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

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T helper (Th)[1] cell-dependent B cell activation is regulated in a number of response systems by T suppressor (Ts) cells (1–6). In many of these pathways of immune regulation, the activation and/or effector function of Ts cells is under strict genetic control (1–3, 6). Recent studies have analyzed the cell/cell interactions that are involved in Ts cell regulation of the major histocompatibility complex (MHC)-restricted activation of B cells by Th cells (4–6). It was demonstrated that accessory cells as well as antigen-primed T cells are required for in vitro activation of these Ts cells. Moreover, recognition by T cells of *I-A*-encoded determinants expressed on these accessory cells is required for in vitro activation of Ts cells, and this interaction determines the subsequent *I-A* restriction of Ts cell effector activity (6). It was found that the *I-A* restriction in this Ts function appears to act in a unique and highly specific manner to interfere with the MHC-restricted interaction between Th cells and the B cells and/or accessory cells with which they interact (6). The precise mechanism of this Ts cell function, however, has not yet been resolved.

To further analyze the mechanism of T cell-mediated immune suppression, cloned Ts cells have been generated and are characterized in the present report. These cloned Ts cells were shown to express a Lyt-1^+2^- phenotype and to function as final effectors of suppression. These MHC-restricted and antigen-specific cloned Ts cells act to suppress only those responses supported by Th cells with apparently identical MHC restriction and antigen specificity.

Materials and Methods

Animals

C57BL/10 (B10), B10.A, B10.BR, (B10 × B10.BR)F_1, (B10 × B10.A)F_1, and (C57BL/6 × C3H/HeJ)F_1 (B6C3F1) mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

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[1] *Abbreviations used in this paper*: ATS; rabbit anti-mouse thymocyte serum; C; complement; FGG; fowl gamma globulin; FITC; fluorescein isothiocyanate; KLH; keyhole limpet hemocyanin; MHC; major histocompatibility complex; MIg; mouse immunoglobulin; PFC; plaque-forming cells; RAMB; rabbit anti-mouse brain serum; Th; T helper; TNP; trinitrophenyl; Ts; T suppressor.

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Chimeras

Chimeras used in this study were prepared as previously described (7) by the transfer of 15×10^6 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 cells (<5%) 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 (8). The degree of substitution was 20 trinitrophenyl (TNP) residues/100,000 daltons KLH (TNP-KLH) and 9 TNP residues/100,000 daltons FGG (TNP-FGG).

Antibodies

Monoclonal hybridoma anti-Lyt-1.2 antibody (lot FPB 031) and anti-Lyt-2.2 antibody (lot FPA 179) were purchased from New England Nuclear, Boston, MA, and used as cytotoxic reagents as described (4). Monoclonal hybridoma anti-Thy-1.2 antibody was a generous gift from Dr. P. Lake, Georgetown University, Washington, DC. Rabbit anti-mouse thymocyte serum (ATS) was obtained from M. A. Bioproducts, Walkersville, MD. Fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-Thy-1.2 antibody (30.H12), monoclonal anti-Lyt-1 antibody (53.7.3), and monoclonal anti-Lyt-2 antibody (53.6.7) were purchased from Becton, Dickinson & Co., Sunnyvale, CA.

Immunization

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

Derivation of Antigen-reactive Clones

KLH-primed $B6C3F_1$ spleen cells were depleted of cell surface immunoglobulin-positive cells by adherence to anti-mouse immunoglobulin (MIg)-coated plastic dishes as previously described (8). The T cells obtained were stimulated in vitro for 1 wk with 50 μg/ml KLH, and then restimulated weekly with 50 μg/ml KLH, unprimed 3,000 rad-irradiated B10 or B10.BR spleen cells, and 5% T cell growth factor, made as the supernatant of 24-h concanavalin A-stimulated BALB/c spleen cells to which alpha methyl D-mannoside had been added to 0.15 M. Cloning of KLH-reactive T cells was accomplished by limiting dilution methods. Cells were plated at a concentration of 0.3 cell/well. Under these conditions, 10–19 positive wells were scored per 96-well microtiter plate. FGG-specific clones were similarly generated from FGG-primed B10 spleen cells.

