T CELL REGULATION OF B CELL ACTIVATION

Lyt-1$^+$,2$^-$ T Cells Modify the MHC-restricted Function of Heterogeneous and Cloned T Suppressor Cells

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The activation of antigen-specific B cell responses can be modulated by a number of T cell regulatory influences. It was first observed (1–3) that B cell responses to many antigens require the participation of T helper (Th)[1] cells. Studies (4, 5) using both heterogeneous and, more recently, cloned Th populations have shown that distinct B cell activation pathways exist in which Th cell–B cell interactions may be either major histocompatibility complex (MHC)-restricted or -unrestricted. It has also been shown (6–14) that these B cell responses are subject to the regulatory effects of T suppressor (Ts) cells, and that these suppressive effects may also involve antigen-specific and genetically restricted cell interactions. The overall complexity of T cell regulation is further increased by the activation of both augmenting (Ta) (15, 16) and contrasuppressor (Tcs) (17) T cells, which appear to further modify T cell–dependent (TD) B cell activation.

Previous studies (4, 5) have shown that both cloned and heterogeneous populations of Th cells are capable of activating B cells in a manner that requires both MHC-restricted Th cell–B cell interaction and carrier-hapten linkage. The MHC-restricted activation of B cells by cloned Th cells was found (16) to be enhanced substantially by a population of MHC-restricted Lyt-1$^+$,2$^-$ Ta cells present in unprimed spleen. In addition, suppression of TD B cell responses in vitro has been shown using both heterogeneous (14, 18, 19) and cloned (20) populations of Ts cells.

Given the potential complexity of interactions that may occur among T cell subpopulations present in heterogeneous T cells, the exclusive use of highly selected or monoclonal T cell populations offers a significant advantage to studies of T cell regulation. Further studies were therefore initiated to investigate the interaction of cloned Th and cloned Ts cells in an effort to define the mechanisms of their actions on B cell activation. Recently (21), we showed that cloned Ts

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[1] *Abbreviations used in this paper:* Acc, accessory; C, complement; FGG, fowl gamma globulin; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; MHC, major histocompatibility complex; PFC, plaque-forming cell; RAMB, rabbit anti–mouse brain; Ta, T augmenting; Tcs, T contrasuppressor; TD, T cell–dependent; Th, T helper; TNP, trinitrophenyl; Ts, T suppressor.

cells are capable of suppressing the B cell responses supported by cloned Th cells in the absence of other T cell populations, and that a tripartite antigen-mediated interaction of Ts cells, Th cells, and responding B cells was necessary to effect antigen-specific suppression. This study was undertaken to analyze the genetic restrictions involved in the function of Ts cells under conditions in which the modifying effects of heterogeneous T cells were eliminated. In contrast to previous findings obtained when heterogeneous T cells were also present in culture, we found that suppression by enriched or cloned Ts cells of B cell responses generated by cloned Th cells required MHC-restricted interaction only for Ts cell activation, and that the effector function of Ts cells was genetically unrestricted under these conditions. Moreover, the data presented here indicate that a heterogeneous population of MHC-restricted Lyt-1$^+$,2$^-$ T cells is capable of modifying the genetic constraints on Ts function in these responses, resulting in suppression that was MHC restricted at its effector level. The findings of these studies suggest that the observed MHC restriction in Ts cell function is the result of complex interactions involving Ts cells, Th cells, and at least one additional MHC-restricted T cell population, which has augmenting and contrasuppressor-like activity.

Materials and Methods

Animals

C57BL/10 (B10), B10.A, B10.BR, (B10 × B10.BR)F_1, (B10 × B10.A)F_1, (C57BL/6 × A/J)F_1 (B6A F_1), and (C57BL/6 × C3H/HeJ)F_1 (B6C3 F_1) mice were purchased either from The Jackson Laboratory, Bar Harbor, ME, or the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. B10.A(3R), B10.A(5R), B10.A(4R), and B10.MBR mice were generously provided by Dr. D. H. Sachs, National Institutes of Health.

Chimeras

Chimeras used in this study were prepared as previously described (22), by the transfer of 1.5×10^7 T cell–depleted bone marrow cells into lethally irradiated (950 rad) recipients, and are designated as bone marrow donor → irradiated recipient. Chimeras were immunized no earlier than 8 wk after irradiation and reconstitution. The chimera spleen cells used in these studies were typed by indirect immunofluorescence, and were of donor origin without detectable (<5%) cells of host origin.

Antigens

Keyhole limpet hemocyanin (KLH) (Calbiochem-Behring Corp., San Diego, CA) and fowl gamma globulin (FGG) (Cappel Laboratories, Cochranville, PA) were conjugated with 2,4,6-trinitrobenzene sulfate (Pierce Chemical Co., Rockford, IL) as previously described (4). The degree of substitution was 20 trinitrophenyl (TNP) residues per 100 kilodaltons (kD) KLH (TNP-KLH), and 9 TNP residues per 100 kD FGG (TNP-FGG).

Antibodies

Monoclonal hybridoma anti-Lyt-1.2 antibody (mAb) (lot FPB031) and anti-Lyt-2.2 mAb (lots FPA179 and FPB126) were purchased from New England Nuclear, Boston, MA, and used as cytotoxic reagents as previously described (18).

Immunization

Mice were immunized with 100 μg of KLH, FGG, or TNP-KLH, in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) intraperitoneally 3–8 wk before use.

Derivation of MHC-restricted and Antigen-specific Cloned Th Cells and Cloned Ts Cells

MHC-restricted and antigen-specific Th and Ts cloned cells were derived and tested for their function as previously described (20).

Generation of Heterogeneous Ts Cells In Vitro

Heterogeneous Ts cells were induced in vitro as previously described (14). Briefly, KLH-primed T cells (see below) were passed over Sephadex G-10 columns twice to deplete accessory (Acc) cells (14, 23). Acc cell–depleted T cells were cultured for 3 d in the presence of additional Acc cells and 100 μg/ml KLH. Cultured cells were extensively washed, and tested for their suppressive activities.

