pPTL.  
Titrations on two additional experimental mice were technically unsatisfactory.  

T-depleted mice were divided into four groups at four months of age. Mice in  
groups B and D were given 0.1 cm³ 1:10 MB ascites i.p. Nine d later, all mice  
were given a source of T cell help. Mice in groups A and B were given 7 × 10⁶  
TKLH i.v.; mice in groups C and D were given 5 × 10⁶ Lyt-2⁻ TKLH i.v. These  
doses were chosen so that all groups received the same number of Lyt-2⁻ TKLH.  
2 d later all mice were immunized with 0.1 mg Ar-KLH in CFA. 10 d after a  
second immunization with Ar-KLH (in IFA), the mice were bled and titers of  
Id MB and anti-Ar antibodies determined. As shown in Table II, all mice responded  
to Ar-immunization. Anti-Ar antibodies of mice that did not receive MB expressed Id MB, as did the anti-Ar antibodies of similarly immunized control mice.  
Anti-Ar antibodies of mice that were treated with MB failed to express Id MB  
even when T cell help was provided as Lyt-2⁻ TKLH.

Discussion

We examined several characteristics of Id suppression induced by the homol-  
gous monoclonal antidiotope, MB. A single intraperitoneal inoculation of MB  
resulted in prolonged, profound, idiotope suppression in all of the mice exam-  
ined. Abrogation of Id MB expression was more complete and more long lasting  
than idiotope suppression reported in some other anti-hapten responses (14).  
This could represent suppression mediated by long-lived suppressor cells. How-  
ever, an apparent suppressed state of similar longevity was produced by adoptive  
transfer of splenic B cells from Id MB suppressed mice to lightly irradiated  
recipients. Transfer of "suppression" by B cells has been explained on the basis  
of clonal dominance of the immune response by secondary B lymphocytes (3, 4),  
and in the present case this explanation is supported by the fact that RAMIg  
adherent spleen cells yielded the same result as Thy-1.2⁻ spleen cells. Attempts  
to induce idiotope suppression by adoptive transfer of splenic T cells from Id MB  
suppressed donors yielded inconsistent results (data not shown).  
The fact that suppression of Id MB expression was transferable by B cells alone

| Group: | A | B | C | D | Normal control |
|-------|---|---|---|---|----------------|
| MB:   | - | + | - | + | -              |
| TKLH: | + | + | Lyt-2⁻ | Lyt-2⁻ | -           |
| Id MB | 803 | <1² | 224 | <1 | 269 |
| Titers* | 85.0 | <1 | 85.6 | <1 | 56 |
|       | 340 | <1 | 3.2 | <1 |           |
|       | <1 | 269 | <1 |           |   |
|       | <1 | 37.1 | <1 |           |   |
| Anti-Ar | 680 | 468 | 568 | 704 | 676 |
| Titers* | 1,120 | 117 | 239 | 287 | 551 |
|       | 5,990 | 599 | 640 | 266 |   |
|       | 456 | 992 | 557 |       |
|       | 284 | 262 | 210 |       |

*²See Table 1.
suggested that idiotope-specific Ts were not required to perpetuate idiotope suppression, once established. This led us to question the need for Ts at the time of initial induction of idiotope suppression. To address this question, adult A/J mice were depleted of mature T cells, before administration of MB. T-depleted mice given a source of T help and immunized with Ar-KLH produced anti-Ar antibody that expressed IdMB. On the contrary, T-depleted mice given MB 9 d before adoptive transfer of TKLH and Ar-immunization did not express IdMB, even when the source of T help was Lyt-2-TKLH (c.f. group D, Table II). Although we were not able to directly assess T depletion of splenic and lymph node populations, our limiting dilution analysis of peripheral blood lymphocytes suggests that these mice were severely T cell deficient before adoptive transfer of TKLH. This did not affect the ability of MB to induce idiotope suppression.

In a series of reports, Nisonoff and his colleagues (2) demonstrated that A/J mice suppressed by heterologous (rabbit) antiidiotypic antiserum and subsequent hyperimmunization with Ar-KLH contained in their spleens T lymphocytes capable of transferring idiotype suppression to naive, lightly irradiated recipients. While the present results do not rule out the participation of small numbers of Ts, they suggest that suppression induced by MB results from direct interaction between antiidiotope and Id+B cells without the intervention of idiotope-specific Ts, in keeping with previous work reported from this laboratory for the anti-Ar response (7) and results reported by Bona et al. for a T-independent response (15). These differences may result from the nature of the reagents used to induce idiotype suppression. In particular, monoclonal antiidiotopes are more restricted in their interactions than antisera, and homologous monoclonal antibodies (such as MB) lack carrier determinants and so may result in tolerance by receptor blockade (16). On the other hand, the two sets of results may not be at variance, since Ts may result from, but not be responsible for, suppression of Id+B cells (17). Further work will be needed to clarify these issues.

Summary

The homologous, monoclonal antiidiotope, MB, induced idiotope suppression that was remarkably stable and could be transferred by B lymphocytes. Marked depletion of T cell function, confirmed by limiting diluting analysis, did not affect the ability of MB to suppress the corresponding idiotope. Suppression induced by MB appears to result from direct interaction with idiotope-positive B cells, without the intervention of idiotope-specific T suppressor cells.

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