T CELL DEVELOPMENT IN B CELL–DEFICIENT MICE
V. Stopping Anti-μ Treatment Results in Igh-Restricted Expansion of the T Suppressor Cell Repertoire Concomitant with the Development of Normal Immunoglobulin Levels

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Igh restriction, a requirement for functional identity between responding lymphocyte populations at the Ig heavy chain allotype, has been a subject of intense investigation in recent years. Igh-restricted cell interactions have been shown in T cell responses to azobenzenearsonate (ABA)1 (1, 2), PC (3, 4), 3-hydroxy-4-nitrophenyl (NP) (5), larger antigens such as KLH (6), and SRBC (7, 8), among others. In the simple hapten systems, such interactions are commonly characterized by the demonstration of idiotypic structures on the responding T cells that are crossreactive with those found on antibodies of the same nominal specificity. Idiotype expression by T cells, and the consequent functional restrictions they dictate, are more common with regulatory T cells (Th and Ts) than effector T cells, such as CTL, further suggesting a role for such structures in immunoregulation. As such, these findings are indicative of a role for idiotype–anti-idiotype interactions in the maintenance of immunologic homeostasis and regulation of responsiveness to both self and foreign antigens, not just at the level of antibody as initially proposed (9), but potentially among all classes of lymphocytes.

In spite of our recognition of the role of such network interactions in the mature animal, our understanding of the means by which such tightly restricted interactions are established during ontogeny remains unclear. Why T lymphocyte clones of a given idiotype come to dominate the response in one strain while remaining virtually silent in others is a subject of considerable controversy. Similarly, the means by which the mature T cell repertoire comes to be dominated by lymphocytes expressing idiotypic receptors that are complementary to those of the B cell repertoire and thus displays idiotypically restricted activity in T–T as well as T–B interactions remains a puzzle. Our laboratories have focused on

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1 Abbreviations used in this paper: ABA, azobenzenearsonate; ABA-SC, ABA-coupled normal spleen cells; DTH, delayed-type hypersensitivity; Igh, Ig heavy chain loci; NP, 3-hydroxy-4-nitrophenyl; slg+, surface Ig+ cells; TNBS, trinitrobenzene sulfonic acid solution; Ts, idiotype positive suppressor/inducer cell.
the T cell repertoire, its generation, and functional restrictions for some years. Recently, we have been interested in how the mechanisms by which the composition of T cell regulatory networks and ultimately the composition of the T cell repertoire as a whole are established. Specifically, we have examined the role played by idiotypes expressed on Ig-bearing B cells in suppressor T cell ontogeny.

Chronic treatment of neonatal mice with a variety of Ig heavy chain–specific antibodies can result in suppression or virtual abrogation of antibody production (10–12), apparently by functionally depleting surface Ig–expressing B cells. Treatment with most isotype-specific antibodies (anti-γ, anti-α, and anti-ε) results in specific depletion of that isotype (13–15), but treatment with anti-μ causes the suppression of all isotypes, presumably due to the presence of IgM on B cell precursors of most or all isotypes (16–18). The strategy of anti-μ treatment, initially developed to study B cell ontogeny in chickens (19) and later in mice (13, 20) has been successfully applied to the study of other questions in recent years. We have used anti-μ-treated mice to examine the role of Igh-encoded idiotypic determinants expressed by B cells during T cell ontogeny.

Anti-μ mice display virtually no (<2%) surface Ig⁺ lymphocytes in spleen or lymph nodes, cannot generate responses to the B cell mitogen LPS, and have been reported to have undetectable or severely reduced levels of serum Ig (13, 20–22). Previously, we examined the influence of Ig upon the ultimate composition of mature suppressor T cell repertoires by comparing the genetic restrictions of anti-μ treated mice with those of normal and normal rabbit Ig–treated mice. In these studies the ABA-specific suppressor T cell network was used. The well characterized interactions between idiotype positive suppressor/inducer cell (Ts), anti-idiotype (TsF), and idiotype positive effector cells (TsF) are functionally restricted by the idiotypes characteristic of the Igh allotype of that strain (reviewed in 23). Thus, C.AL-20 (Igh-1b) Ts, or its soluble factor, TsF₁, acts in syngeneic mice but not in the Igh congenics BALB/c (Igh-1a) or CB-20 (Igh-1b). We found that development of Ts in the virtual absence of B cells and their products leads to profound changes in the T cell idiotypic repertoire (24, 25). Anti-μ C.AL-20 Ts₁ or TsF₁ fail to suppress normal C.AL-20 mice but are suppressive in syngeneic anti-μ-treated C.AL-20 mice and, surprisingly, in normal Igh congenic BALB/c ABA-specific responses. A shift in the idiotypic composition of the anti-μ C.AL-20 Ts repertoire was directly shown (26), accounting for these functional changes. The clear implication of these data was that B cells and Ig expressing the idiotypes characteristic of that strain’s Igh allotype were acting to influence the composition of at least some components of the T cell repertoire, in this case Ts. Such a hypothesis is consistent with studies carried out to examine Th repertoires developed in normal or Ig-deficient mice (27–31).