Preparation of Cells

T cells. KLH-primed T cells were obtained as spleen cells nonadherent to anti–mouse Ig–coated dishes, as described previously (4). These populations were then used as Th cells, or as a precursor population for the generation of Ts cells. Ta cells were prepared as T cells from unprimed animals (17).

(B + Acc) cells. (B + Acc) cells were prepared by depleting TNP-KLH-primed spleen cells of T cells by treatment with a T cell–specific cytotoxic rabbit anti–mouse brain (RAMB) serum plus complement (C) (4).

Acc cells. Unprimed spleen cells were treated with RAMB + C to deplete T cells, and were used as a source of Acc cells after 3,000-rad irradiation.

Assay of Suppressive Activity

Cloned Ts cells or precultured heterogeneous T cells were assayed for suppressive activity by adding them to responding cell mixtures of $3–4 \times 10^6$ TNP-primed (B + Acc) cells that had been cocultured with cloned Th cells or KLH-primed heterogeneous Th cells in the presence or absence of 10^6 unprimed T cells (as a source of Ta cells). Cultures were carried out as previously described (4). Cells were harvested and assayed for TNP-specific hemolytic plaque-forming cells (PFC).

PFC Assay

Sheep erythrocytes were conjugated with TNP, and direct PFC (IgM), as well as total PFC (facilitated by rabbit anti–mouse IgG) were assayed on these conjugated target cells (4). IgG PFC were calculated as total PFC − direct PFC. All points shown represent the arithmetic means of triplicate cultures, expressed as IgG anti-TNP PFC responses per culture.

Results

Restriction Specificities of Cloned Th Cells and Cloned Ts Cells. The precise restriction specificities of cloned Th cells were first determined by assessing the ability of these cells to cooperate with T-depeleted TNP-primed (B + Acc) cells derived from MHC congenic and recombinant strains (Table I, exp. 1). Clone 8-5 provided help to B cells of B10, B10.A(3R), and B10.A(5R) origin, but not to the other B cells employed, mapping the specificity of 8-5 to $I\text{-}A^b$. On the other hand, clone 9-16 provided help to B cells of B10.A(3R), B10.A(5R), B10.A, B10.MBR, and B10.BR origin, but not to B10 or B10.A(4R), consistent with the previously mapped restriction specificity of 9-16 to $I\text{-}E^k$. Next, the MHC restriction specificity for Ts cell function was evaluated. 8-4 and 9-5 cloned Ts cells were added to responding B10, B10.A(3R), B10.A(5R), B10.A(4R), B10.A, B10.MBR, or B10.BR spleen cells (Table I, exp. 2). Clone 8-4 Ts cells suppressed the responses of B10, B10.A(3R), and B10.A(5R), but not those of B10.A(4R),

TABLE I

Restriction Specificities of Cloned Th and Cloned Ts Cells

Responding cells*	IgG anti-TNP PFC/culture‡				
	Exp. 1		Exp. 2		
	Clone 8-5	Clone 9-16	control	Clone 8-4	Clone 9-5
B10	915 ± 8	0	3,933 ± 546	352 ± 38	3,907 ± 132
B10.A(3R)	1,302 ± 97	1,724 ± 532	2,780 ± 137	475 ± 109	2,288 ± 341
B10.A(5R)	660 ± 69	1,196 ± 242	2,481 ± 80	387 ± 35	1,918 ± 98
B10.A(4R)	0	0	1,663 ± 30	1,874 ± 198	96 ± 61
B10.A	0	1,425 ± 343	1,161 ± 118	1,372 ± 54	132 ± 76
B10.MBR	0	2,235 ± 275	2,024 ± 243	2,024 ± 118	211 ± 129
B10.BR	0	1,689 ± 80	1,320 ± 84	1,381 ± 46	61 ± 23

* H-2 (KABJECD) of responding cells are B10 (bbbbbbb), B10.A(3R) (bbbbkdd), B10.A(5R) (bbbkkdd), B10.A(4R) (kkbbbbb), B10.A (kkkkddd), B10.MBR (bkkkkkq), and B10.BR (kkkkkkk).

‡ 4×10^6 TNP-KLH-primed spleen cells of the indicated strain of origin were treated with RAMB plus C (exp. 1) or were untreated (exp. 2), and were cocultured with 10^4 of the indicated cloned T cells. Cultures were stimulated with 1 ng/ml TNP-KLH. In exp. 1 (B plus Acc), cells gave no significant PFC response. In exp. 2, responses without cloned T cells were presented as control values.

TABLE II

Origin, Specificity, and Function of Cloned T Cells

T cell clone	Origin	Specificity	Function
8-4	B6C3 F_1	I-A^b + KLH	Ts
8-5	B6C3 F_1	I-A^b + KLH	Th
9-5	B6C3 F_1	I-A^k + KLH	Ts
9-16	B6C3 F_1	I-E^k + KLH	Th
2-19-2	B10	I-A^b + FGG*	Th*

* Data not shown (16).

B10.A, B10.MBR, and B10.BR. In contrast, 9-5 Ts cells suppressed the responses of B10.A(4R), B10.MBR, B10.A, and B10.BR, but not those of B10, B10.A(3R), and B10.A(5R). These results indicate that 8-4 Ts cell function is restricted to I-A^b and 9-5 Ts cell function to I-A^k. Although antigen specificity was not evaluated in Table I, all clones used in these studies expressed strict antigen specificity, as previously reported (20). MHC restriction specificities and antigen specificities of the clones employed in these studies are summarized in Table II.

