T CELL DEVELOPMENT IN B CELL–DEFICIENT MICE

II. Serological Characterization of Suppressor T Cell Factors (TsF_1) 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 (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

[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; TsF_1, first order suppressor T cell factor(s).

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expression of idiotypes by ABA-specific Ts-1 and TsF_1, 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 TsF_1 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, TsF_1 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, TsF_1 obtained from anti-μ–treated C.AL-20 mice developed the ability to suppress BALB/c mice. Moreover, TsF_1 from anti-μ–treated C.AL-20 mice was found not to express the major CRI determinants normally associated with C.AL-20 TsF_1 (4). However, to our surprise, ABA-TsF_1 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-TsF_1 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 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 $CRI_{(A)}$ (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 antiidiotypic antibodies against $CRI_{(A)}$ and $CRI_{(C)}$ has enabled us to establish that TsF_1 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 TsF_1, 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-2^d, Igh-1^a) and C.AL-20 (H-2^d, Igh-1^d) 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 ^{51}Cr 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 ^{51}Cr release = ([^{51}Cr release from targets in presence of effector cells] − [spontaneous ^{51}Cr release])/([maximum ^{51}Cr 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, 5×10^8 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 immunoadsorbent columns were prepared and characterized as described (2). TsF was fractionated on immunoadsorbents 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 antiserum. 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 5×10^8 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 TsF_1 Obtained from C.AL-20 and BALB/c Mice. Our previous work (1) has demonstrated that TsF_1 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 TsF_1 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 TsF_1 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 TsF_1 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 TsF_1, 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 TsF_1 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 TsF_1 and anti-$CRI_{(A)}$ immunoabsorbent columns. In C.AL-20 mice, it is evident that most, if not all of the TsF_1 activities can be retained by anti-$CRI_{(A)}$ columns. The failure of anti-$CRI_{(C)}$ column to bind all the BALB/c TsF_1 is not due to over-saturation of the immunoadsorbent column, since repassage of the unbound fraction over the anti-$CRI_{(C)}$ column also failed

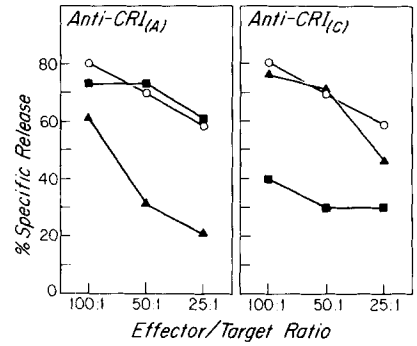

FIGURE 1. Normal C.AL-20 TsF can be retained by anti-$CRI_{(A)}$ but not anti-$CRI_{(C)}$ column. Normal C.AL-20 TsF_1 was prepared by intravenous injection of 5×10^7 ABA-SC; 7 d later TsF_1 was prepared as described. 2×10^8 cell-equivalents of TsF were passed through an anti-$CRI_{(A)}$ or anti-$CRI_{(C)}$ 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 3×10^7 ABA-SC only (○). TsF was given to the experimental groups, beginning on the day of immunization, for five successive days at 2×10^7 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.

