The cellular interactions in the anti-\(\text{p-azobenzenearsonate}\) (ABA)\(^1\) suppressor T cell pathway, for the inhibition of ABA-specific delayed-type hypersensitivity and cytotoxic T cell responses, are restricted by Igh-linked genes (reviewed in 1). The inducer, Ts-1 (T suppressor cell), and the effector, Ts-3, suppressor T cells, and one of their factors (TsF\(_1\)) bear the major crossreactive idiotypic (CRI) determinants recognized by rabbit antiidiotypic antibodies prepared by immunizing rabbits with purified anti-ABA from appropriate strains of mice (2, 3).

The presence of these Ig idiotypic specificities on T cells is not the result of the expression of Ig heavy chain variable region genes in T cells, but rather reflects the degree to which the repertoire of these regulatory T cells is influenced by the Ig idiotypes on B cells during their differentiation or induction. We therefore proposed (4) that clonal expansion of a B cell subpopulation bearing a particular idiotypic specificity stimulates the clonal expansion of corresponding antiidiotypic T or B cells. These antiidiotypic T or B cells, in turn, select and trigger the expansion of a population of idiotype-bearing T cells. Therefore, the detection of Ig idiotypes on T cells may merely reflect a serological or conformational crossreactivity, and represent internal images of B cell idiotypic specificities rather than a true genetic identity.

This hypothesis is supported by findings indicating that certain T cell activities appear to depend on B cells for their expression (reviewed in 5). These include idiotype-specific helper T cells (6, 7), isotype-specific helper T cells (8), and antigen-specific proliferating T cells (9, 10). Moreover, it has recently been shown (11) that B cell–deficient mice are unable to produce one of the two chains comprising the T cell–derived, sheep red blood cell–specific inducer-suppressor factor.

Taking advantage of our previous observations (1) of the expression of anti-CRI–defined idiotype by Ts-1 cells and their factors (TsF\(_1\)) in the ABA-system, we studied whether the presence of Ig-bearing B cells is required for the...
expression of idiotypes by ABA-specific Ts-1 and TsF1, and for the Igh-1 linked genetic restrictions normally associated with the activity of this factor (4). We reasoned that B cell-deficient mice, produced as a result of treatment with rabbit anti-mouse IgM antibodies (anti-μ) starting within 24 h of birth (13, 14), would develop ABA-specific Ts-1 and TsF1 lacking the appropriate idiootypic determinants if, in fact, B cells expressing idiotypes are ontogenically required for the generation of idiootype-positive T cells. We expected that ABA suppressor T cells and their factors, when taken from mice developed without B cells, would no longer show Igh restrictions, and would be active in all strains, irrespective of their genotypes. In agreement with this prediction, TsF1 obtained from anti-μ-treated BALB/c mice gained the capacity of suppressing the DTH and cytotoxic T lymphocyte (CTL) responses of normal C.AL-20 mice. Similarly, TsF1 obtained from anti-μ-treated C.AL-20 mice developed the ability to suppress BALB/c mice. Moreover, TsF1 from anti-μ-treated C.AL-20 mice was found not to express the major CRI determinants normally associated with C.AL-20 TsF1 (4). However, to our surprise, ABA-TsF1 from anti-μ-treated BALB/c or C.AL-20 mice were not active in other strains, such as H-2-identical B10.D2 (12). Furthermore, while ABA-TsF1 from anti-μ-treated BALB/c and C.AL-20 mice reciprocally lost their Igh restrictions for each other, they also lost their ability to suppress normal mice of their own respective strains. This study was designed to explore the parameters that normally limit the effects that Ig-bearing B cells have on the T cell repertoire, as illustrated above. We made use of the relationships that have been shown to exist between the CRI expressed by anti-ABA antibodies of A or AL/N mice and BALB/c mice (15-18) to explain our earlier results.

In the antibody response to ABA-KLH (keyhole limpet hemocyanin), all mice of the A or AL/N strain, including allotype-congenic C.AL-20 mice, produce anti-ABA antibodies that bear the CRI specificity CRI(A). In general, 20-70% of the anti-ABA population carries CRI(A) determinants (15, 16). In addition, a second idiootypic family has been described, which comprises a minor portion (10-15%) of the anti-ABA antibodies produced in the A strain of mice, and which is serologically distinct from CRI(A) (17, 18). It is of considerable interest that this minor idiotype in the A strain corresponds to the major idiotype associated with anti-ABA antibodies of BALB/c mice CRI(C).

The availability of antidiotypic antibodies against CRI(A) and CRI(C) has enabled us to establish that TsF1 obtained from anti-μ-treated C.AL-20 mice, functional in BALB/c but not in C.AL-20 mice, indeed bears the CRI(C) determinants. And TsF1, obtained from anti-μ-treated BALB/c, suppresses C.AL-20 but not BALB/c mice, and expresses CRI(A) determinants. The significance of these findings will be discussed with respect to the role of B cells in the generation of the suppressor T cell repertoire.