Antigen-specific Suppression of Cloned Th Cell Responses by Cloned Ts Cells Requires MHC-restriction Only for Ts Cells. Cloned Ts cells have previously been shown (27) to suppress antibody responses mediated by heterogeneous populations of MHC-restricted Th cells in an MHC-restricted and antigen-specific manner. To further characterize the MHC restriction requirements for Ts cell activation and function in the absence of heterogeneous T cell populations, cloned Ts cell activity was tested on B cell responses mediated by cloned Th cells (Table III). B10 B cell responses mediated by I-A^b-restricted Th clone 8-5 were suppressed by I-A^b-restricted Ts clone 8-4, but not by I-A^k-restricted Ts clone 9-5. In contrast, the responses of B10.BR B cells mediated by I-E^k-restricted Th clone 9-16 were suppressed by I-A^k-restricted Ts clone 9-5 but not by I-A^b-

TABLE III
Interaction of Ts Cells with (B + Acc) Cells Is MHC-restricted

Cloned Ts	IgG anti-TNP PFC/culture*			
	B10 (B + Acc)	B10.BR (B + Acc)	B10.A(3R) (B + (Acc)	
	8-5 (A^b) Th	9-16 (E^k) Th	8-5 (A^b) Th	9-16 (E^k) Th
Not added	3,572 ± 299	3,291 ± 484	2,939 ± 299	3,889 ± 428
8-4 (A^b)	52 ± 30	3,080 ± 456	88 ± 8	220 ± 103
9-5 (A^k)	3,669 ± 268	0	2,692 ± 26	4,408 ± 60

* 4×10^6 TNP-primed (B + Acc) cells of indicated origin were cocultured with 10^4 cloned Th cells, 8-5 or 9-16. 10^4 cloned Ts cells were added to the culture. Cultures were stimulated with 1 ng/ml TNP-KLH.

TABLE IV
Ts Cell Interaction with Acc Cells Is MHC-restricted, Whereas Ts Cell Function Is MHC-unrestricted

Cloned Ts	IgG anti-TNP PFC/culture			
	B10 (B + Acc)		B10.BR (B + Acc)	
	8-5 (A^b) Th	8-5 (A^b) + BR Acc	9-16 (E^k) Th	9-16 (E^k) + B10 Acc
Not added	1,160 ± 105	1,504 ± 217	1,249 ± 122	1,848 ± 219
8-4 (A^b)	17 ± 17	52 ± 29	1,249 ± 57	70 ± 46
9-5 (A^k)	1,134 ± 152	342 ± 20	0	0

4×10^6 TNP-primed B10 or B10.BR (B + Acc) cells were cocultured with 10^4 cells of the indicated Th clone in the presence or absence of 10^6 additional Acc cells. 10^4 cloned Ts cells were added to the culture. Cultures were stimulated with 1 ng/ml TNP-KLH.

restricted Ts clone 8-4. The fact that both 8-5 (I-A^b-restricted) and 9-16 (I-E^k-restricted) Th clones could efficiently cooperate with B10.A(3R) B cells made it possible to analyze the MHC-restriction requirements for Ts cell function by determining the ability of Ts clones 8-4 and 9-5 to suppress the responses of B10.A(3R) B cells mediated by either 8-5 or 9-16 Th cells. Ts clone 8-4 (I-A^b-restricted) was found to suppress the responses mediated by either 8-5 or 9-16 cloned Th cells, whereas Ts clone 9-5 (I-A^k-restricted) did not affect these responses (Table III), suggesting that cloned Ts cells are MHC-restricted in their suppression of B cell responses mediated by cloned Th cells. However, these experiments did not define whether this restriction occurs at the level of Ts cell activation, Ts effector function, or both.

This question was approached by determining whether MHC-restricted Ts cell activation with appropriate Acc cells was sufficient to allow suppression of the Th cell–mediated activation of B cells expressing either appropriate or inappropriate MHC specificities. In the absence of added Acc cells, B10 B cell responses mediated by Th clone 8-5 (I-A^b-restricted) were suppressed by Ts clone 8-4 (I-A^b-restricted) but not Ts clone 9-5 (I-A^k-restricted) (Table IV). However, the failure of Ts clone 9-5 to suppress the response of B10 B cells was

overcome by the addition of B10.BR Acc cells to the culture. Similarly, the responses of B10.BR B cells mediated by Th clone 9-16 (I-E^k-restricted) were suppressed by Ts clone 8-4 (I-A^b-restricted) only in the presence of added B10 Acc cells. These results showed that an MHC-restricted interaction of Ts cells with Acc cells is sufficient for Ts cell function, and that, subsequent to activation, cloned Ts cells were capable of suppressing B cell responses in an MHC-unrestricted fashion.

Next, we determined whether Ts cells that had been activated in an MHC-restricted and antigen-specific manner were antigen-specific in their effector function (Table V). 8-4 and 9-5 Ts cell function was tested on the responses of (B10 × B10.BR)F_1 B cells mediated by Th clone 2-19-2 (I-A^b + FGG), 8-5 (I-A^b + KLH), or 9-16 (I-E^k + KLH). In the presence of F_1 (B + Acc) cells, the MHC-restricted activation requirements of both I-A^b-restricted and I-A^k-restricted T cells are satisfied. Under these conditions, the responses mediated by both KLH-specific cloned Th cells were completely suppressed by either KLH-specific Ts clone (Table V). In contrast, the responses mediated by FGG-specific cloned Th cells were not suppressed by either Ts clone. In addition, this failure of suppression was not overcome when the antigen-specific activation requirement of cloned Ts cells was satisfied by the addition of either free KLH (Table V) or TNP-KLH to the culture, despite the fact that these responses were susceptible to suppression by FGG-specific Ts cells (20, 21, and data not shown). Thus, although cloned Ts cells, following appropriate activation, suppress B cell responses regardless of the MHC restriction of responding cloned Th cells, the antigen-specificity of Ts effector function is conserved.