Assay of Proliferative Response

1×10^4 antigen-reactive T cells were stimulated in flat-bottomed microtiter wells (Costar 3596; Costar, Cambridge, MA) with antigen in the presence of 5×10^5 irradiated spleen (filler) cells for 48 h. One μCi of [^{3}H]thymidine was added per well, cultures maintained for an additional 6 h, and incorporated radioactivity counted. Results are expressed as the geometric mean of triplicate cultures.

Preparation of Cells for In Vitro Antibody Responses

(B plus accessory) cells. T-depleted TNP-KLH–primed spleen (B plus accessory) cells were prepared by treatment with a T cell-specific cytotoxic rabbit anti-mouse brain serum (RAMB) plus complement (C) as previously described (8). In selected experiments, more rigorous T cell depletion was accomplished by pretreating in vivo with 0.5 ml of a 1/10 dilution of ATS and then treating spleen cells sequentially in vitro with RAMB plus C, monoclonal anti-Thy-1.2, monoclonal anti-Lyt-1.2, and monoclonal anti-Lyt-2.2 plus C.

Th cells. KLH-primed Th cells were obtained as spleen cells nonadherent to anti-MIg–

coated plastic dishes as previously described (8).

Accessory cells. Unprimed spleen cells were T depleted by treatment with RAMB plus C followed by 3,000 rad irradiation, and were used as a source of accessory cells.

Cloned T cells. Cloned T cells were assayed for suppressive activities either on TNP-KLH– or TNP-FGG–primed spleen cells or on a mixture of TNP-primed (B plus accessory) cells and KLH-primed Th cells.

Culture Conditions for In Vitro Antibody Response

Cultures were performed as previously described in 2-ml wells incubated for 5 d at 37°C in 5% CO_2-humidified air (8). For assays of suppressive activity, titrated numbers of cloned Ts cells were added to either 3 or 4 $\times$ 10^6 TNP-KLH– or TNP-FGG–primed spleen cells per culture or a mixture of 3 $\times$ 10^6 TNP-primed (B + accessory) cells and 1 $\times$ 10^6 KLH-primed Th cells per culture. Cells were harvested, washed, and assayed for plaque-forming cells (PFC) on TNP-conjugated sheep erythrocytes (8). All points shown in each experiment represent the arithmetic mean IgG PFC responses of triplicate cultures.

Immunofluorescence Staining and Flow Microfluorometry

Cloned cells were stained with reagents in amounts predetermined to be saturating. Flow microfluorometry analysis of the cloned cells was performed with a fluorescence-activated cell sorter (Dual Laser FACS II; B-D FACS Systems, Sunnyvale, CA). Fluorescence data were collected using logarithmic amplification as previously described (9) on 5 $\times$ 10^4 viable cells as determined by both forward light scatter intensity and propidium iodide exclusion (10) and are displayed as a graph of logarithmic fluorescence intensity versus cell number. Logarithmic amplification was provided by a three decade logarithmic amplifier constructed from a National Institutes of Health-modified design of R. Hiebert, Los Alamos Scientic Laboratory, Los Alamos, NM.

Results

Cloned T Cells Are Antigen Specific and MHC Restricted. The antigen specificities of cloned cells were assessed by measuring antigen-specific proliferative responses (Table I). Clones 8-4 and 8-5 proliferated to KLH in the presence of B10 but not B10.BR accessory cells, whereas clones 9-5, 9-9, and 9-16 proliferated to KLH in the presence of B10.BR but not B10 accessory cells. None of these clones proliferated to FGG. In contrast, clones 2-15-5 and 2-19-2 responded to FGG but not KLH plus B10 accessory cells. These results demonstrated that each of these clones responded to specific antigen in an MHC-restricted manner.