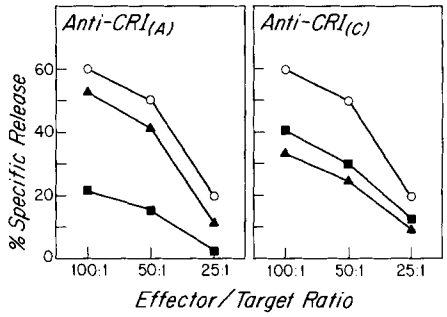

FIGURE 2. Majority of BALB/c TsF_1 can be retained by anti-$CRI_{(C)}$ but not by anti-$CRI_{(A)}$ column. The experimental protocols used in these experiments were exactly identical to experiments presented in Fig. 1, except normal BALB/c TsF_1 was used instead of normal C.AL-20 TsF_1. (○) Control mice were normal BALB/c mice immunized subcutaneously with 3×10^7 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 TsF_1 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 TsF_1 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 TsF_1 from normal BALB/c mice appears to be idiotypically somewhat more heterogeneous than C.AL-20 TsF_1 raised an important issue regarding the Igh restriction specificity of the fraction of normal BALB/c TsF_1 that does not express $CRI_{(C)}$ determinants. It is possible that the small fraction of BALB/c TsF_1 that was $CRI_{(C)}^-$ may not be Igh restricted in its function. Therefore, we enriched the $CRI_{(C)}^-$ TsF by

TABLE I

Igh Restriction Specificity of $CRI_{(C)}$-bearing and Non–$CRI_{(C)}$-bearing BALC/c TsF_1

Strain	Treatment*	Specific killing‡ at effector/target ratios of:		
		100:1	50:1	25:1
			%	
BALB/c	—	40	32	19
	Anti-$CRI_{(C)}$–passed TsF_1:			
	Filtrate	21	15	8
	Eluate	13	10	2
C.AL-20	—	57	40	28
	Anti-$CRI_{(C)}$–passed TsF_1:			
	Filtrate	52	39	26
	Eluate	49	42	37

Normal BALB/c or C.AL-20 were immunized subcutaneously with 3×10^7 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 2×10^7 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.

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 $CRI_{(C)}$ 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

TsF_1 from Anti-μ-treated C.AL-20 Mice Can Be Retained by Anti-$CRI_{(C)}$ Column but Not by Anti-$CRI_{(A)}$ Column

Treatment*	Specific killing‡ at effector/target ratios of:		
	100:1	50:1	25:1
		%	
—	85	60	41
Anti-$CRI_{(A)}$-passed TsF_1:			
Filtrate	41	40	18
Eluate	82	52	37
Anti-$CRI_{(C)}$-passed TsF_1:			
Filtrate	62	48	30
Eluate	45	31	22

Normal BALB/c mice were immunized subcutaneously with 3×10^7 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.

* TsF_1 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.

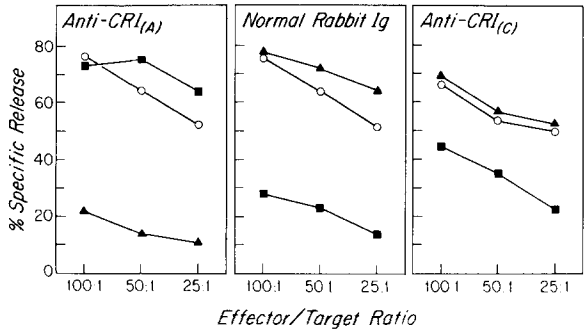

FIGURE 3. ABA-specific TsF_1 from anti-μ-treated BALB/c mice can be retained by anti-$CRI_{(A)}$ but not by anti-$CRI_{(C)}$ column. ABA-specific TsF_1 was prepared from anti-μ-treated BALB/c mice, as described. The factor(s) was then passed through an anti-$CRI_{(A)}$, normal rabbit Ig, or anti-$CRI_{(C)}$ 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 3×10^7 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.

TsF_1 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-μ–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-μ–treated C.AL-20 mice, TsF_1 obtained from anti-μ–treated BALB/c mice displays the opposite idiotypic specificities normally associated with BALB/c TsF_1. Anti-μ 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-μ–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-μ–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-$CRI_{(C)}$ 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 TsF_1 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, TsF_1 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 TsF_1 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 TsF_1 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 TsF_1 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 TsF_1 obtained from A/J mice, treatment of BALB/c mice with anti-$CRI_{(A)}$ monoclonal antibodies rendered them susceptible to the suppressive effect of TsF_1 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_1 from C.AL-20 mice bears $CRI_{(A)}$ determinants, and most, but not all of the TsF_1 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_1 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_1. 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_1) 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_1 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_1 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_1 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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