Lyt-1$^+$,2$^-$ T Cells Modify MHC-restriction of Cloned Ts Function. Previously reported findings (20) had demonstrated that cloned Ts cells were MHC restricted in their suppression of responses mediated by heterogeneous Th cell populations. In contrast, the results presented above show that the same cloned Ts cells are MHC unrestricted in effector function against cloned Th cells. Therefore, the suppressive activities of cloned Ts cells were directly compared in the responses of (B10 × B10.A)F_1 (B + Acc) cells mediated by heterogeneous or cloned Th cell populations. F_1 B cell responses mediated by $F_1 \rightarrow$ B10 chimeric Th cells were suppressed by Ts clone 8-4 (I-A^b-restricted), but not by Ts clone

TABLE V

Effector Function of Cloned Ts Cells Is Antigen-specific

Cloned Ts cells	IgG anti-TNP PFC/culture (B10 × B10.BR)F_1 (B + Acc)*		
	8-5 (A^b + KLH) Th‡	(E^k + KLH) Th‡	2-19-2 (A^b + FGG) Th§
Not added	1,091 ± 253	1,003 ± 92	1,232 ± 287
8-4 (A^b + KLH)	0	26 ± 15	1,276 ± 114
9-5 (A^k + KLH)	44 ± 31	39 ± 32	1,056 ± 76

* 4 × 10^6 TNP-primed (B10 × B10.BR)F_1 (B + Acc) cells were cocultured with 10^4 cells of the indicated Th clone in the presence or absence of 10^4 cloned Ts cells.

‡ Cultures were stimulated with 1 ng/ml TNP-KLH.

§ Cultures were stimulated with 1 ng/ml TNP-FGG + 1 ng/ml KLH.

9-5 (I-A^k-restricted), whereas F_1 B cell responses mediated by $F_1 \rightarrow$ B10.A chimeric Th cells were suppressed by 9-5 but not by 8-4 Ts cells (Table VI, exp. 1). In striking contrast, F_1 B cell responses mediated by cloned Th cells, either 8-5 or 9-16, were effectively suppressed by either 8-4 or 9-5 Ts cells (Table VI, exp. 2). When titrated numbers of Ts cells were added under these conditions, no preferential effect of cloned Ts cells on either of the cloned Th populations was observed (data not shown).

One potential difference between these two situations was the presence of additional (noncloned) T cells in the cultures containing heterogeneous Th cells. To determine whether the presence of these "other" T cells could affect the MHC-restriction requirements for suppression, cloned Ts cell activity was tested on cloned Th cells in the presence or absence of additional unprimed T cells. Ts cell activity was tested on the responses of (B10 × B10.A)F_1 (B + Acc) cells mediated by cloned Th cells in the presence or absence of added F_1, $F_1 \rightarrow$ B10, or $F_1 \rightarrow$ B10.A T cells. As before, the B cell responses mediated by 8-5 Th cells were suppressed by either 8-4 or 9-5 Ts cells (Table VII), and once again, titration of Ts cell numbers demonstrated an equivalent effect of the two cloned Ts populations (data not shown). Responses mediated by the I-A^b-restricted Th clone 8-5 were augmented in the presence of unprimed F_1 T cells (Table VII) as previously described, and the phenotype of the functioning Ta cells was found to be Thy-1^+, Lyt-1^+,2^- (data not shown) (20). Addition of the I-A^b-restricted Ts clone 8-4 to these cultures suppressed the augmented response completely, whereas the I-A^k-restricted Ts clone 9-5 only partially inhibited the response. Conversely, the augmented responses mediated by 9-16 Th cells were only partially inhibited by 8-4 Ts cells, but were completely suppressed by clone 9-5 Ts cells (Table VII). The phenotype of the subpopulation of unprimed F_1 T cells that mediated this effect on Ts–Th cell interactions was determined to be Lyt-1^+,2^- (data not shown). Responses supported by Th clone 8-5 were also augmented by chimeric T cells of $F_1 \rightarrow$ B10 origin, but not by cells of $F_1 \rightarrow$ B10.A origin, whereas responses by Th clone 9-16 were augmented by $F_1 \rightarrow$ B10.A cells, but not by $F_1 \rightarrow$ B10 T cells, indicating that the observed augmentation is mediated by an MHC-restricted interaction (16). Moreover, the augmented responses generated by Th clone 8-5 and $F_1 \rightarrow$ B10 chimeric T cells were again completely suppressed by Ts clone 8-4 but only partially inhibited by

TABLE VI

Comparison of Ts Cell Function on Heterogeneous and Monoclonal Th Cell Populations

Cloned Ts	IgG anti-TNP PFC/culture (B10 × B10.A)F_1 (B + Acc)*				
	Exp. 1		Exp. 2		
	$F_1 \rightarrow$ B10	$F_1 \rightarrow$ B10.A	$F_1 \rightarrow$ B10	8-5 (A^b)	9-16 (E^k)
Not added	1,909 ± 172	1,883 ± 375	1,346 ± 388	1,698 ± 215	4,400 ± 156
8-4 (A^b)	387 ± 43	2,393 ± 188	475 ± 85	202 ± 98	132 ± 40
9-5 (A^k)	1,716 ± 52	466 ± 68	1,240 ± 265	422 ± 169	105 ± 60

* 4×10^6 TNP-primed (B10 × B10.A)F_1 (B + Acc) cells were cocultured with either 10^6 KLH-primed chimeric T cells or 10^4 cloned Th cells in the presence or absence of 10^4 cloned Ts cells. Cultures were stimulated with 1 ng/ml TNP-KLH.

TABLE VII

Unprimed T Cells Function to Modify MHC-unrestricted Suppression

Cloned Ts	Unprimed T cells	IgG anti-TNP PFC/culture using cloned Th cells	
		8-5 (A^b) Th	9-16 (E^k) Th
Not added	Not added	1,232 ± 101	2,393 ± 115
8-4 (A^b)	Not added	52 ± 21	13 ± 10
9-5 (A^k)	Not added	79 ± 21	0
Not added	(B10 × B10.A)F_1	4,752 ± 189	5,420 ± 352
8-4 (A^b)	(B10 × B10.A)F_1	92 ± 10	1,988 ± 115
9-5 (A^k)	(B10 × B10.A)F_1	1,980 ± 214	0
Not added	F_1 → B10	4,523 ± 413	1,953 ± 109
8-4 (A^b)	F_1 → B10	105 ± 42	79 ± 64
9-5 (A^k)	F_1 → B10	1,478 ± 53	0
Not added	F_1 → B10.A	1,777 ± 17	5,649 ± 447
8-4 (A^b)	F_1 → B10.A	132 ± 40	1,812 ± 258
9-5 (A^k)	F_1 → B10.A	281 ± 76	0

* 4×10^6 TNP-primed (B10 × B10.A)F_1 (B + Acc) cells were cocultured with 10^4 cloned Th cells in the presence or absence of 10^4 cloned Ts cells. 10^6 unprimed T cells of the indicated strain were added to the culture. Cultures were stimulated with 1 ng/ml TNP-KLH.