The cell surface phenotypes of clones 8-4, 8-5, 9-5, and 9-16 as well as clone 5.6 (a cytotoxic T cell clone, kindly provided by Dr. J. A. Bluestone, National Institutes of Health (11), were analyzed by fluorescence-activated cell sorter. One example of the fluorescence profiles obtained in the present studies is shown in Fig. 1, and the findings are summarized in Table II. Clones 8-4, 8-5, 9-5, and 9-16 are all strongly Thy-1^+ and Lyt-2^- (whereas clone 5.6 is strongly Lyt-2^+) and are all Lyt-1^+ although varying in their quantitative levels of Lyt-1 expression.

Distinct T Cell Clones Mediate MHC-restricted Help or MHC-restricted Suppression. To determine whether the antigen-reactive clones described above provide helper function for B cell activation, clones were co-cultured with TNP-primed B10 or B10.BR (B plus accessory) cells and stimulated with 0.001 μg/ml TNP-KLH. Two out of seven clones derived by stimulation with B10 accessory cells plus KLH, and two out of nine clones derived by stimulation with B10.BR accessory cells plus KLH functioned as helper cells. Representative results are

TABLE I
Proliferative Responses of Cloned Cells

Expt.	Cloned T cells‡	Strain of origin	[^{3}H]TdR uptake*					
			B10 accessory cells			B10.BR accessory cells		
			Medium	KLH	FGG	Medium	KLH	FGG
			cpm					
1	8-4	B6C3F$_1$	1,507	37,082	615	662	1,451	2,182
			(1.46)	(1.06)	(1.54)	(2.29)	(2.62)	(3.78)
	8-5	B6C3F$_1$	3,345	29,126	4,520	1,215	1,360	3,665
			(1.31)	(1.03)	(1.17)	(1.57)	(1.54)	(2.18)
	9-16	B6C3F$_1$	3,106	1,535	3,124	4,695	85,937	4,240
			(1.19)	(1.63)	(1.30)	(1.39)	(1.09)	(1.07)
	2-19-5	B10	1,459	1,674	68,562	1,252	1,191	1,378
			(1.37)	(1.10)	(1.03)	(1.68)	(1.10)	(1.39)
2	9-5	B6C3F$_1$	3,159	9,865	2,046	9,319	84,735	10,748
			(3.87)	(3.70)	(1.80)	(1.17)	(1.05)	(1.46)
	9-9	B6C3F$_1$	761	399	160	848	98,932	1,031
			(2.77)	(2.08)	(1.94)	(1.18)	(1.05)	(2.39)
	2-15-5	B10	2,646	1,398	58,297	1,883	4,605	735
			(1.47)	(1.82)	(1.10)	(2.03)	(1.94)	(1.98)

* Geometric mean and standard error of triplicate cultures.
‡ 1×10^4 cloned cells were stimulated with 50 μg/ml of the indicated antigen and 5×10^5 accessory cells.

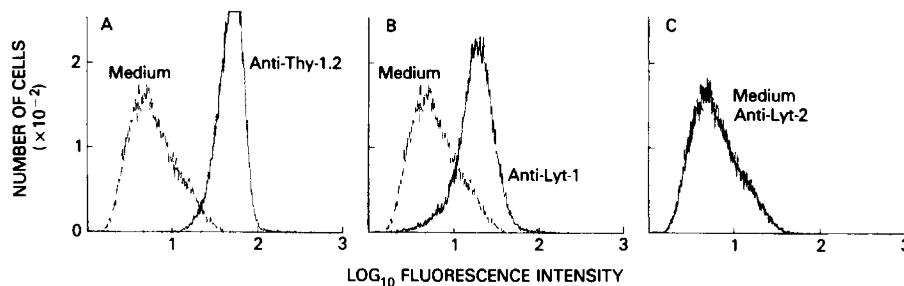

FIGURE 1. Fluorescence-activated cell sorter profile of clone 8-4. Clone 8-4 cells were incubated with FITC-conjugated monoclonal anti-Thy-1.2 (*A*), monoclonal anti-Lyt-1 (*B*), or monoclonal anti-Lyt-2 (*C*), and were analyzed by fluorescence-activated cell sorter. Fluorescence intensity was measured and expressed on a logarithmic scale.