Given the relationship between the virtual absence of Igh-encoded gene products in the anti-μ mice and the disruption of the normal T cell repertoire that was observed, we postulated that the subsequent exposure of these T cells to appropriate Igh-encoded determinants (Ig) would result in specific changes in the T cell repertoire. The present study was carried out to test this hypothesis.

In these experiments, chronic treatment of C.AL-20 mice with anti-μ was discontinued at 6–8 wk of age and the gradual development of B cell maturity was followed. Simultaneously, the composition of the Ts repertoire was exam-
ined. We observed that the Ts repertoire underwent a precisely defined expansion, as shown by the acquisition of an additional Ts restriction specificity. This idiotypically restricted specificity is the same as that of Ts developed in normal C.AL-20 mice. That is, previously anti-μ-treated C.AL-20 mice retain the capacity to generate Ts that suppress CTL responses in BALB/c (Igh-1b) mice, while they acquire the capacity to suppress responses in normal C.AL-20 (Igh-1a) mice within 2 wk of major increases in the levels of surface Ig+ B cells and serum Ig. The resulting, functionally chimeric Ts repertoire, which is characterized by expression of parallel idiotypically restricted Ts networks, is demonstrable for at least 13 wk. The findings provide further evidence of a significant role for Ig-encoded determinants in determining the composition of the T cell repertoire and, ultimately, the composition of immunologic networks as a whole.

Materials and Methods

Animals. BALB/c (H-2b and Igh-1b) mice were obtained from Charles River Breeding Laboratories, Wilmington, MA. C.AL-20 (H-2a and Igh-1d) and C.B-20 (H-2a and Igh-1b) mice were obtained from breeding colonies maintained at Harvard Medical School. Anti-μ C.AL-20 mice were prepared by treating newborns with rabbit anti-mouse μ chain antibodies, as previously described (24, 25). Briefly, within 24 h after birth and three times weekly thereafter, C.AL-20 mice were injected intraperitoneally with 100 μl of concentrated antibody consisting of 700–1,000 μg of specific anti-mouse IgM activity. At 6 wk of age, chronic treatment of anti-μ mice was discontinued and mice were thereafter bled at weekly intervals, until killed.

All animals were maintained in accordance with 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 (NIH publication No. 78-23, revised 1978).

Preparation of Antigen and Antigen-Coupled Cells. A 40 mM solution of diazonium salt of p-arsanilic acid ABA (Eastman Kodak Co., Rochester, NY) was activated and conjugated to single cell suspensions of erythrocyte-free syngeneic spleen cells (10 mM final concentration). After washing in HBSS, these ABA-coupled spleen cells (ABA-SC) cells were irradiated (1,500 rad) and used to prime for delayed-type hypersensitivity (DTH) and CTL responses (32). TNP-conjugated spleen cells (TNP-SC) were prepared in a similar fashion by incubating normal spleen cells in a 10 mM trinitrobenzene sulfonic acid solution (TNBS) (Eastman Kodak Co.), pH 7.4, in a 37°C waterbath for 10 min; they were washed extensively and irradiated as described above (33).

Delayed Hypersensitivity. Mice were primed by subcutaneous injection of 3 × 107 ABA-coupled or TNP-coupled spleen cells. 6 d later, they were challenged by injecting 25 μl of 10 mM ABA diazonium salt solution or 10 mM TNBS solution into the left hind footpad. 24 h later, footpad swelling was compared with the contralateral foot.

Induction and Elicitation of CTL Responses. To prime for the induction of ABA-specific CTL activity, groups of at least two normal, two normal rabbit Ig-treated, or two anti-μ-treated mice were injected subcutaneously with 3 × 107 ABA-coupled spleen cells. 7 d later spleens were removed from each group of animals and single cell suspensions prepared for in vitro stimulation of CTL secondary responses. Briefly, 6 × 106 irradiated ABA-SC were cocultured with 7 × 106 responder spleen cells in 16-mm Linbro tissue culture wells (Linbro Chemical Co., Hamden, CT) in a total volume of 2 ml/well. Culture medium consisted of RPMI 1640 supplemented with 100 μg/ml of penicillin, 100 μg/ml of streptomycin, 0.25 μg/ml of fungizone, 2 mM glutamine, 5 × 10−5 M 2-Me, and 10% preselected, heat-inactivated FCS. Cultures were incubated for 5 d in 5% CO2 at 37°C with saturated humidity. Chromium release assays were carried out using ABA-coupled or unhaptenated 125I-labeled Con A-activated syngeneic blasts or P815 tumor cells as targets.
Preparation and Testing of Suppressor Factor (TsF₁). To induce Ts₁, mice were injected intravenously with $5 \times 10^7$ irradiated ABA-SC (1,500 rad) and killed 7 d later. A snap freeze-thaw procedure (using spleen cell suspensions prepared from these mice) followed by high speed centrifugation (34) yielded cell-free lysates which were designated TsF₁.

To test the capacity of TsF₁ to inhibit development of DTH or CTL responses, $2 \times 10^7$ cell equivalents were administered intravenously on the day of immunization and for 4 succeeding days. On the sixth day mice were footpad-challenged to assess DTH responsiveness. 24 h later, footpad swelling was measured and the mice were killed to set up in vitro, secondary CTL cultures, as described above. The genetic restrictions characteristic of TsF₁ that we obtained from different groups of mice were evaluated by their capacity to induce suppression in syngeneic and Igh congenic, MHC identical mice.