Ts clone 9-5; conversely, the augmented response of Th clone 9-16 and F_1 → B10.A T cells was more effectively inhibited by Ts clone 9-5 than by Ts clone 8-4. These results show that unprimed Lyt-1$^+$,2$^-$ T cells modify the suppressive effect of cloned Ts cells on the responses mediated by cloned Th cells. The resulting suppression shows a substantial preference for suppression of Th cells expressing an MHC restriction similar to that of the Ts cells. It thus appears that these Lyt-1$^+$,2$^-$ T cells function to modify only the MHC-unrestricted component of suppression observed when cloned Ts cells act upon cloned Th cells expressing a different MHC restriction.

Lyt-1$^+$,2$^-$ T Cells Modify MHC-unrestricted Suppression Mediated by Heterogeneous Ts Cell Populations. Further experiments were carried out to determine whether the cellular interactions elucidated using cloned Ts cells could also be detected in the function of heterogeneous populations of Ts cells. Heterogeneous MHC-restricted Ts cell populations were generated as previously described (14). KLH-primed (B10 × B10.BR)F_1 T cells were precultured with either B10 or B10.BR Acc cells in the presence of KLH, and the induced Ts cell activity was tested on the responses of (B10 × B10.BR)F_1 (B + Acc) cells, and Th cells of either F_1 → B10 or F_1 → B10.BR origin. The responses mediated by F_1 → B10 Th cells were suppressed by F_1 Ts cells induced with B10 Acc cells, but not by F_1 Ts cells induced with B10.BR Acc cells. The responses mediated by F_1 → B10.BR Th cells showed the reciprocal pattern (Table VIII, exp. 1). In addition, the heterogeneous Ts cells induced by this protocol showed the identical suppression pattern on the responses mediated by cloned Th cells 8-5 and 9-16 (Table VIII, exp. 2). Thus, the activity of the Ts cells induced in heterogeneous F_1 T cell populations was found to be MHC-restricted, as previously shown (14).

TABLE VIII

Heterogeneous F_1 Ts Cell Populations Function to Suppress Responses Mediated by Either Heterogeneous or Monoclonal Th Cells in an MHC-restricted Manner

Exp.	Th cells added	IgG anti-TNP PFC/culture with added Ts cells*		
		None	H-2^b-restricted	H-2^k-restricted
1	$F_1 \rightarrow$ B10	1,399 ± 45	114 ± 31	1,038 ± 207
	$F_1 \rightarrow$ B10.BR	1,487 ± 8	1,232 ± 38	457 ± 31
2	$F_1 \rightarrow$ B10	884 ± 32	396 ± 80	1,152 ± 116
	8-5 (A^b)	1,091 ± 83	409 ± 10	990 ± 32
	9-16 (E^k)	1,135 ± 30	1,311 ± 46	255 ± 35

* 4×10^6 TNP-primed (B10 × B10.BR)F_1 (B + Acc) cells were cultured with either 10^6 KLH-primed chimeric heterogeneous or 10^4 cloned monoclonal Th cells in the presence or absence of KLH-primed heterogeneous F_1 Ts cell populations. Cultures were stimulated with 1 ng/ml TNP-KLH. Ts cells were generated by culturing KLH-primed (B10 × B10.BR)F_1 T cells for 3 d with 100 μg/ml KLH and B10 or B10.BR Acc cells.

Ts cells were then induced in (B10 × B10.A)F_1 T cells, and were treated with either anti-Lyt-1 or anti-Lyt-2 mAb + C. The remaining Ts cell activity was tested on the responses of B6A F_1 (B + Acc) cells and 8-5 Th clones in the presence or absence of unprimed T cells of (B10 × B10.A)F_1, $F_1 \rightarrow$ B10, or $F_1 \rightarrow$ B10.A origin, with or without treatment with anti-Lyt-1 or anti-Lyt-2 mAb + C (Table IX). Ts cells, which were induced by preculturing with B10 Acc cells and antigen, suppressed the F_1 responses mediated by 8-5 Th clone (Table IX, group 7), and the treatment of such suppressor cells with anti-Lyt-2 but not anti-Lyt-1 + C eliminated the suppressive function of Ts cells (groups 8 and 9), indicating that the functioning Ts cells are Lyt-1^-,2^+ phenotype, as previously reported (14, 19). Addition of unprimed F_1 T cells to the culture did not reverse the suppression (Table IX, group 10), although F_1 T cells augmented the responses mediated by cloned Th cells (group 2). Ts cells that had been induced by preculturing with B10.A Acc cells and antigen did not suppress the response generated by Th clone 8-5 (Table IX, group 13). However, elimination of Lyt-1^+ cells from this Ts population resulted in the ability of residual Lyt-1^-,2^+ Ts cells to suppress (in MHC-unrestricted fashion) responses of clone 8-5 Th cells (group 14). The addition of normal Lyt-1^+,2^- F_1 T cells to these cultures, however, reversed the suppression mediated by Lyt-1^-,2^+ Ts cells (Table IX, groups 15–17). This effect of unprimed T cells was mediated by MHC-restricted T cells, since $F_1 \rightarrow$ B10 but not $F_1 \rightarrow$ B10.A chimeric T cells reversed the MHC unrestricted suppressive function of heterogeneous Lyt-2 Ts cells (Table IX, groups 18 and 19). These results indicate that MHC-restricted Lyt-1 T cells modify the MHC-unrestricted suppression mediated by heterogeneous as well as cloned Ts cell populations.