presented in Table III. Clones 8-5 and 9-16 provided help to T-depleted TNP-primed B10 or B10.BR (B plus accessory) cells, respectively, for responses to TNP-KLH (Table III). In contrast, five out of seven *H-2^b*-restricted KLH-specific clones (including 8-4) and seven out of nine *H-2^k*-restricted and KLH-specific clones (including 9-5 and 9-9) failed to provide any help under the experimental conditions tested (Table III).

Next, it was asked whether those clones that have no helper function were able to suppress antibody responses. One out of five *H-2^b*-restricted nonhelper clones and three out of seven *H-2^k*-restricted nonhelper clones functioned as suppressor cells. In the experiment shown (Table IV), the suppressive activities of clones 8-4, 9-5, and 9-9 were measured by adding graded numbers of those

TABLE II

Summary of Fluorescence-activated Cell Sorter Analysis

Clones	Strain of origin	Fluorescein-conjugated reagent*			
		Medium‡	Anti-Thy-1.2	Anti-Lyt-1	Anti-Lyt-2
8-4	$B6C3F_1$	244	2,037	791	247
8-5	$B6C3F_1$	468	3,196	5,958	461
9-5	$B6C3F_1$	373	3,098	899	398
9-16	$B6C3F_1$	206	1,918	564	211
5.6§	$B6.CH\text{-}2^{bm10}$	103	1,543	317	3,685

* Fluorescence intensity was measured using logarithmic amplification as shown in Fig. 1. For purpose of comparison, data in this table were converted to linear units using a calibration standard curve for the logarithmic amplifier used.

‡ Unstained cells. Staining with FITC-conjugated mouse myeloma protein (MOPC-460) gave equivalent values.

§ A cytotoxic T cell clone (11).

TABLE III

Helper Function of Cloned T Cells

Cloned T cells*	Restriction‡	Anti-TNP IgG PFC/Culture	
		B10	B10.BR
Not added	—	0	0
8-5	*H-2^b^*	1,224 ± 58	0
9-16	*H-2^k^*	0	1,267 ± 399
8-4	*H-2^b^*	0	0
9-5	*H-2^k^*	14 ± 11	79 ± 31
9-9	*H-2^k^*	21 ± 17	7 ± 5

* 1×10^4 cloned T cells were co-cultured with 3×10^6 TNP-primed B10 or B10.BR (B + accessory) cells. Cultures were stimulated with 0.001 μg/ml TNP-KLH.

‡ Determined from proliferative responses (see Table I).

TABLE IV

MHC-restricted Suppression by Cloned T Cells

Cloned T cells	Restriction	Anti-TNP IgG PFC/Culture*		
		B10 ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	B10.A ($H\text{-}2^a$)
Not added	—	3,291 ± 220	1,469 ± 61	2,288 ± 132
8-4 (3×10^3)	$H\text{-}2^b$	1,504 ± 15	1,927 ± 190	2,534 ± 132
(10×10^3)		572 ± 101	1,751 ± 483	2,393 ± 206
9-5 (3×10^3)	$H\text{-}2^k$	3,722 ± 42	536 ± 220	616 ± 215
(10×10^3)		3,080 ± 396	0	0
9-9 (3×10^3)	$H\text{-}2^k$	3,423 ± 619	897 ± 251	1,047 ± 235
(10×10^3)		3,699 ± 500	290 ± 84	264 ± 76