**Materials and Methods**

**Animals.** BALB/c (H-2d, Igh-1a) and C.AL-20 (H-2d, Igh-1a) mice were bred and maintained in our colony in accordance with the guidelines of the Committee on Animals, of the Harvard Medical School, and those prepared by the Committee on Care and Use of Laboratory Animals, of the Institute of Laboratory Animal Resources, National Research Council (Department of Health and Human Services publication, National Institutes of Health 78-23, revised 1978).
Preparation of Anti-μ Antibodies, and Treatment of Newborn Mice. Anti-μ were prepared by immunizing NZW rabbits with purified MOPC 104E (λ) or TEPC 183 (κ) (Bionetics Laboratory Products, Charleston, SC) in 0.5–1 mg complete Freund's adjuvant (CFA) per immunization. Hyperimmune antisera were pooled, absorbed with mouse red blood cells, and precipitated twice with 50% ammonium sulfate. The final preparations were then concentrated and dialyzed extensively with phosphate-buffered saline (pH 7.2). The amount of anti-μ-specific antibody was quantitated by a quantitative precipitin test. Each mouse received from 700 μg to 1 mg of rabbit anti-μ per injection. Newborn mice were injected with 50 μl of anti-μ (0.7–1 mg) i.p. within 24 h three times per week (Monday, Wednesday, Friday), until they were sacrificed. All experiments were carried out when animals reached the age of 6–7 wk. All mice were housed in cages with filters and acidified water (pH 2.8).

Preparation of Hapten-conjugated Syngeneic Spleen Cells. The diazonium salt of p-arsanilic acid (Kodak) was prepared as previously described (2). Briefly, a 40 mM solution of ABA-diazonium salt was prepared from arsanilic acid. The ABA solution was activated and conjugated to single-cell suspensions of erythrocyte-free spleen cells at a final concentration of 10 mM ABA. After washing twice in Hank's balanced salt solution (HBSS), the ABA-coupled spleen cells (ABA-SC) were used to prime for ABA-specific CTL in vivo. A total of 3 × 10^7 viable ABA-SC were injected subcutaneously into two separate sites on the dorsal flanks of mice. Each group consisted of at least two mice.

In Vitro Induction of CTL. 7 d after subcutaneous immunization, spleen cells were prepared, and pooled for use as responder cells for in vitro culture. The culture conditions used to generate CTL have been described in detail elsewhere (4). Briefly, 7 × 10^6 spleen cells from primed or suppressed animals were cocultured with 6 × 10^6 ABA-coupled irradiated syngeneic spleen cells in 16 mm Linbro tissue culture wells (Linbro Chemical Co., Hamden, CT) in a volume of 2 ml of medium per well. Culture medium consisted of RPMI 1640 supplemented with 100 U/ml Penicillin, 100 μg Streptomycin, 0.25 μg/ml Fungizone, 2 mM glutamine, 5 × 10^-5 M 2-mercaptoethanol, and 5–10% preselected heat-inactivated fetal calf serum. Cultures were incubated for 5 d in 5% CO_2 at 37°C, with saturated humidity.

Chromium-release Assay. This assay has been described in detail previously (4). Briefly, 3 × 10^7, concanavalin A (Con A)-induced blasts were labeled with 0.5–1.0 mCi of 51Cr for 90 min, washed, coupled with hapten as described above, and used as targets in the assay. Con A blasts were prepared by culturing 4 × 10^6 spleen cells/ml with 2 μg/ml of Con A for 48 h in RPMI 1640 medium, supplemented with serum, as described above. Cytotoxicity was calculated on the basis of the formula: percent specific 51Cr release = ([51Cr release from targets in presence of effector cells] - [spontaneous 51Cr release])/([maximum 51Cr release in presence of detergent] - [spontaneous release]). The spontaneous release of Con A blast targets ranged from 20 to 30% in the 4 h assay.

Preparation of Suppressor T Cell Factor. Normal or anti-μ-treated mice were given intravenous injections of 5 × 10^7 ABA-SC, and irradiated (1,500 rad). 7 d later, the mice were sacrificed, and the spleens teased into single-cell suspensions. Suppressor factors were prepared using a snap-freezing and thawing method, as described (11). Briefly, 5 × 10^6 washed spleen cells in 1 ml of HBSS were subjected to alternate snap freezing at -70°C and thawing at 37°C. This was repeated four times, and was followed by centrifugation at 10,000 g for 60 min. The supernatants were then frozen at -70°C until use. To test the ability of TsF to inhibit ABA-specific CTL response, 2 × 10^7 cell-equivalents/day of TsF were injected intravenously into ABA-SC–primed normal mice, beginning at the day of immunization with 3 × 10^7 ABA-SC, and for five successive days. 2 d after the last injection, the animals were killed and their spleens were removed to set up the CTL assay.