Fluorescence Analysis of Surface Ig⁺ Cells. The percentage of spleen or lymph node cells expressing cell surface (murine) Ig was determined by staining NH₄Cl-treated spleen cells with FITC-conjugated rabbit anti-mouse Ig (recognizing murine μ, γ, α, κ, and λ chains) or rabbit anti-goat Ig as a control. After extensive washing, these cells were fixed and stored in a solution of 2% paraformaldehyde (Sigma Chemical Co., St. Louis, MO) and 1% BSA until analyzed. The number of surface Ig⁺ cells and their relative fluorescence intensity were determined using a FACS II (Becton Dickinson & Co., Mountain View, CA). 5,000–10,000 cells were analyzed per sample. A duplicate preparation of each sample was independently stained and analyzed.

Determination of Serum Ig Levels. Total serum IgM and IgG concentrations were measured by solid-phase RIA. Flexible, 96-well plates (Falcon 3911, Becton Dickinson & Co., Oxnard, CA) were coated overnight with affinity-purified goat anti-mouse Ig (1 mg/ml). The plates were washed with PBS and blocked with a PBS (1% BSA, 1% Tween 20) solution for 2–4 h. After washing, duplicate serial dilutions of mouse sera or purified myeloma protein standards (MOPC 104E μ,λ; TEPC 185 μ,κ [Litton Bionetics, Kensington, MD], mouse IgG, [Miles Laboratories Inc., Naperville, IL]) were added for 6 h. The plates were washed and ¹²⁵I-labeled rabbit anti-mouse μ chain, γ chains or κ chain antibodies were added. Sp act of these reagents was determined for each labeling and ranged from 19–25 × 10⁶ cpm/μg. The optimal dilution of radiolabeled Ig was determined for each reagent, 1–3 × 10⁵ cpm/well being used. After overnight incubation at 4°C the wells were washed and counted. In each assay, the curve obtained with a myeloma protein of known concentration was used as a standard. The sensitivity of the assays allowed detection of IgM down to 30 ng/ml serum and IgG to 10–20 ng/ml.

Results

Recovery of the B Cell Repertoire upon Stopping Chronic Anti-μ Treatment. Continuous treatment of mice with rabbit anti-mouse IgM antibodies beginning within 24 h of birth abrogates the development of functional B cell repertoires. Anti-μ mice contain no detectable mature B cells in spleen or lymph nodes as measured by the presence of surface Ig expression, no proliferative response to the B cell mitogen LPS, and greatly reduced levels of serum Ig are detectable.

It is known that stopping anti-μ treatment in adulthood ultimately allows the development of mature B cells and normal serum Ig levels (16). To characterize the rate of appearance of mature B cell repertoires in such mice, chronic anti-μ treatment was discontinued for 6–8-wk-old anti-μ C.AL-20 mice. At the indicated times thereafter, mice were bled and in some cases killed to determine LPS responsiveness and the extent of surface Ig expression on spleen cells (Table I). It can be seen that during treatment and for up to several weeks thereafter anti-μ mice have no demonstrable surface Ig⁺ spleen cells and cannot respond to LPS. Beginning about the fourth to sixth week after discontinuing anti-μ treatment (anti-μ + 4 wk; anti-μ + 6 wk), significant numbers of Ig-expressing lymphocytes can be found in the spleens of these animals. The number of B cells
Kinetics of Mature B Cell Development after Stopping Chronic Anti-μ Treatment

| Mice Tested* | Killed at: | LPS response* | sIg+ cells% | Normal control% |
|--------------|------------|---------------|-------------|-----------------|
| Normal C.AL-20 (>25) | 0 | 100 | 29-42 | 100 |
| Anti-μ C.AL-20 (>25) | 0 | <5 | <2 | <2 |
| Anti-μ C.AL-20 (10) | 1 | <5 | <2 | <2 |
| Anti-μ C.AL-20 (8) | 2 | <5 | <2 | <2 |
| Anti-μ C.AL-20 (8) | 3 | <5 | 2-8 | <2-23 |
| Anti-μ C.AL-20 (14) | 4 | ND | <2-26 | <2-66 |
| Anti-μ C.AL-20 (6) | 6 | 50-90 | 10-24 | 33-75 |
| Anti-μ C.AL-20 (6) | 7 | 86-118 | 18-31 | 50-100 |
| Anti-μ C.AL-20 (4) | 9 | 70-115 | 20-52 | 40-100 |

* Spleen cells from normal or anti-μ-treated C.AL-20 mice.
† Relative capacity of spleen cells to generate proliferative responses to LPS as described in Materials and Methods. Normal controls generated LPS responses of 70–166 × 10⁵ cpm.
‡ Percentage of spleen cells positive for surface immunoglobulin by FACS analysis using FITC-conjugated rabbit anti-mouse Ig. Nonspecific staining with an irrelevant FITC-conjugated antibody (FITC-rabbit anti-goat Ig) yielded 3–8% staining in the experiments shown and has been subtracted from the data presented.
§ Calculated percentage of surface Ig+ cells observed in normal spleen cells stained that day.

