T CELL DEVELOPMENT IN B CELL–DEFICIENT MICE
II. Serological Characterization of Suppressor T Cell Factors (TsF₁)
Produced in Normal Mice and in Mice Treated Chronically with Rabbit
Anti–Mouse IgM Antibodies

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The cellular interactions in the anti-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 (TsF₁) bear the major crossreactive idiotypic (CRI)
determinants recognized by rabbit antiidiotypic antibodies prepared by immu-
nizing 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 idiototypes on T cells may merely reflect a serological or confor-
mational crossreactivity, and represent internal images of B cell idiotypic speci-
ficities 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 (TsF₁) in the ABA-system,
we studied whether the presence of Ig-bearing B cells is required for the

¹ Abbreviations used in this paper: ABA, p-azobenzenearsonate; ABA-SC, ABA-coupled syngeneic
spleen cells; anti-μ, rabbit anti-mouse IgM; Con A, concanavalin A; CFA, complete Freund's adjuvant;
CRI, crossreactive idiotype; CTL, cytotoxic T lymphocyte; HBSS, Hank's balanced salt solution; Igh,
Ig heavy chain gene loci; Ts, T suppressor cell; TsF₁, first order suppressor T cell factor(s).
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 idiotypic determinants if, in fact, B cells expressing idiotypes are ontogenically required for the generation of idiotype-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 CRIcA. In general, 20-70% of the anti-ABA population carries CRIcA determinants (15, 16). In addition, a second idiotypic 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 CRIcA (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 CRIcC.

The availability of antidiotypic antibodies against CRIcA and CRIcC 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 CRIcC determinants. And TsF1, obtained from anti-μ--treated BALB/c, suppresses C.AL-20 but not BALB/c mice, and expresses CRIcA 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-1b) 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% CO₂ 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 = [(91Cr 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 × 10^9 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 90 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-CRI antisera. Rabbit anti-CRI(A) 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 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) immunoabsorbent 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 immunoabsorbent column, since repassage of the unbound fraction over the anti-CRI(c) column also failed.
T CELL DEVELOPMENT IN B CELL-DEFICIENT MICE

Figure 1. Normal C.AL-20 TsF1 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 x 10^7 ABA-SC; 7 d later TsF1 was prepared as described. 2 x 10^6 cell-equivalents of TsF1 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 x 10^7 ABA-SC only (Ο). TsF1 was given to the experimental groups, beginning on the day of immunization, for five successive days at 2 x 10^6 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. (Ο) Control mice were normal BALB/c mice immunized subcutaneously with 3 x 10^7 ABA-SC. (□) Animals treated with unbound (filtrate) fraction. (▲) Animals treated with bound (eluate) fractions.

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
passing normal BALB/c TsF₁ over an anti-CRI₉ column and assaying for the \( \text{Igh} \) restriction of the unbound TsF₁. The results of such an experiment are shown in Table I. As can be seen, normal BALB/c TsF₁ can be divided into CRI₉-bearing and non-CRI₉-bearing fractions; both of these fractions are suppressive in normal BALB/c mice. More importantly, both the non-CRI₉-bearing and CRI₉-bearing fractions remain nonfunctional in C.AL-20 mice, indicating that even though some of BALB/c TsF₁ may not express CRI₉ determinants, they are still \( \text{Igh} \) restricted in their function.

**TsF₁ Obtained from Anti-\( \mu \)-treated C.AL-20 Mice Expressed CRI₉ but not CRI₄ Determinants.** Since TsF₁ obtained from anti-\( \mu \)-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 TsF₁ bear CRI₄ specificities. ABA-specific TsF₁ was prepared from anti-\( \mu \)-treated C.AL-20 mice. This TsF₁ was then passed through an anti-CRI₄ 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), TsF₁ obtained from anti-\( \mu \)-treated C.AL-20 mice no longer expresses CRI₄ determinants. Therefore, when passed through a rabbit anti-CRI₄ column, the suppressor activities reside mainly in the filtrate, not in the acid eluate. In contrast, the identical TsF₁, when passed through an anti-CRI₉ column, yields a filtrate with minimal suppressor activity; significant suppressor
TABLE II

TsF₁ from Anti-μ-treated C.AL-20 Mice Can Be Retained by Anti-
CRI(c) Column but Not by Anti-CRI(a) Column

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<th>Treatment*</th>
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<td></td>
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<td>Eluate</td>
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<tr>
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<td>Eluate</td>
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Normal BALB/c mice were immunized subcutaneously with 3 \times 10^7
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 \(^{51}\)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.

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. (○) Control mice were normal
C.AL-20 mice immunized subcutaneously with 3 \times 10^7 ABA-SC. (□) Animals treated with
unbound (filtrate) fractions. (▲) Animals treated with bound (eluate) fractions.

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 corre-
sponding 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 congeneic 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\(_{C}\) anti-
bodies, 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 Slaoui 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. Slaoui, 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 from C.AL-20 mice bears CRI(A) determinants, and most, but not all of the TsF 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)+ T cells, or conversely, the presence of antiidiotypic TsF 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. 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 (TsF1) from C.AL-20 and BALB/c mice revealed a significant difference between TsF from these two strains of mice. The idiotypic composition of TsF1 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 TsF1 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, TsF1 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.
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