Affinity Chromatography of TsF. Solid-phase immunoabsorbent columns were prepared and characterized as described (2). TsF was fractionated on immunoabsorbents in the following manner: A 5-ml plastic column containing antibody-conjugated Sepharose 4 B (Pharmacia Fine Chemicals, Piscataway, NJ) was prepared by using an IgG fraction of anti-μRII antisera. Rabbit anti-μRII, antibodies were prepared by repeatedly injecting rabbits subcutaneously with specifically purified A/J anti-ABA antibodies in CFA. These
antibodies were rendered specific for idiotypic determinants by repeated absorption with normal A/J Ig. Antiidiotypic antibodies against the BALB/c major idiotypic family, CRI(c), was prepared by immunizing rabbits with a monoclonal anti-ABA antibody, 36–60, which is the major idiotypic family in BALB/c mice, and represents a minor component in A/J mice; 36–60 is derived from A/J mice (18). These antibodies were kindly provided to us by Dr. M. Gelfter of Massachusetts Institute of Technology, Boston, MA.

A control column was similarly prepared, using an IgG fraction of normal rabbit serum in place of the anti-CRI antiserum. The adsorption of the factor is carried out at 4°C by allowing 5 × 10⁶ cell-equivalent of TsF in a 1 ml volume to enter the gel matrix. The TsF was then allowed to remain in the column at least 60 min at 4°C. The column was then washed with at least 5× its own void volume, using cold PBS (pH 7.2). Such effluents were termed filtrates. Materials that remained in the column were eluted with five bed-volumes of a glycine-HCl buffer (pH 2.8). The collected eluates were immediately neutralized to pH 7.0 with 1 N NaOH as the material emerged from the column. Both the filtrates and eluates were concentrated to the original volume by negative pressure dialysis at 4°C, and were thereafter frozen at −70°C. Such materials were thawed immediately before use.

Results

Serological Characterization of TsF₁ Obtained from C.AL-20 and BALB/c Mice. Our previous work (1) has demonstrated that TsF₁ obtained from C.AL-20 mice, bears the major CRI determinants that are serologically crossreactive with those present in anti-ABA antibodies from appropriate strains of CRI(A) mice. We would expect, accordingly, that TsF₁ from normal BALB/c mice should bear the corresponding CRI(c) determinant normally associated with anti-ABA antibodies of BALB/c mice. Therefore, we investigated whether antiidiotypic antibodies prepared against the CRI(c) determinant will react with TsF₁ from normal BALB/c mice.

ABA-specific TsF was prepared from normal BALB/c and C.AL-20 mice. These TsF were then passed through an anti-CRI(A) or anti-CRI(c) column. Both the filtrate (unbound) and the acid eluate (bound) were then tested on BALB/c or C.AL-20 mice, respectively, for their ability to suppress priming for ABA-specific CTL responses. The results of such an experiment are shown in Figs. 1 and 2. When TsF₁ obtained from normal C.AL-20 was passed over an anti-CRI(A) column, no suppressive activities could be found in the filtrate, and all the suppressive activity could be recovered in the acid eluate. In contrast, when this factor was passed through an anti-CRI(c) column, all the suppressor activity remained in the filtrate, and the eluate was devoid of any suppressor activity (Fig. 1). On the other hand, the suppressor activity of normal BALB/c TsF₁, when passed through an anti-CRI(A) column, was found in the filtrate, not in the acid eluate (Fig. 2). This provides direct evidence that TsF₁ from normal BALB/c mice lacks CRI(A) specificities. When normal BALB/c TsF was passed through an anti-CRI(c) column (Fig. 2), most of the suppressor activities could be recovered in the acid eluate. Nevertheless, there was significant suppressor activity that failed to bind to the anti-CRI(c) column. This observation differs significantly from our findings with C.AL-20 TsF₁ and anti-CRI(A) immunoadsorbent columns. In C.AL-20 mice, it is evident that most, if not all of the TsF₁ activities can be retained by anti-CRI(A) columns. The failure of anti-CRI(c) column to bind all the BALB/c TsF₁ is not due to over-saturation of the immunoadsorbent column, since repassage of the unbound fraction over the anti-CRI(c) column also failed...
FIGURE 1. Normal C.AL-20 TsF can be retained by anti-CRI(A) but not anti-CRI(c) column. Normal C.AL-20 TsF1 was prepared by intravenous injection of $5 \times 10^7$ ABA-SC; 7 d later TsF1 was prepared as described. $2 \times 10^4$ cell-equivalents of TsF were passed through an anti-CRI(A) or anti-CRI(c) immunoabsorbent column. Both the unbound fraction (filtrate) and the bound fraction (eluate) were tested in immunized C.AL-20 mice. Control mice were normal C.AL-20 mice immunized subcutaneously with $3 \times 10^7$ ABA-SC only (O). TsF was given to the experimental groups, beginning on the day of immunization, for five successive days at $2 \times 10^4$ cell-equivalents/day in 0.2 ml volumes. 2 d after the last injection, spleen cells from each group were removed to set up ABA-specific CTL culture. ABA-CTL assays were done 5 d later, as described in Materials and Methods. Percent specific killing represents killing of ABA-conjugated syngeneic Con A blasts minus killing of unconjugated blasts.