Intriguingly, although the proportion of sIg+ B cells and LPS responses are usually comparable to those of control mice.

A similar picture emerges from examination of total serum IgM and IgG levels (Fig. 1). Serum IgM is not detectable in anti-μ mice for some time after stopping treatment (sensitivity limit of assay, 30 ng IgM/ml mouse serum). Indeed, depending upon the particular cohort of mice examined, serum IgM was sometimes not detectable for 20–25 d after stopping anti-μ treatment. By 4–6 wk after stopping anti-μ treatment, significant amounts of IgM were detectable, rising rapidly from much less than 1% to 40–80% of control values in untreated or normal rabbit Ig-treated mice.

The development of serum IgG levels follows essentially the same pattern with one noteworthy exception. Although serum IgG levels in anti-μ mice are consistently 2–3 logs lower than those in normal mice, IgG is not totally undetectable, averaging 10–40 ng/ml (0.01–0.1% of normal serum concentrations). As with IgM, serum IgG is much less than 1% of control levels from the first 2–3 wk after stopping anti-μ treatment. Between 4 and 6 wk, total IgG concentrations rise to levels approaching those of normal controls.

Total serum Ig levels measured in RIA using ¹²⁵I-labeled anti-K reagents were found to confirm the pattern of Ig expression presented above individually for IgM and IgG isotypes (data not shown).

If the composition of the functional peripheral T cell repertoire is at least in part dependent upon the nature of the idiotype expressing Ig present during T cell ontogeny, then the development of B cells in adult, anti-μ mice might be
expected to influence the composition of the T cell repertoire. To examine this possibility, antigen-specific Ts were induced in chronically treated anti-μ C.AL-20 mice and mice whose anti-μ treatment had been stopped for 3, 7, or 9 wk. The genetic restriction pattern of the cell-free lysate (TsF1) or the spleen cells (Ts1) from these mice was assessed by examining their capacities to suppress development of ABA-specific DTH (Fig. 2). The data shown, a compilation of four experiments, indicate a major change in the Ts repertoire of these mice. We reported previously that TsF1 from anti-μ C.AL-20 mice cannot suppress ABA-specific DTH, IL-2 generation, or CTL responses in normal or normal rabbit Ig-treated C.AL-20 mice. Anti-μ C.AL-20 TsF1 (or Ts1) does induce antigen-specific suppression of ABA responses in anti-μ C.AL-20 recipients and, interestingly, normal Igh congenic BALB/c mice (24). The data presented in Fig. 2 confirm the capacity of TsF1 from anti-μ C.AL-20 mice to suppress DTH responses in BALB/c but not normal C.AL-20 mice. In addition, TsF1 induced 3 wk after stopping anti-μ injections (anti-μ + 3 wk), a time at which serum Ig levels are extremely low, was found to display the same functional genetic restrictions as anti-μ C.AL-20 TsF1. However, TsF1 produced by C.AL-20 mice 7–9 wk after stopping anti-μ treatment, a time by which serum Ig levels have
greatly increased (from <<1% to 40–80% of control levels), shows an Igh-restricted expansion of the Ts repertoire by its suppression of DTH responses in normal C.AL-20 mice. At the same time, the ability of TsF₁ from these mice to suppress development of DTH in BALB/c mice is maintained. The DTH responses of MHC-identical Igh-1b C.B-20 mice are not affected by treatment with any of these TsF₁ preparations (data not shown), indicating that a specific (Igh-1b-restricted) acquisition rather than a total loss of Igh restriction has occurred in these mice.

These findings were confirmed and extended by assessing the capacity of TsF₁ to inhibit establishment of CTL responses (Fig. 3). Normal C.AL-20 and BALB/c mice were primed with ABA-coupled spleen cells, treated with TsF₁ from the donors indicated, challenged for DTH, then used to measure secondary anti-ABA CTL responses. It is apparent that while TsF₁ from anti-μ C.AL-20 + 3 wk mice suppressed DTH and CTL responses in BALB/c mice, it had no impact upon the ability of normal C.AL-20 mice to generate DTH or CTL responses. Thus, it exhibits the same characteristic restrictions as anti-μ C.AL-20 TsF₁. In contrast, TsF₁ produced by anti-μ C.AL-20 + 7 wk mice displays the capacity to suppress BALB/c responses and has acquired the ability to suppress normal C.AL-20 DTH and CTL responses as well.

Expansion of the functional Ts repertoire is antigen-specific (TNP-specific responses are not affected, data not shown) and Igh-1b-restricted, as shown by the failure of these TsF₁ to suppress CTL or DTH responses of C.B-20 (Igh-1b) mice (data not shown).