* 3×10^6 TNP-KLH-primed B10, B10.BR, or B10.A spleen cells were cultured in the presence or absence of the indicated cloned T cells. Cultures were stimulated with 0.001 μg/ml TNP-KLH.

clones to responding cultures of TNP-KLH-primed B10 (H-2^b), B10.BR (H-2^k), or B10.A (H-2^a) spleen cells that were stimulated with 0.001 μg/ml TNP-KLH. Clone 8-4, which proliferated to KLH plus B10 accessory cells, suppressed the responses of B10 spleen cells but was without effect on responses of B10.BR or B10.A spleen cells (Table IV). In contrast, clones 9-5 and 9-9, which proliferated to KLH plus B10.BR accessory cells, suppressed the responses of B10.BR and B10.A but not B10 (Table IV). Addition of Th clones 8-5 or 9-16 did not suppress the responses of B10 or B10.BR spleen cells to TNP-KLH (data not shown). These results demonstrated that clones 8-4, 9-5, and 9-9 do have haplotype-specific suppressive function. Since all of these Ts clones were generated in B6C3F$_1$ [(H-2^b × H-2^k)F$_1$] T cell populations, and were neither "autoreactive" to B10 or B10.BR cells in the absence of specific antigen, nor alloreactive to B10.A cells (data not shown), the existence of allogeneic-like effects mediated by these Ts clones was effectively excluded. However, the possibility remained that allogeneic effects might be mediated by T cells in the responding B10, B10.BR, or B10.A spleen cell population that recognized, for example, residual accessory cells in the cloned Ts cell population. Additional experiments were therefore carried out in which responding populations consisted of F$_1$ Th cells (nonreactive to F$_1$ or parental cells present in cultures) and T cell-depleted parental (B plus accessory) cells. Under these conditions, where all allogeneic effects would appear to be excluded, MHC-restricted suppressor activity was still observed (data not shown; also see Table VI). Thus, the observed MHC restriction in cloned Ts cell activity appears to be related to the MHC restriction of functioning Th cells and/or to the MHC type of responding (B plus accessory) cells, and does not result from T cell-mediated allogeneic effects.

Suppressive Effect of Cloned Ts Cells Is Antigen Specific. Since it was previously demonstrated that both antigen-specific and antigen-nonspecific Ts cell populations can be generated by stimulation of primed T cells with specific antigen and that both Ts populations function in a haplotype-specific manner (5, 6), it was next determined whether the cloned Ts cells described above are antigen specific or nonspecific in their effector function. Cloned Ts cells were added to TNP-KLH– or TNP-FGG–primed responding B10 or B10.BR spleen cells and the cultures stimulated with 0.001 μg/ml TNP-KLH or TNP-FGG, respectively (Table V). Clone 8-4 suppressed the responses of B10 spleen cells to TNP-KLH but did not inhibit the responses of B10 cells to TNP-FGG or of B10.BR cells to either TNP-KLH or TNP-FGG. In contrast, clones 9-5 and 9-9 suppressed the responses of B10.BR spleen cells to TNP-KLH but inhibited neither the response of B10.BR spleen cells to TNP-FGG nor the responses of B10 spleen cells to TNP-KLH or TNP-FGG. In addition, these cloned cells failed to suppress responses to TNP-FGG even in the presence of 0.001 μg/ml KLH or TNP-KLH (Table V, experiment 2). It was demonstrated in parallel experiments that responses to TNP-FGG were in fact suppressible by Ts cells of appropriate specificity (reference 5 and data not shown). These results demonstrated that the cloned Ts cells described above function through a pathway that is both haplotype and antigen specific.