FIGURE 2. Majority of BALB/c TsF1 can be retained by anti-CRI(c) but not by anti-CRI(A) column. The experimental protocols used in these experiments were exactly identical to experiments presented in Fig. 1, except normal BALB/c TsF1 was used instead of normal C.AL-20 TsF1. (O) Control mice were normal BALB/c mice immunized subcutaneously with $3 \times 10^7$ ABA-SC. (■) Animals treated with unbound (filtrate) fraction. (▲) Animals treated with bound (eluate) fraction.

to demonstrate any additional binding (data not shown). From this experiment we can conclude that, as expected, normal C.AL-20 TsF1 bears determinants that crossreact with CRI(A) specificities, and does not crossreact with CRI(c) specificities. In contrast, most, but not all of the normal BALB/c TsF1 expresses determinants crossreactive with CRI(c), but not with CRI(A).

Igh Restriction Specificities of BALB/c Factors that Failed to Bind to Anti-CRI(c) Immunoabsorbent Column. Our observation that ABA-specific TsF1 from normal BALB/c mice appears to be idiotypically somewhat more heterogeneous than C.AL-20 TsF1 raised an important issue regarding the Igh restriction specificity of the fraction of normal BALB/c TsF1 that does not express CRI(c) determinants. It is possible that the small fraction of BALB/c TsF1 that was CRI(c) may not be Igh restricted in its function. Therefore, we enriched the CRI(c) - TsF by
Normal BALB/c or C.AL-20 were immunized subcutaneously with $3 \times 10^7$ ABA-conjugated syngeneic spleen cells. 7 d later, spleen cells from control and treated animals were cultured in vitro for 5 d in the presence of ABA-SC as stimulators for the generation of ABA-specific CTL response.

* ABA-specific TsF$_1$ from BALB/c mice were passed over an anti-CRI$_{c}^{-}$ immunoabsorbent column, as described in Materials and Methods. Both the unbound (filtrate) or bound (acid eluate) fractions were injected into BALB/c or C.AL-20 mice, beginning on the day of immunization, for five successive days at $2 \times 10^7$ cell-equivalents/day, in 0.2 ml volumes. 2 d after the last injections, spleen cells from each group were removed to set up ABA-specific CTL culture.

** A standard 4-h $^{51}$Cr-release assay was done after 5 d in culture. Percent specific killing represents killing on ABA-conjugated syngeneic Con A blasts, minus killing on uncoupled Con A blasts.

---

### Table I

**Igh Restriction Specificity of CRI$_{(c)}$-bearing and Non-CRI$_{(c)}$-bearing BALC/c TsF$_1$**

| Strain     | Treatment* | Specific killing$^2$ at effector/target ratios of: |
|------------|------------|--------------------------------------------------|
|            |            | 100:1   | 50:1  | 25:1  |
| BALB/c     | —          | %       |       |       |
|            | Anti-CRI$_{(c)}$-passed TsF$_1$: |         |       |       |
|            | Filtrate   | 40      | 32    | 19    |
|            | Eluate     | 21      | 15    | 8     |
| C.AL-20    | —          | 57      | 40    | 28    |
|            | Anti-CRI$_{(c)}$-passed TsF$_1$: |         |       |       |
|            | Filtrate   | 52      | 39    | 26    |
|            | Eluate     | 49      | 42    | 37    |

---

passing normal BALB/c TsF$_1$ over an anti-CRI$_{(c)}$ column and assaying for the Igh restriction of the unbound TsF. The results of such an experiment are shown in Table I. As can be seen, normal BALB/c TsF$_1$ can be divided into CRI$_{(c)}$-bearing and non-CRI$_{(c)}$-bearing fractions; both of these fractions are suppressive in normal BALB/c mice. More importantly, both the non-CRI$_{(c)}$-bearing and CRI$_{(c)}$-bearing fractions remain nonfunctional in C.AL-20 mice, indicating that even though some of BALB/c TsF$_1$ may not express CRI$_{(c)}$ determinants, they are still Igh restricted in their function.

**TsF$_1$ Obtained from Anti-µ-treated C.AL-20 Mice Expressed CRI$_{(c)}$ but not CRI$_{(a)}$ Determinants.** Since TsF$_1$ obtained from anti-µ-treated C.AL-20 mice inhibits the development of ABA-specific CTL responses in normal BALB/c mice but not in C.AL-20 mice, we next examined whether these TsF$_1$ bear CRI$_{(a)}$ specificities. ABA-specific TsF$_1$ was prepared from anti-µ-treated C.AL-20 mice. This TsF$_1$ was then passed through an anti-CRI$_{(a)}$ or an anti-CRI$_{(c)}$ column. Both the filtrate and acid eluate were then tested in BALB/c mice.