**Correlation between Serum Ig Expression and Expansion of the Ts Repertoire.** The relationship between the development of normal C.AL-20 B cell repertoires in anti-μ mice and the acquisition of normal C.AL-20 Ts₁ repertoires was strengthened by the series of experiments presented in Table II. While it was consistently found that TsF₁ prepared ≤3 wk after stopping anti-μ treatment (a time at which

| TsF₁ Donor | CAL-20 | BALB/c |
|-----------|--------|--------|
| None      |        |        |
| Anti-μ C.AL-20 |        |        |
| Anti-μ C.AL-20 + 3 wk |        |        |
| Anti-μ C.AL-20 + 7 wk |        |        |
| Anti-μ C.AL-20 + 9 wk |        |        |

*Figure 2. Altered genetic restrictions of TsF₁ produced by anti-μ C.AL-20 mice at various times after stopping chronic anti-μ treatment. DTH is expressed as a percentage of positive control footpad swelling by pooling data obtained in four separate experiments. Significant suppression (p < 0.05) of the capacity to mount DTH reactions is indicated (*). The magnitude of the positive controls ranged from 45–70 (± 5–8) x 10⁻⁴ mm.*
significant B cell expansion had not yet occurred) was functionally identical to that of anti-μ mice still under treatment, and that TsF1 prepared from anti-μ C.AL-20 mice ≥7 wk after stopping chronic anti-μ treatment consistently suppressed ABA-specific responses in both BALB/c and C.AL-20 mice, the functional restrictions of TsF1 prepared 5 wk after stopping anti-μ injections yielded

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**TABLE II**

**Correlation between Appearance of Surface Ig-Expressing Spleen Cells and Igh-Restricted Expansion of the Ts Repertoire**

| Exp. | TsF1 donor      | Killed at: | sIg* cells | Suppression* |
|------|-----------------|------------|------------|--------------|
|      |                 | wk | %       | C.AL-20 | BALB/c     |
| 1    | Anti-μ C.AL-20  | 5  | <2      | 0      | 30-45      |
| 2    | Anti-μ C.AL-20  | 5  | 6       | 0-9    | 45-82      |
| 3    | Anti-μ C.AL-20  | 5  | 37      | 41-80  | 40-97      |
| 4    | Anti-μ C.AL-20  | 5  | 23      | 90-93  | 79-84      |

* Percentage of spleen cells from TsF1 donors that stain with FITC-conjugated rabbit anti-mouse Ig. Data are shown after subtraction of nonspecific staining with rabbit anti-goat Ig (2-6% in these assays).

* Suppression of ABA-specific CTL responses in normal C.AL-20 and BALB/c mice relative to positive controls upon treatment with TsF1 from the donors indicated.

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**FIGURE 3.** Genetic restrictions of TsF1 from anti-μ C.AL-20 mice generated 3 or 7 wk after stopping chronic anti-μ treatment. Normal C.AL-20 and normal BALB/c mice were primed with $3 \times 10^7$ ABA-coupled syngeneic spleen cells and treated with TsF1 produced by anti-μ C.AL-20 mice + 3 wk (O), anti-μ C.AL-20 + 7 wk (Δ), or were left untreated (●). Their capacity to mount CTL and DTH responses was assessed as described in Materials and Methods.
variable results. In some experiments anti-μ C.AL-20 + 5 wk TsF₁ displayed Igh restrictions characteristic of anti-μ mice (Table II, Experiment 1 and 2), while in others an Igh-restricted expansion of the Ts repertoire had occurred (Experiment 3 and 4), as shown by its capacity to suppress CTL responses in both Igh-1^a and Igh-1^d haploypical mice. Similar findings were obtained with the capacity of these TsF₁ to suppress the development of DTH responses (data not shown). To our great interest, analysis of the Ts₁ donor spleens in these experiments revealed a strict association between the presence of mature B cells and the expansion of the Ts₁ repertoire. That is, TsF₁ obtained from anti-μ + 5 wk mice with significant reconstitution of the B cell repertoire displayed a parallel expansion of the (id⁺) Ts₁ repertoire, while cohorts of mice where the B cell repertoire had not yet recovered showed restrictions in the Ts₁ repertoire characteristic of anti-μ mice still under treatment.

**Prolonged Maintenance of Functionally Chimeric Suppressor T Cell Repertoires.** In light of our findings that stopping anti-μ treatment of C.AL-20 mice leads to the development of dual Ts₁ repertoires, it was of interest to determine (a) if a similar expansion of the suppressor cell repertoire is demonstrable for Ts₂ and Ts₃ cells after stopping anti-μ treatment and (b) if the existence of dual Ts repertoires is a transient state.

In these experiments, the ability of Igh-1^a-restricted or Igh-1^d-restricted TsF₁ to suppress CTL responses in anti-μ C.AL-20 mice and anti-μ C.AL-20 + 10 wk or + 20 wk mice was assessed. Anti-μ C.AL-20 mice were primed for ABA-specific CTL responses 10 and 20 wk after stopping chronic anti-μ treatment, and the capacity of normal BALB/c or normal C.AL-20 TsF₁ to induce suppression in these mice was determined. Unlike either normal or anti-μ-treated mice, anti-μ C.AL-20 + 10 wk or + 20 wk mice are receptive to TsF₁ obtained from both Igh-1^a and Igh-1^d donors (Fig. 4). This is reminiscent of what was found for the Ts₁ repertoires in anti-μ + 7 or + 9 wk mice, which exhibited dual suppressor cell repertoires at the Ts₁ level. Igh-1^b-restricted TsF₁ failed to induce
suppression (data not shown), indicating that the Ts<sub>2,3</sub> repertoires in these mice, though activated by TsF<sub>1</sub> from either Igh-1<sup>a</sup> or Igh-1<sup>d</sup> mice, display two distinct Igh restrictions rather than a nonspecific loss of Igh restriction.