Suppressive Function of Cloned Ts Cells Is Not Due to Nonspecific Effects on B Cells,

TABLE V

Antigen-specific and MHC-restricted Suppression by Cloned T Cells

Expt.	Cloned Ts cells‡	Restriction	Anti-TNP IgG PFC/Culture*			
			TNP-KLH		TNP-FGG	
			B10	B10.BR	B10	B10.BR
1	Not added	—	3,590 ± 601	1,386 ± 75	1,073 ± 68	853 ± 107
	8-4	$H\text{-}2^b$	356 ± 53	1,372 ± 64	1,108 ± 69	985 ± 35
	9-5	$H\text{-}2^k$	4,012 ± 193	145 ± 10	871 ± 45	730 ± 83
	9-9	$H\text{-}2^k$	3,851 ± 171	66 ± 32	976 ± 146	756 ± 8

Expt.	Cloned Ts cells‡	Restriction	TNP-FGG		TNP-FGG + KLH		TNP-FGG + TNP-KLH	
			B10	B10.BR	B10	B10.BR	B10	B10.BR
2	Not added	—	915 ± 68	1,478 ± 107	853 ± 76	1,491 ± 53	862 ± 53	1,337 ± 288
	8-4	$H\text{-}2^b$	985 ± 83	ND	950 ± 114	ND	959 ± 38	ND
	9-5	$H\text{-}2^k$	ND	1,082 ± 40	ND	1,047 ± 101	ND	1,258 ± 123

* In experiment 1, 3×10^6 TNP-KLH- or TNP-FGG-primed B10 or B10.BR spleen cells were cultured in the presence or absence of cloned Ts cells. Cultures were stimulated with 0.001 μg/ml TNP-KLH or TNP-FGG, respectively. In experiment 2, 4×10^6 TNP-FGG-primed B10 or B10.BR spleen cells were cultured in the presence or absence of cloned Ts cells. Cultures were stimulated with 0.001 μg/ml TNP-FGG, 0.001 μg/ml TNP-FGG + 0.001 μg/ml KLH, or 0.001 μg/ml TNP-FGG + 0.001 μg/ml TNP-KLH. ND, not done.

‡ 1×10^4 cloned Ts cells were added to the cultures.

Accessory Cells, or Th Cells Expressing a Given MHC Haplotype. To determine what cell population is the target of these cloned Ts cells, further experiments were carried out. KLH-primed (B10 × B10.A)F_1 Th cells were co-cultured with TNP-primed B10, B10.A, or (B10 × B10.A)F_1 (B plus accessory) cells in the presence of 0.001 μg/ml TNP-KLH and in the presence or absence of (B10 × B10.A)F_1 accessory cells. Graded doses of cloned Ts cells were then added to these cultures and their suppressive activities measured (Table VI). Clone 8-4 suppressed the responses of B10 but not B10.A (B plus accessory) cells even in the presence of (B10 × B10.A)F_1 accessory cells (Table VI) that were independently shown to be functional (data not shown). In contrast, clones 9-5 and 9-9 suppressed the responses of B10.A but not B10 (B plus accessory) cells. Once again, addition of (B10 × B10.A)F_1 accessory cells did not overcome the failure of these cloned Ts cells to suppress the responses of B10 (B plus accessory) cells (Table VI). Although cloned Ts cells completely suppressed the responses of appropriate parental (B plus accessory) cells, no single population of parent-restricted cloned Ts cells completely suppressed the responses of (B10 × B10.A)F_1 (B plus accessory) cells. In addition, a mixture of 9-5 and 9-9 also failed to completely suppress the (B10 × B10.A)F_1 responses. In contrast, however, a mixture of 8-4 and 9-5, or of 8-4 and 9-9 cloned Ts cells completely inhibited the (B10 × B10.A)F_1 responses (Table VI). Thus, neither $H\text{-}2^k$-restricted Ts cells nor $H\text{-}2^b$-restricted Ts cells alone completely suppressed the responses of F_1 responding cells, while a mixture of $H\text{-}2^k$- and $H\text{-}2^b$-restricted Ts cells did suppress completely. These results suggested that the suppressive function of the cloned Ts cells characterized here is not due to nonspecific inhibitory effects on B cells, accessory cells, or Th cells expressing a given MHC haplotype. Rather these results suggested that cloned Ts cells function by exerting a specific effect on the MHC-restricted interactions of Th cells with (B plus accessory) cells.