The results of a representative experiment are shown in Table II. As we have reported earlier (4), TsF$_1$ obtained from anti-µ-treated C.AL-20 mice no longer expresses CRI$_{(a)}$ determinants. Therefore, when passed through a rabbit anti-CRI$_{(a)}$ column, the suppressor activities reside mainly in the filtrate, not in the acid eluate. In contrast, the identical TsF$_1$, when passed through an anti-CRI$_{(c)}$ column, yields a filtrate with minimal suppressor activity; significant suppressor
TABLE II

T cell development in B cell-deficient mice

| Treatment* | Specific killing* at effector/target ratios of: |
|------------|-----------------------------------------------|
|            | 100:1  | 50:1  | 25:1  |
|            | %      |       |       |
| ---        |        |       |       |
| Anti-CRI(a)-passed TsF₁: | | | |
| Filtrate  | 41     | 40    | 18    |
| Eluate    | 82     | 52    | 37    |
| Anti-CRI(c)-passed TsF₁: | | | |
| Filtrate  | 62     | 48    | 30    |
| Eluate    | 45     | 31    | 22    |

Normal BALB/c mice were immunized subcutaneously with 3 x 10⁷ ABA-conjugated syngeneic spleen cells. 7 d later, spleen cells from controls and treated groups were cultured in vitro for 5 d in the presence of ABA-SC as stimulators for the generation of ABA-specific CTL response.

* TsF₁ was obtained from anti-μ-treated C.AL-20 mice and passed through either an anti-CRI(a) or anti-CRI(c) column. Both the filtrate and eluate from each column was tested in BALB/c mice, as described.

** A standard 4-h ³Cr-release assay was done after 5 d in culture. Percent specific killing represents killing on ABA-conjugated syngeneic Con A blasts, minus killing on uncoupled Con A blasts.

Figure 3. ABA-specific TsF₁ from anti-μ-treated BALB/c mice can be retained by anti-CRI(a) but not by anti-CRI(c) column. ABA-specific TsF₁ was prepared from anti-μ-treated BALB/c mice, as described. The factor(s) was then passed through an anti-CRI(a), normal rabbit Ig, or anti-CRI(c) column. Both the filtrate and acid eluate were tested in normal C.AL-20 mice, as described in Materials and Methods, and in Fig. 1. (C) Control mice were normal C.AL-20 mice immunized subcutaneously with 3 x 10⁷ ABA-SC. ( □) Animals treated with unbound (filtrate) fractions. ( ▲) Animals treated with bound (eluate fraction).

Activity can then be recovered in the acid eluate. This experiment suggests that the reason for the ability of ABA-specific TsF from anti-μ-treated C.AL-20 mice to work in BALB/c is directly related to their acquisition of the CRI(c) specificities.

TsF₁ Obtained from Anti-μ-treated BALB/c Mice Express CRI(a) Determinants but Not CRI(c) Specificities. Since anti-μ-treated BALB/c TsF was shown to suppress C.AL-20 mice, we wished to know whether TsF from anti-μ-treated BALB/c
mice acquires the capacity to express the CRI$_{(A)}$ determinants. Experiments were done using an identical protocol. ABA-specific TsF$_1$ was prepared from anti-$\mu$-treated BALB/c mice. This TsF was then passed through an anti-CRI$_{(A)}$ or anti-CRI$_{(C)}$ column. Both the filtrate and acid eluate were then tested in C.AL-20 mice. The results of a typical experiment are shown in Fig. 3. It was clearly shown that, as with TsF$_1$ obtained from anti-$\mu$-treated C.AL-20 mice, TsF$_1$ obtained from anti-$\mu$-treated BALB/c mice displays the opposite idiotypic specificities normally associated with BALB/c TsF$_1$. Anti-$\mu$ BALB/c TsF$_1$ expressed the CRI$_{(A)}$ specificities associated with normal C.AL-20 TsF$_1$. None of the factors bound to the normal rabbit Ig control column, since all the suppressor activity was detected in the filtrate, and none in the acid eluate.

Discussion

The presence of Ig idiotypes on T cells, and of Igh-controlled restrictions in the suppressor T cell cascade led us to propose that these idiotypes and corresponding Igh restrictions they determine are the reflection of the influence that the major B cell idiotypes impose on the T cell repertoire during T cell development or antigen-specific immune responses (4). Our observations, in the ABA-specific T cell suppressor system, that the CRI on TsF$_1$ were dependent on the presence of Ig-bearing B cells expressing these very same idiotypes was in agreement with our hypothesis and provided, in addition, the indication that the network theory of Jerne (19) should be extended to the relationships that it imposes on the respective repertoires of T cells and B cells.

In the course of the experiments carried out with anti-$\mu$-treated mice, we were puzzled by the findings that the loss of the major idiotype of ABA-specific antibodies of C.AL-20 mice CRI$_{(A)}$ by the TsF$_1$ from anti-$\mu$-treated C.AL-20 mice was associated with: (a) New Igh restrictions that favor exclusively BALB/c mice, to the exclusion of other congeneric strains, expressing different Igh genes. (b) Loss of the ability to suppress normal C.AL-20 mice.