It is of particular interest to note that the maintenance of these dual, idiotypically restricted Ts repertoires is long lived, persisting for at least 13–15 wk.

Discussion

We have previously examined the generation of Igh genetic restrictions between lymphocytes and the resulting establishment of idiotype-based networks by comparing the composition of Ts repertoires in normal and anti-μ-treated (B cell-deficient) mice. This report represents a different approach to the study of the role of Igh-linked determinants in T cell development. It has been established (13, 16) that stopping anti-μ treatment of adult mice results in a gradual decrease in circulating rabbit anti-mouse μ antibodies, followed by maturation of pre-B cells, and ultimately, generation of functional B cell repertoires and normal levels of serum Ig.

In the present report we have investigated the consequences of this natural reconstitution of the B cell repertoire upon the composition of the Ts repertoire.

Mature B cell activity, virtually absent in mice under chronic anti-μ treatment, recovers substantially within 4–6 wk after stopping anti-μ treatment. Igh-restricted expansion of the Ts repertoire consistently follows within 2 wk of major increases of splenic B cells and serum Ig concentrations. At the same time, the already established Ts repertoire that is characteristic of anti-μ mice under treatment is fully retained. Thus, by 7 wk after stopping anti-μ treatment, the Ts repertoire of anti-μ C.AL-20 mice is functionally expanded.

TsF<sub>1</sub> from these mice suppress DTH and CTL responses in Igh-1<sup>a</sup> (BALB/c) and Igh-1<sup>d</sup> (C.AL-20), but not control Igh-1<sup>b</sup> (C.B.20) mice. This change in the Ts<sub>1</sub> repertoire is reflected in a similar fashion in the Ts<sub>2</sub> and Ts<sub>3</sub> repertoires of these mice. The acquisition of a complete, parallel, Igh-restricted suppressor cell network in these mice is governed by Igh restrictions characteristic of the expressed Ts repertoire in normal (Ig-containing) C.AL-20 mice. Thus, the Ts repertoire characteristic of anti-μ C.AL-20 mice under chronic treatment is maintained and a new, previously silent Ts repertoire, complementary to the developing B cell repertoire, is expanded.

It would be tempting to conclude that the association between the development of B cell maturity in previously anti-μ treated C.AL-20 mice and the strictly limited expansion of the Ts repertoire that follows 2 wk later is causally related. There are indeed several relevant points that would provide support for such an interpretation: (a) The inflection point in the development of B cell maturity comes ~5 wk after stopping anti-μ treatment. While some cohorts of mice develop a functional B cell repertoire somewhat more rapidly and others more slowly, it is striking that those mice in which the B cell repertoire is significantly reconstituted within 5 wk display a corresponding expansion of the Ts repertoire, while those mice in which substantial B cell activity has not yet emerged display no detectable change in Ts restrictions; (b) total serum Ig levels in individual cohorts of mice consistently revealed sharp increases 10–20 d before Ts<sub>1</sub> induction in those mice that displayed chimeric Ts repertoires. Mice in which such increases
were not observed did not have any detectable changes in the composition of the Ts repertoire (data not shown).

It is interesting that the present experimental design mimics, in a natural fashion, the previously described experimental manipulations in which transfer of peripheral T cells (Igh-1\textsuperscript{a}-derived, Thy-1.2\textsuperscript{+}) to an irradiated Igh congenic environment (Igh-1\textsuperscript{b}, Thy-1.1) leads to "reeducation" of the donor Ts repertoire. TsF\textsubscript{1} produced by cells parked in an Igh congenic (Igh-1\textsuperscript{b}) environment for 12 d then showed the capacity to suppress responses in Igh-1\textsuperscript{b}, as well as Igh-1\textsuperscript{a} but not control Igh-1\textsuperscript{a} recipients (35).

A prominent difference between this and the present report is that the former involves T cell enrichment, adoptive transfer to an Igh congenic environment, and other procedures to ensure that the resultant TsF\textsubscript{1} is truly derived from donor T cells and not residual host T cells. In the present report, T cells were left in situ to be naturally exposed to significant concentrations of novel Ig determinants with a minimum of experimental manipulation. Results from both approaches show that exposure of the T cell repertoire to normal concentrations of murine Ig is associated with Igh-restricted expansion of the Ts repertoire. In both cases, this expansion is functionally limited to T cells responsive to idiotypes characteristic of that particular Igh allotype, and not others. We postulate, but have not yet shown that these functionally chimeric Ts repertoires (Igh-1\textsuperscript{a}-/Igh-1\textsuperscript{b}-restricted) are composed of distinct Igh-1\textsuperscript{a}-restricted and Igh-1\textsuperscript{b}-restricted Ts populations.

The finding that a dual functional repertoire is maintained for at least 13 wk is of considerable interest. It suggests that once established, the Ts repertoire is remarkably stable. Given the appropriate environmental stimuli one may expand previously silent T cell clones, thereby adding to the expressed repertoire, but it appears that components of the T cell repertoire expanded soon after birth may remain stable and functional indefinitely.