Discussion

These studies were carried out to assess the mechanism of suppression mediated by MHC-restricted and antigen-specific cloned and heterogeneous Ts cells. Previous studies had characterized both heterogeneous Lyt-1^-,2^+ Ts populations

TABLE IX

Lyt-1$^+$,2$^-$ T Cells Function to Modify MHC-unrestricted Suppresson Mediated by Heterogeneous Ts Cell Populations

Group	F_1 Ts cells added		Unprimed T cells added		IgG PFC/culture; B6A F_1 (B + Acc) + 8-5 (A^b) Th
	Restriction specificity	Treatment	Origin	Treatment	
1	Not added		Not added		1,383 ± 144
2	Not added		B6A F_1	None	2,966 ± 166
3	Not added		B6A F_1	Anti-Lyt-1 + C	1,116 ± 185
4	Not added		B6A F_1	Anti-Lyt-2 + C	2,933 ± 175
5	Not added		$F_1 \rightarrow$ B10	None	3,033 ± 158
6	Not added		$F_1 \rightarrow$ B10.A	None	1,116 ± 142
7	B10 ($H\text{-}2^b$)	None	Not added		300 ± 152
8	B10 ($H\text{-}2^b$)	Anti-Lyt-2 + C	Not added		1,388 ± 59
9	B10 ($H\text{-}2^b$)	Anti-Lyt-1 + C	Not added		133 ± 133
10	B10 ($H\text{-}2^b$)	Anti-Lyt-1 + C	B6A F_1	None	350 ± 49
11	B10 ($H\text{-}2^b$)	Anti-Lyt-1 + C	$F_1 \rightarrow$ B10	None	66 ± 66
12	B10 ($H\text{-}2^b$)	Anti-Lyt-1 + C	$F_1 \rightarrow$ B10.A	None	183 ± 101
13	B10.A ($H\text{-}2^a$)	None	Not added		1,833 ± 104
14	B10.A ($H\text{-}2^a$)	Anti-Lyt-1 + C	Not added		91 ± 91
15	B10.A ($H\text{-}2^a$)	Anti-Lyt-1 + C	B6A F_1	None	1,316 ± 59
16	B10.A ($H\text{-}2^a$)	Anti-Lyt-1 + C	B6A F_1	Anti-Lyt-1 + C	200 ± 76
17	B10.A ($H\text{-}2^a$)	Anti-Lyt-1 + C	B6A F_1	Anti-Lyt-2 + C	1,233 ± 191
18	B10.A ($H\text{-}2^a$)	Anti-Lyt-1 + C	$F_1 \rightarrow$ B10	None	1,425 ± 137
19	B10.A ($H\text{-}2^a$)	Anti-Lyt-1 + C	$F_1 \rightarrow$ B10.A	None	291 ± 166

4×10^6 TNP-primed B6A F_1 (B + Acc) cells were cocultured with 10^4 clone 8-5 Th cells in the presence or absence of 0.5×10^6 KLH-primed heterogeneous F_1 Ts cells, which were treated as indicated. 0.5×10^6 unprimed T cells of indicated origin were added after treatment. Cultures were stimulated with 1 ng/ml TNP-KLH.

(14, 19) and cloned Lyt-1$^+$,2$^-$ (20) populations that function to suppress the in vitro TD antibody responses of primed B cells. Both heterogeneous and cloned Ts cells, although differing in cell surface phenotype, exhibited similar requirements for antigen specificity and MHC restriction, both at the level of Ts cell activation and at the level of Ts effector function following activation. The observed MHC restrictions in Ts function were rather unique, and appeared to consist of a requirement for similarity in MHC restriction specificity between Ts cells and the Th cells functioning to generate a given B cell response (14, 20). Although these genetic restrictions were observed in the function of both cloned and heterogeneous Ts cells, previous experiments were carried out under conditions in which some source of heterogeneous T cells was present in culture. The possibility therefore existed that the observed MHC restriction in Ts function was influenced by more complex interactions among the T cell subpopulations present in heterogeneous populations. To better assess the nature of the regulatory T cell interactions involved in the control of TD B cell activation, these studies were performed under conditions in which only cloned or highly selected populations of Ts cells and Th cells were present, and in which the influence of additional T cell populations could be directly evaluated.

Our results show that the activation of cloned Ts cells remained both antigen specific and MHC restricted. After appropriate activation, the effector function of cloned Ts cells was antigen specific, even when only cloned populations of Th and Ts cells were used (21). However, these activated Ts cells suppressed the responses supported by cloned Th cells in an apparently MHC-unrestricted fashion, i.e., in the absence of any requirement for similarity between the restriction specificities of the cloned Ts and Th cells. This property was not unique to cloned Ts cells, since heterogeneous Lyt-1^-,2^+ Ts cells, which had been depleted of Lyt-1^+,2^- cells, suppressed the response of cloned Th cells in a similarly unrestricted manner. In contrast, when heterogeneous unprimed Lyt-1^+,2^- T cells were added back to these in vitro cultures, a substantial degree of MHC restriction in the effector function of both cloned and heterogeneous Ts cells was reconstituted. Thus, cloned Ts cells or Lyt-1^+-depleted heterogeneous Lyt-2^+ Ts cells, which are restricted to one parental MHC haplotype (H-2^A), suppressed antibody responses by (A × B)F_1 B cells equally well when the cloned Th cells were either H-2^A-restricted or H-2^B-restricted, and thus exhibited MHC-unrestricted effector function. However, in the presence of heterogeneous Lyt-1^+,2^- F_1 T cells, H-2^A-restricted Ts cells functioned in an MHC-restricted fashion, and preferentially suppressed the responses of H-2^A-restricted Th cells. Moreover, this function of heterogeneous Lyt-1^+,2^- T cells was itself MHC restricted, and this modifying effect on suppression occurred only when the Lyt-1^+,2^- T cells were similar in MHC restriction specificity to the functioning Th cells. Specifically, we showed that H-2^b-restricted, but not H-2^a-restricted heterogeneous Lyt-1^-,2^+ Ts preferentially suppressed responses by I-A^b-restricted cloned Th cells in the presence of heterogeneous Lyt-1^+,2^- F_1 T cells. Similarly, in the presence of Lyt-1^+,2^- T cells, I-A^b-restricted cloned Ts cells (8-4) suppressed (B10 × B10.A)F_1 B cell responses preferentially when I-A^b-restricted (8-5), rather than I-E^k-restricted (9-16) Th cells were used. A complicating finding in this regard was the observation that I-A^k-restricted cloned Ts cells (9-5) under these conditions preferentially suppressed the response of I-E^k- (9-16) rather than I-A^b-restricted (8-5) cloned Th cells. Although this latter finding remains unexplained, it is possible that I-A^k and I-E^k products are recognized with significant crossreactivity by either Ts clone 9-5 or Th 9-16.