We reasoned that these results, in addition to their demonstration of the influence of Ig idiotypes on the suppressor T cell repertoire, were conditioned by the interesting reciprocal relationships that exist in the idiotypes of anti-ABA antibodies of A and C.AL-20 mice, and of BALB/c mice. A and C.AL-20 mice, which display CRI$_{(A)}$ as their major idiotypes, also display CRI$_{(C)}$ specificities, characteristic of BALB/c mice, on a minor population of their ABA-specific antibodies, and that BALB/c mice, which normally express the CRI$_{(C)}$ idiotype, can be induced, under certain circumstances, to express the CRI$_{(A)}$ idiotype (20). We report that a large fraction of the TsF$_1$ from BALB/c mice display CRI$_{(C)}$, even though a significant fraction, 15–40%, is CRI$_{(C)}^-$. The failure of our anti-CRI$_{(C)}$ column to bind all of the BALB/c TsF$_1$ is probably not due to the fact that the antiidiotypic antibodies used were prepared by immunizing rabbits with monoclonal anti-ABA antibodies (18), since a second anti-CRI$_{(C)}$ immunoabsorbent column prepared with rabbit anti-CRI$_{(C)}$ antibodies, which were generated by immunizing rabbits with purified anti-ABA antibodies from BALB/c mice (kindly provided by Dr. A. Brown of St. Jude Children’s Research Hospital, Memphis, TN), also revealed the presence of CRI$_{(C)}$-bearing and non–CRI$_{(C)}$-bearing TsF$_1$ in BALB/c mice (data not shown).
Moreover, since none of the BALB/c TsF can be retained by the anti-CRI(A) immunoabsorbent column, the non-CRI(c)-bearing TsF does not bear CRI(A) determinants either. Furthermore, since both the CRI(c)-bearing and the non-CRI(c)-bearing TsF show similar restriction specificity for BALB/c mice, these factors must play a role in the ABA-specific suppressor pathway in BALB/c mice.

Our experiments have also shown that TsF1 from anti-μ-treated C.AL-20 mice not only acquired the capacity to work in BALB/c mice, but more importantly, also expressed the CRI(c) specificities. Similarly, TsF1 from anti-μ-treated BALB/c mice is functional in C.AL-20 mice, and bears the CRI(A) determinants. The results obtained from anti-μ-treated BALB/c mice deserve further comment, since almost all of the TsF1 prepared from anti-μ-treated BALB/c mice can be retained by anti-CRI(A) column. Therefore, we must conclude that both the CRI(c)-bearing and non-CRI(c)-bearing TsF1 normally associated with normal BALB/c mice were replaced by CRI(A)-bearing TsF in anti-μ-treated mice.

These observations suggest that the effect of anti-μ treatment is complex. Since <1% of Ig-bearing B cells can be detected in our anti-μ-treated mice (data not shown), the effect of these B cells in determining T cell idiotype specificities is expected to be minimal. Yet, the removal of most B cells now enables a minor TsF1 idiotypic specificity to become dominant, by a mechanism that remains to be elucidated. It has been reported by Kim and her colleagues (21) that even though no serum IgM can be detected in anti-μ-treated mice, detectable amounts of total Ig, IgG1, and IgG2 in these mice were 1,000-fold, 100-fold, and 5,000-fold less, respectively, than those of control mice. These residual Ig molecules may play a role in the alteration of IgH restriction patterns in our experiment model. However, it should be noted that in the previous studies (21), spleen cells from anti-μ-treated mice contained 2–5% Ig+ B cells, and the serum samples were pooled, not from individual mice. In our anti-μ-treated mice, the B cell level never reached higher than 2%, and no Ig was detected in their serum. Thus, it is not clear by what mechanisms T cells in anti-μ-treated C.AL-20 mice acquire CRI(c) determinants, and T cells from anti-μ-treated BALB/c mice acquire CRI(A) specificities. It has been reported by Slaou and his associates (20) that treatment of BALB/c mice, which normally do not express CRI(A) specificities with monoclonal anti-CRI(A) antibodies, causes them to express these determinants when immunized against ABA. More recently, it was found that, while BALB/c mice normally are insensitive to the suppressor effect of TsF1 obtained from A/J mice, treatment of BALB/c mice with anti-CRI(A) monoclonal antibodies rendered them susceptible to the suppressive effect of TsF1 from A/J mice (M. Slaou, personal communication). Since proper idiotypic and antiidiotypic interaction is absolutely required for the completion of the ABA-specific suppressor pathway, we can conclude from these experiments that the CRI(A) idiotype is indeed present in the potential repertoire of BALB/c mice, both in the T and B cell compartment, but that during a normal humoral or cell-mediated response to ABA, these CRI(A)-bearing clones remain silent or suppressed. This dominant trait can be broken either by treatment with anti-μ, starting at birth, as in our experiments, or by treatment with monoclonal antibodies in adulthood.