Cloned Ts Cells Function by Specifically Inhibiting the MHC-restricted Interaction of

TABLE VI
MHC-restricted Ts Cell Function Is Not Overcome by Addition of F_1 Accessory Cells

Cloned Ts cells	Anti-TNP IgG PFC/Culture*				
	(B + accessory) cells				
	B10		B10.A		(B10 × B10.A)F_1
	Without F_1 acc cells	With F_1 acc cells	Without F_1 acc cells	With F_1 acc cells	Without F_1 acc cells
Not added	2,235 ± 221	2,499 ± 280	1,336 ± 63	1,548 ± 197	1,575 ± 70
8-4 (3 × 10^3)	413 ± 98	ND	1,531 ± 212	1,795 ± 80	1,372 ± 295
(10 × 10^3)	35 ± 35	ND	1,390 ± 236	1,302 ± 215	545 ± 91
9-5 (3 × 10^3)	2,244 ± 279	2,560 ± 423	123 ± 46	ND	1,152 ± 53
(10 × 10^3)	2,182 ± 312	2,103 ± 122		ND	668 ± 92
9-9 (3 × 10^3)	2,261 ± 280	2,666 ± 250	422 ± 52	ND	1,469 ± 242
(10 × 10^3)	2,050 ± 167	2,340 ± 48	35 ± 35	ND	862 ± 146
8-4 (10 × 10^3) + 9-5 (10 × 10^3)	ND	ND	ND	ND	17 ± 17
8-4 (10 × 10^3) + 9-9 (10 × 10^3)	ND	ND	ND	ND	88 ± 48
9-5 (10 × 10^3) + 9-9 (10 × 10^3)	ND	ND	ND	ND	528 ± 80

* 1 × 10^6 KLH-primed (B10 × B10.A)F_1 Th cells were co-cultured with 3 × 10^6 TNP-primed (B + accessory) cells of the indicated strain in the presence or absence of 1 × 10^6 (B10 × B10.A)F_1 accessory (acc) cells. Cultures were stimulated with 0.001 μg/ml TNP-KLH. ND, not done.

Th Cells with (B Plus Accessory) Cells. To further characterize the mechanism of action of the haplotype-restricted and antigen-specific cloned Ts cells described in this report, experiments were carried out using Th cells from chimeric mice. Clones 8-4 and 9-5 were tested for their suppressive activities on the responses of either KLH-primed (B10 × B10.A)F_1 → B10 Th cells or KLH-primed (B10 × B10.A)F_1 → B10.A Th cells and TNP-primed (B10 × B10.A)F_1 (B plus accessory) cells (Table VII, experiment 1). (B10 × B10.A)F_1 → B10 Th cells and (B10 × B10.A)F_1 → B10.A Th cells were MHC restricted in their function, since these Th cells helped host haplotype (B plus accessory) cells but did not provide help to non-host type (B plus accessory) cells (data not shown). The responses generated by (B10 × B10.A)F_1 → B10 Th cells and (B10 × B10.A)F_1 (B plus accessory) cells were specifically suppressed by H-2^b-restricted clone 8-4 Ts cells but not by H-2^k-restricted clone 9-5 Ts cells (Table VII, experiment 1). In contrast, the responses generated by (B10 × B10.A)F_1 → B10.A Th cells and (B10 × B10.A)F_1 (B plus accessory) cells were specifically suppressed by H-2^k-restricted clone 9-5 Ts cells but not by H-2^b-restricted clone 8-4 Ts cells. Additional experiments using (B10 × B10.BR)F_1 (B plus accessory) cells and (B10 × B10.BR)F_1 → parent Th cells demonstrated the same restriction pattern (Table VII, experiment 2). Thus, when chimeric Th cells were restricted to recognizing parent_A but not parent_B determinants on (A × B)F_1 (B plus accessory) cells, the responses generated by these populations were suppressed by H-2^a-restricted but not by H-2^b-restricted cloned Ts cells. These results indicated that these cloned Ts cells do not act to nonspecifically inhibit the function of Th cells,