The precise cellular mechanism of the observed T cell interactions described here has not yet been defined. The data presented confirm the previously described activity of MHC-restricted Lyt-1^+,2^- T cells to augment the responses of cloned Th cells in the absence of Ts (16). It is not yet certain, however, whether the cells mediating this augmenting effect are identical to those that influence the MHC restriction of suppression. Several possible mechanisms exist by which Lyt-1^+,2^- T cells might modify suppression: (*a*) It is possible that cells in the unprimed Lyt-1^+,2^- population acquire intrinsic Th activity during in vitro culture, and that this helper activity is resistant to MHC unrestricted suppression. Attempts to directly isolate functioning Th cells from Lyt-1^+,2^- populations involved in these mixed cultures are currently in progress. (*b*) It is also possible that Lyt-1^+,2^- T cells might function to render cloned Th cells resistant to MHC-unrestricted suppression. This mechanism of action is analogous to that described recently (17) for the effect of Tcs cells, which render Th

cells resistant to subsequent Ts-mediated effects. Indeed, the effects characterized herein could be interpreted as a form of contrasuppression that acts to selectively block a pathway of MHC-unrestricted Ts activity, while leaving intact the susceptibility to MHC-restricted Ts function. Alternatively, (*c*) Lyt-1$^+$,2$^-$ Tcs cells might act on Ts cells to interfere with unrestricted Ts effects, or (*d*) might directly alter B cell susceptibility to unrestricted suppression. Experiments involving preincubation of Lyt-1$^+$,2$^-$ T cells with isolated Th, Ts, or B cell populations may distinguish among these alternative sites of action.

The form of MHC-restricted suppression observed in these and previous studies (14, 20, 21) is consistent with a competitive mechanism in which positive (helper) and negative (suppressive) regulatory signals compete in their interactions with responding B cells and/or accessory cells. Previous results (14, 20, 21) suggested that such competition occurred at the level of MHC-restricted Ts and Th cell recognition of the same Ia-antigen complexes presented on responding cells. Our findings indicate that if such a mechanism is involved in the T cell–mediated suppression observed here, then this competitive interaction between MHC-restricted regulatory T cells is critically influenced by an Lyt-1$^+$,2$^-$ subpopulation of MHC-restricted T cells that function, directly or indirectly, to oppose MHC-unrestricted suppression. A more precise identification and characterization of the Lyt-1$^+$,2$^-$ T cells mediating the effects described here would permit a better understanding of the mode of action of these cells. They are clearly MHC restricted in some phase of their activation or function, but since active cells are present in unprimed T cell populations, their antigen specificity is unclear. A screening of available Lyt-1$^+$,2$^-$ antigen-specific or autoreactive T cell clones has, to date, failed to identify any monoclonal population with this Tcs-like activity. The use of phenotypic markers previously shown to be expressed on Ta (16) or Tcs (17) populations may prove informative in future studies.

A modifying effect of Lyt-1$^+$,2$^-$ T cells on Ts function was observed here not only upon the function of cloned Ts cells, but also upon the function of heterogeneous Lyt-1$^-$,2$^+$ Ts cells. It is therefore conceivable that such effects are functioning in other T cell regulatory systems as well. As has been the case for the recently described Tcs cells, such effects may easily be overlooked. In the present instance, it was only in the absence of any source of heterogeneous Lyt-1$^+$,2$^-$ T cells that this effect was appreciated. A source of such T cells contributed by the responding populations in a given immune response, or by the Ts population itself, would obscure the existence of such a regulatory population in an experimental system, and previous studies using heterogeneous populations of (Lyt-1$^+$,2$^-$) Th cells in fact failed to detect their effect (20). It would therefore be useful to reconsider the potential influence of such regulatory cells in other systems of regulatory T cell interaction.

Summary

Previous studies have shown the existence of both heterogeneous Lyt-1$^-$,2$^+$ suppressor (Ts) cells and cloned Lyt-1$^+$,2$^-$ Ts cells which, despite the difference in their Lyt phenotypes, functioned in a similar antigen-specific and major histocompatibility complex (MHC)-restricted fashion to suppress the antibody responses generated by cloned helper T (Th) cells and hapten-primed B cells.