The mechanism responsible for the preferential expansion of CRI(c) clones at the expense of CRI(A) clones in normal BALB/c mice is not clear. In the C.AL-
20 mice, it is known that CRI(c) specificities represent a minor portion of the idiotypic families (10-15%), while the majority (20-70%) of anti-ABA antibodies bear CRI(a) specificities. Therefore, in C.AL-20 mice, in contrast to BALB/c mice, CRI(a) clones rather than CRI(c) clones are preferentially expanded. Whatever the mechanism responsible for the preferential clonal expansion of CRI(a) B cells in C.AL-20 mice, and of CRI(c) B cells in BALB/c mice, based on our results, the development of idiotypic specificities on suppressor T cells appears to parallel that in B cells. Thus, the majority of the TsF<sub>1</sub> from C.AL-20 mice bears CRI(a) determinants, and most, but not all of the TsF<sub>1</sub> from BALB/c mice bear CRI(c) determinants. It is possible that TsF obtained from C.AL-20 mice contain a small portion that bears CRI(c), and that a minor fraction of BALB/c TsF expresses CRI(a) specificities, but due to limitations in the sensitivity of our in vivo assay, we may not be able to detect them.

The exact mechanisms responsible for the dominance of one idiotypic family over another one in TsF repertoire is unknown, but it is clear that the establishment of a hierarchy in the expression of a particular idiotypic specificity in TsF results in the Igh restriction specificity of that TsF. It is possible that explanations that have been suggested for the phenomenon of idiotypic dominance in antibody responses may be also applicable to the dominance of idiotypic family TsF repertoire. These include affinity for antigen, clonal size, or regulatory mechanisms, either in the form of helper or suppressor T cells (22-24). For example, in normal C.AL-20 mice, the presence of antiidiotypic helper T cells specific for CRI(a) could promote the clonal expansion of CRI(a)<sup>+</sup> T cells, or conversely, the presence of antiidiotypic TsF<sub>1</sub> specific for CRI(c) in C.AL-20 mice could prevent the emergence of CRI(c)-bearing T cells in these mice. If this is true, one could postulate a breakdown of the idiotypic hierarchy in anti-μ-treated mice, resulting in the appearance of the minor idiotypic specificities over a major idiotypic family in the TsF<sub>1</sub>. Nevertheless, the mechanisms by which B cells influence the hierarchy of the suppressor T cell idiotypic family still remain a mystery.

Summary

Serological analysis of idiotypic specificities present in azobenzenearsonate (ABA)-specific first-order suppressor T cell factors (TsF<sub>1</sub>) from C.AL-20 and BALB/c mice revealed a significant difference between TsF from these two strains of mice. The idiotypic composition of TsF<sub>1</sub> from BALB/c mice appears to be more heterogeneous, and at least two different fractions can be readily identified. One bears the characteristic BALB/c-associated CRI(c) (crossreactive idiotype) determinants, and the other is non-CRI(c)-bearing. Analysis of ABA-specific TsF<sub>1</sub> from animals lacking B cells uncovered a fundamental change in the expression of their idiotypic specificities. TsF from rabbit anti-mouse IgM (anti-μ)-treated C.AL-20 mice failed to express the characteristic CRI(a) determinants. Instead, they express CRI(c) specificities. Similarly, TsF<sub>1</sub> from anti-μ-treated BALB/c mice did not express their characteristic CRI(c) specificities, but rather express CRI(a) determinants. These experiments provide strong evidence that the Igh restriction specificity of TsF is dictated by the particular idiotypic specificities expressed. They also clearly demonstrate that B cells and their products play an important role in establishing the idiotypic composition and repertoire of suppressor T cells.
We gratefully acknowledge the excellent technical assistance of Leonard Colarusso, and we thank Mary Jane Tawa for her assistance in the preparation of the manuscript.

Received for publication 16 January 1985.