This result displays some similarities with observations made for the TNP-specific Th repertoire. Normal and anti-\mu-treated BALB/c mice both display functional but idiotypically distinct TNP-specific Th repertoires (36). It was recently determined that mice that are chronically anti-\mu-treated for the first 3-4 wk of life and then stopped contain TNP-specific Th repertoires that are unchanged (at 9 wk of age) from those of anti-\mu mice still under treatment (37). These results were interpreted to suggest that Th repertoires are established early in life and are thereafter stable, a finding that concurs with the above demonstrated stability of the ABA-specific Ts repertoire of anti-\mu mice.

However, the two systems differ in that the Igh-restricted expansion of the Ts repertoire of anti-\mu mice that is observed after spontaneous development of the C.AL-20 B cell repertoire was not observed for the TNP-specific Th repertoire in anti-\mu BALB/c mice. This failure of the Th repertoire to expand was observed up to 9 wk of age (i.e., 4-5 wk after stopping anti-\mu treatment). The time at which the B cell repertoire was reconstituted in these mice remains to be established. It is possible that the apparent discrepancy is a reflection of different rates of B cell reconstitution in the two strains and that the TNP-specific Th repertoire some 7-10 wk after stopping anti-\mu treatment would also be idiotypically expanded. Alternatively, it is possible that Th and Ts repertoires display
different degrees of flexibility, with the helper cell repertoire fixed early in life and the suppressor cell repertoire responsive to changing environmental stimuli, such as idiotype, throughout the lifetime of the animal.

**Summary**

B cell-deficient (anti-μ-treated) mice have proven to be a valuable tool with which to examine the influence of Ig idiotypic determinants upon the development of the Ts repertoire. We have previously reported that ABA-specific Ts repertoires matured in normal and Ig-deficient environments differ from one another in their composition, and consequently, their functionally expressed IgH restrictions.

The present report characterizes the impact of natural development of mature B cell activity upon the composition of the Ts repertoire.

After stopping anti-μ treatment of C.AL-20 mice, ABA-specific Ts repertoires undergo a defined expansion shown by their acquisition of an additional Ts network that displays IgH restrictions characteristic of normal C.AL-20 mice. This IgH-1α-restricted repertoire can be readily shown within 2 wk of major increases in surface Ig spleen cells and total serum Ig levels in these mice. At the same time, the original Ts restriction specificity (IgH-1α-restricted) generated in the Ig-deficient environment of anti-μ C.AL-20 mice, is not lost for at least 20 wk. The resulting dual Ts repertoire, characterized by expression of parallel, idiotypically restricted Ts networks, is demonstrable for at least 13 wk. These findings favor an important role for Ig determinants in determining the makeup of the T cell repertoire, and ultimately, the composition of immunologic networks as a whole.

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**References**

1. 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 group of genes. *J. Exp. Med.* 149:1084.

2. Takaoki, M., M.-S. Sy, B. Whitaker, J. Nepom, R. Finberg, R. N. Germain, A. Nisonoff, B. Benacerraf, and M. I. Greene. 1982. Biologic activity of an idiotype-bearing suppressor T cell factor produced by a long-term T cell hybridoma. *J. Immunol.* 128:49.

3. Bottomly, K., and D. E. Mosier. 1979. Mice whose B cells cannot produce the T15 idiotype also lack an antigen-specific helper T cell required for T15 expression. *J. Exp. Med.* 150:1599.

4. McNamara, M., Kohler, H., and S. Smyk. 1984. Idiotype-specific T cells' role in regulation. In *The Biology of Idiotypes*. M. I. Greene and A. Nisonoff, editors. Plenum Press, New York. 341.

5. Weinberger, J., B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses
to 4-hydroxy-3-nitrophenylacetyl. III. Interactions of effector suppressor T cells are restricted by I-A and Igh-V genes. *J. Exp. Med.* 151:1413.

6. Tada, T., G. Suzuki, R. Abe, Y. Kumagi, K. Hiramatsu, and S. Miyatani. 1983. *In Immunoregulation.* N. Fabris, E. Gerai, J. Hadden, and N. A. Mitchison, editors. Plenum Publishing Corp., New York. 1.

7. Eardley, D. D., F. W. Shen, H. Cantor, and R. K. Gershon. 1979. Genetic control of immunoregulatory circuits. Genes linked to the Ig locus govern communication between regulatory T cell sets. *J. Exp. Med.* 150:44.

8. Flood, P., K. Yamauchi, and R. K. Gershon. 1982. Analysis of the interactions between two molecules that are required for the expression of Ly-2 suppressor cell activity. *J. Exp. Med.* 156:361.

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

10. Manning, D. D., and J. W. Jutila. 1972. Immunosuppression in mice injected with heterologous anti-immunoglobulin antiserum. *J. Immunol.* 108:282.

11. Lawton, A. R., III, R. Asofsky, M. B. Hylton, and M. D. Cooper. 1972. Suppression of immunoglobulin class synthesis in mice. 1. Effects of treatment with antibody to # chain. *J. Exp. Med.* 135:277.

12. Manning, D. D. 1977. Complete humoral immunosuppression of mice by rabbit anti- anti- antibodies passing the murine placenta. *J. Immunol.* 118:1109.