Our studies were carried out to assess in further detail the genetically restricted cell interactions that mediate this immune response suppression. We show that the activation of both heterogeneous and cloned Ts cells is antigen-specific and MHC-restricted under our experimental conditions. After appropriate activation, the effector function of both cloned Lyt-1^+,2^- Ts cells and heterogeneous Lyt-1^-,2^+ Ts cells was also antigen-specific. In contrast, once activated, Ts cells suppressed the responses generated by cloned Th cells and hapten-primed B cells in an MHC-unrestricted fashion. We also showed, however, that a population of unprimed Lyt-1^+,2^- T cells was able to significantly alter the genetic restriction requirements for Ts cell function. The activity of this population was itself MHC-restricted, and was observed only when the unprimed Lyt-1^+,2^- T cells shared the MHC restriction specificity of the cloned Th cells functioning in a given response. When these requirements were satisfied, Lyt-1^+,2^- T cells significantly modified the suppression mediated by both heterogeneous and cloned Ts cells, resulting in suppression that was then MHC restricted in its effector function as well as in its activation requirements. Thus, our findings suggest that the observed MHC restriction in Ts function is the result of a complex interaction involving Ts cells, Th cells, and an additional population of MHC-restricted Lyt-1^+,2^- T cells. This newly characterized activity of Lyt-1^+,2^- T cells functionally resembles that of an MHC-restricted contrasuppressor population that selectively blocks a pathway of MHC-unrestricted Ts activity, while leaving intact susceptibility to MHC-restricted Ts effects.

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References

1. Miller, J. F. A. P., and G. F. Mitchell. 1969. Thymus and antigen reactive cells. *Transplant. Rev.* 1:3.
2. Davies, A. J. S. 1969. The thymus and the cellular basis of immunity. *Transplant. Rev.* 1:43.
3. Claman, H. N., and E. A. Chaperon. 1969. Immunological complementation between thymus and marrow cells—a model for the two cell theory of immunocompetence. *Transplant. Rev.* 1:32.
4. Asano, Y., A. Singer, and R. J. Hodes. 1981. Role of the major histocompatibility complex in T cell activation of B cell subpopulations. Major histocompatibility complex–restricted and –unrestricted B cell responses are mediated by distinct B cell subpopulations. *J. Exp. Med.* 154:1100.
5. Asano, Y., M. Shigeta, C. G. Fathman, A. Singer, and R. J. Hodes. 1982. Role of the major histocompatibility complex in T cell activation of B cell subpopulations. A single monoclonal T helper cell population activates different B cell subpopulations by distinct pathways. *J. Exp. Med.* 156:350.
6. Cantor, H., and R. K. Gershon. 1979. Immunological circuits: cellular composition. *Fed. Proc.* 38:2058.
7. Germain, R. N., and B. Benacerraf. 1981. A single major pathway of T lymphocyte interactions in antigen-specific immune suppression. *Scand. J. Immunol.* 13:1.

8. Kontiainen, S., and M. Feldmann. 1978. Suppressor cell induction in vitro. IV. Target of antigen-specific suppressor factors and its genetic relationship. *J. Exp. Med.* 147:110.
9. McDougal, J. S., F. W. Shen, S. P. Cort, and J. Bard. 1980. Feedback suppression: phenotype of T cell subsets involved in the Ly-1 T cell–induced immunoregulatory circuit. *J. Immunol.* 125:1157.
10. Tada, T., and K. Okumura. 1980. The role of antigen specific T cell factors in the immune response. *Adv. Immunol.* 28:1.
11. Baxevanis, C. N., N. Ishii, Z. A. Nagy, and J. Klein. 1982. H-2-controlled suppression of T cell response to lactate dehydrogenase B. Characterization of the lactate dehydrogenase B suppressor pathway. *J. Exp. Med.* 156:822.
12. Heuer, J., K. Bruner, B. Opalka, and E. Kolsch. 1982. A cloned T-cell line from a tolerant mouse represents a novel antigen-specific suppressor cell type. *Nature (Lond.)* 296:456.
13. Sorensen, C. M., and C. W. Pierce. 1981. Haplotype-specific suppression of antibody responses in vitro. I. Generation of genetically restricted suppressor T cells by neonatal treatment with semiallogeneic spleen cells. *J. Exp. Med.* 154:35.
14. Asano, Y., and R. J. Hodes. 1983. T cell regulation of B cell activation. I-A-restricted T suppressor cells inhibit the major histocompatibility complex–restricted interactions of T helper cells with B cells and accessory cells. *J. Exp. Med.* 157:1867.
15. Tokuhisa, T., M. Taniguchi, K. Okumura, and T. Tada. 1978. An antigen-specific I region gene product that augments the antibody response. *J. Immunol.* 120:414.
16. Asano, Y., and R. J. Hodes. 1984. T cell regulation of B cell activation. MHC-restricted T augmenting cells enhance the B cell responses mediated by MHC-restricted cloned T helper cells. *J. Immunol.* 132:1151.
17. Gershon, R. K., D. D. Eardley, S. Durum, D. R. Green, F.-W. Shen, K. Yamauchi, H. Cantor, and D. B. Murphy. 1981. Contrasuppression. A novel immunoregulatory activity. *J. Exp. Med.* 153:1533.
18. Asano, Y., and R. J. Hodes. 1982. T cell regulation of B cell activation. T cells independently regulate the responses mediated by distinct B cell subpopulations. *J. Exp. Med.* 155:1267.
19. Asano, Y., and R. J. Hodes. 1983. T cell regulation of B cell activation. Antigen-specific and antigen-nonspecific suppressor pathways are mediated by distinct T cell subpopulations. *J. Immunol.* 130:1061.
20. Asano, Y., and R. J. Hodes. 1983. T cell regulation of B cell activation. Cloned Lyt-1$^+$,2$^-$ T suppressor cells inhibit the major histocompatibility complex-restricted interaction of T helper cells with B cells and/or accessory cells. *J. Exp. Med.* 158:1178.
21. Asano, Y., and R. J. Hodes. 1984. T cell regulation of B cell activation. An antigen-mediated tripartite interaction of Ts cells, Th cells, and B cells is required for suppression. *J. Immunol.* 133:2864.
22. Singer, A., K. S. Hathcock, and R. J. Hodes. 1979. Cellular and genetic control of antibody responses. V. Helper T cell recognition of H-2 determinants on accessory cells but not B cells. *J. Exp. Med.* 149:1208.
23. Ly, L., and R. Mishell. 1974. Separation of mouse spleen cells by passage through Sephadex G-10. *J. Immunol. Methods.* 5:239.