References
1. Greene, M. I., J. J. Nelles, M.-S. Sy, and A. Nisonoff. 1982. Regulation of immunity to azobenzenearsonate hapten. Adv. Immunol. 32:253.
2. Bach, B. A., M. I. Greene, B. Benacerraf, and A. Nisonoff. 1979. Mechanisms of regulation of cell-mediated immunity. IV. Azobenzenearsonate-specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to the heavy-chain allotype linkage group of genes. J. Exp. Med. 149:1084.
3. Sy, M.-S., M. H. Dietz, and A. Nisonoff, R. N. Germain, B. Benacerraf, and M. I. Greene. 1980. Antigen- and receptor-driven regulatory mechanisms. V. The failure of idiotype-coupled spleen cells to induce unresponsiveness in animals lacking the appropriate VH gene is caused by the lack of idiotype-matched targets. J. Exp. Med. 152:1226.
4. Sy, M.-S., A. Lowy, K. T. HayGlass, C. A. Janeway, Jr., M. Gurish, M. I. Greene, and B. Benacerraf. 1984. Chronic treatment with rabbit anti-mouse # chain antibodies alters the characteristic immunoglobulin heavy chain restriction of murine suppressor T cell factors. Proc. Natl. Acad. Sci. USA. 81:3846.
5. Janeway, Jr., C. A. 1984. The role of idiotype and of immunoglobulin in T cell differentiation and function. In The Biology of Idiotypes, M. I. Greene and A. Nisonoff editors. Plenum Press, NY. 349.
6. Bottomly, K., C. A. Janeway, Jr., B. J. Mathieson, and D. E. Mosier. 1980. Absence of an antigen-specific helper T cell required for the expression of the T-15 idiotype in mice treated with anti-# antibody. Eur. J. Immunol. 10:159.
7. Janeway, Jr., C. A., R. A. Murgita, F. E. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin dependent antigen-specific helper T cell. Proc. Natl. Acad. Sci. USA. 74:4502.
8. Rosenberg, Y. J., and R. Asofsky. 1981. T cell regulation of isotype expression. The requirement for a second Ig specific helper T cell population for the induction of IgG responses. Eur. J. Immunol. 11:705.
9. Tzehoval, E., P. DeBaetselier, Y. Ron, B. Tartakovsky, M. Feldman, and S Segal. 1983. Splenic B cells function as immunogenic antigen-presenting cells for the induction of effector T cells. Eur. J. Immunol. 10:159.
10. Ron, Y., P. DeBaetselier, J. Gordon, M. Feldman, and S. Segal. 1981. Defective induction of antigen reactive proliferating T cells in B cell deprived mice. Eur. J. Immunol. 11:964.
11. Flood, P. A., C. A. Janeway, Jr., and R. K. Gershon. 1984. B cell deprived mice lack functional expression of certain T suppressor cell subsets. J. Mol. Cell. Immunol. 1:167.
12. HayGlass, K. T., S. J. Naides, B. Benacerraf, and M.-S. Sy. 1985. T cell development in B cell deficient mice. II. Restriction specificity of suppressor T cell factor(s) produced in mice treated chronically with rabbit anti-mouse # chain antibody. J. Mol. Cell. Immunol. In press.
13. Lawton, A. R., R. Asofsky, M. B. Hylton, and M. D. Cooper. 1972. Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to # chain. J. Exp. Med. 135:277.
14. Manning, D. D. 1975. Heavy chain isotype suppression: A review of the immunosup-
pressive effects of heterologous anti-Ig heavy chain anti-sera. *J. Reticuloendothel. Soc.* 18:63.

15. Brown, A. R., P. Estess, E. Lamoyi, L. Gill-Pazaris, P. D. Gottlieb, J. D. Capra, and A. Nisonoff. 1980. Studies of genetic control and microheterogeneity of an idiotype associated with anti-\(\rho\)-azophenylarsonate antibodies of A/J mice. *Prog. Clin. Biol. Res.* 42:231.

16. Slaughter, C. A., and J. D. Capra. 1984. Structural and genetic basis of the major crossreactive idiotype of the A strain mouse. In *The Biology of Idiotypes*. M. I. Greene and A. Nisonoff editors. Plenum Press, NY. 35.

17. Gill-Pazaris, L. A., E. Lamoyi, A. R. Brown, and A. Nisonoff. 1981. Properties of a minor crossreactive idiotype associated with anti-\(\rho\)-azophenylarsonate antibodies of A/J mice. *J. Immunol.* 126:75.

18. Marshak-Rothstein, A., M. N. Margolies, J. D. Benedetto, and M. L. Gefter. 1981. Two structurally distinct and independently regulated idiotypic families associated with the A/J response to azophenylarsonate. *Eur. J. Immunol.* 11:565.

19. Jerne, N. K. 1974. Toward a network theory of the immune system. *Ann. Immunol. (Paris).* 125:373.

20. Slaoui, M., O. Leo, J. Marvel, M. Moser, J. Hiernaux, and J. Urbain. 1984. Idiotypic analysis of potential and available repertoires in the arsonate system. *J. Exp. Med.* 160:1.

21. Kim, J. K., F. Rollwagen, R. Asofsky, and I. Lefkovitz. 1984. The abnormal functional of T cells in chronically anti-\(\mu\) treated mice with no mature B lymphocytes. *Eur. J. Immunol.* 14:476.

22. Sigal, N. H. 1982. Regulation of azophenylarsonate-specific repertoire expression. I. Frequency of cross-reactive idiotype-positive B cells in A/J and BALB/c mice. *J. Exp. Med.* 156:1352.

23. Etlinger, H. M., M. H. Julius, and C. H. Heusser. 1982. Mechanism of clonal dominance in the murine anti-phosphorylcholine response. I. Relationship between antibody avidity and clonal dominance. *J. Immunol.* 128:1685.

24. Woodland, R., and H. Cantor. 1978. Idiotype specific T helper cells are required to induce idiotype positive B memory cells to secrete antibody. *Eur. J. Immunol.* 8:600.