13. Manning, D. D. 1975. Heavy chain isotype suppression. A review of the immunosuppressive effects of heterologous anti-lg heavy chain antisera. *J. Reticuloendothel. Soc.* 18:63.

14. Manning, D. D., J. K. Manning, and N. D. Reed. 1976. Suppression of reaginic antibody (IgE) formation in mice by treatment with anti- anti- antiserum. *J. Exp. Med.* 144:288.

15. Bazin, H., B. Platteau, A. Beckers, and R. Pauwels. 1978. Differential effects of neonatal injections of anti- # or anti- anti- antibodies on the synthesis of IgM, IgD, IgE, IgA, IgG1, IgG2a, IgG2b, and IgG2c immunoglobulin classes. *J. Immunol.* 121:2083.

16. Manning, D. D. 1974. Recovery from anti-lg-induced immunosuppression: implications for a model of Ig secreting cell development. *J. Immunol.* 113:455.

17. Raff, M. D., J. J. T. Owen, M. D. Cooper, A. R. Lawton, M. Megson, and W. E. Gathings. 1975. Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin-induced immunoglobulin suppression in vitro. Possible implications of B-cell tolerance to self. *J. Exp. Med.* 142:1052.

18. Gordon, J., R. Murgita, and R. B. Tomasi, Jr. 1975. The immune response of mice treated with anti- anti- antibodies. The effect on antibody forming cells, their precursors and helper cells assayed in vitro. *J. Immunol.* 114:1808.

19. Kincade, P. W., A. R. Lawton, D. E. Bockman, and M. D. Cooper. 1970. Suppression of immunoglobulin G synthesis as a result of antibody-mediated suppression of immunoglobulin M synthesis in chickens. *Proc. Natl. Acad. Sci. USA.* 67:1918.

20. Cooper, M. D., J. F. Kearney, W. E. Gathings, and A. R. Lawton. 1980. Effects of anti-lg antibodies on the development and differentiation of B cells. *Immunol. Rev.* 52:29.

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

22. Snider, D. P., J. Gordon, M. R. McDermott, B. J. Underdown. 1985. Chronic giardia muris infection in anti-IgM treated mice. 1. Analysis of immunoglobulin and parasite-specific antibody in normal and immunoglobulin-deficient animals. *J. Immunol.* 134:4153.
23. Greene, M. I., M. J. Nelles, M.-S. Sy, and A. Nisonoff. 1982. Regulation of immunity to the azobenzene arsonate hapten. Adv. Immunol. 32:254.

24. 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 μ chain antibody alters the characteristic immunoglobulin heavy chain restriction of murine suppressor T-cell factors. Proc. Natl. Acad. Sci. USA. 81:3846.

25. HayGlass, K. T., S. J. Naides, B. Benacerraf, and M.-S. Sy. 1985. T cell development in B cell deficient mice. III. Restriction specificity of suppressor T cell factor(s) produced in mice treated chronically with rabbit anti-mouse μ chain antibody. J. Mol. Cell. Immunol. 2:107.

26. Sy, M.-S., K. T. HayGlass, and B. Benacerraf. 1985. T cell development in B cell−deficient mice. II. Serological characterization of suppressor T cell factor(s) produced in normal mice and in mice treated chronically with rabbit anti-mouse IgM antibodies. J. Exp. Med. 161:1402.

27. Janeway, C. A., Jr., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. Proc. Natl. Acad. Sci. USA. 74:4582.

28. 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-μ antibody. Eur. J. Immunol. 10:159.

29. 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.

30. Martinez, A. C., P. Pereina, B. Bernabe, and A. Bandeina, E. L. Larsson, P. A. Cazenave, and A. Coutinho. 1984. Internal complementarities in the immune system. Regulation of the expression of helper T cell idiotypes. Proc. Natl. Acad. Sci. USA. 81:4520.

31. Janeway, C. A., Jr. 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 Publishing Corp., New York. 349.

32. Sherman, L., S. Burakoff, and B. Benacerraf. 1978. The induction of cytotoxic T lymphocytes with specificity for p-azophenylarsenate-coupled syngeneic cells. J. Immunol. 121:1432.

33. Shearer, G. M. 1974. Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Eur. J. Immunol. 4:527.

34. Greene, M. I., B. A. Bach, and B. Benacerraf. 1979. Mechanisms of regulation of cell-mediated immunity. III. The characterization of Azobenzene arsonate-specific suppressor T-cell-derived-suppressor factors. J. Exp. Med. 149:1069.

35. HayGlass, K. T., S. J. Naides, B. Benacerraf, and M. S. Sy. 1984. Suppressor T cell factor(s) (TsF) display an altered pattern of Igh genetic restriction when developed in an Igh congenic host. Proc. Natl. Acad. Sci. USA. 82:2133.

36. Martinez, A. C., P. Pereina, R. Bernabe, A. Bandeina, E. L. Larsson, P. A. Cazenave, and A. Coutinho. 1984. Internal complementarities in the immune system: regulation of the expression of helper T cell idiotypes. Proc. Natl. Acad. Sci. USA. 81:4520.

37. Martinez, A. D., R. R. Bernabe, P. Pereina, P. A. Cazenave, and A. Coutinho. 1985. Establishment of idiotypic helper T cell repertoires early in life. Nature (Lond.). 317:721.