TABLE VII

MHC-restricted Interaction of Th Cells with (B + Accessory) Cells Is Suppressed by Cloned Ts Cells

Cloned Ts cells‡	Anti-TNP IgG PFC/Culture*		
	Experiment 1		Experiment 2
	$F_1 \rightarrow$ B10 Th	$F_1 \rightarrow$ B10.A Th	$F_1 \rightarrow$ B10 Th
Not added	1,909 ± 172	1,883 ± 375	1,346 ± 388
8-4	387 ± 43	2,393 ± 188	475 ± 85
9-5	1,716 ± 52	466 ± 68	1,240 ± 265

* Experiment 1: 1×10^6 KLH-primed (B10 × B10.A)$F_1 \rightarrow$ B10 Th or (B10 × B10.A)$F_1 \rightarrow$ B10.A Th cells were co-cultured with 3×10^6 TNP-primed (B10 × B10.A)F_1 (B + accessory) cells. Experiment 2: 1×10^6 KLH-primed (B10 × B10.BR)$F_1 \rightarrow$ B10 Th cells were co-cultured with 3×10^6 TNP-primed (B10 × B10.BR)F_1 (B + accessory) cells. Cultures were stimulated with 0.001 μg/ml TNP-KLH.

‡ 3×10^3 (experiment 1) or 1×10^4 (experiment 2) cloned Ts cells were added to the cultures.

TABLE VIII

Cloned Ts Cells Act as Suppressor Effectors to Inhibit the Responses Mediated by Cloned Th Cells

Cloned Ts cells‡	Anti-TNP IgG PFC/Culture*	
	B10 (B + acc) cells	B10.BR (B + acc) cells
	Th clone 8-5	Th clone 9-16
Not added	844 ± 64	1,108 ± 85
8-4	0	1,056 ± 128
9-5	932 ± 38	0
9-9	836 ± 61	0

* 1×10^4 cloned Th cells were co-cultured with 3×10^6 TNP-primed B10 or B10.BR (B + accessory [acc]) cells that were treated with ATS in vivo and then were treated with RAMB + C, anti-Thy-1.2 + anti-Lyt-1.2 + anti-Lyt-2.2 + C in vitro. Clone 8-5 is specific for *H-2^b^* + KLH and clone 9-16 is specific for *H-2^k^* + KLH. Cultures were stimulated with 0.001 μg/ml TNP-KLH.

‡ 1×10^6 cloned Ts cells were added to the cultures.

B cells, or accessory cells that genotypically express a given MHC phenotype. Rather these antigen-specific and MHC-restricted Ts cells act to inhibit in a highly specific manner the MHC-restricted interaction between Th cells and (B plus accessory) cells.

Cloned Ts Cells Act as Suppressor Effectors to Inhibit the Responses Mediated by Cloned Th Cells. Next, it was determined whether the cloned Ts cells characterized in this report can act as effector cells or function only through the induction of suppressors in other T cells present in cultures (1, 12, 13). To minimize the possibility that other T cells were participating in suppression, cloned Th cells were used as a source of help, and (B plus accessory) populations were extensively depleted of T cells. (B plus accessory) cells were prepared from TNP-primed spleen cells by pretreating in vivo with ATS followed by sequential in vitro treatment with RAMB plus C, and with anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2.2 plus C. TNP-primed B10 or B10.BR (B plus accessory) cells were cultured with KLH-specific Th clone 8-5 ($H\text{-}2^b$ restricted) or Th clone 9-16 ($H\text{-}2^k$ restricted), respectively. 1×10^4 cloned Ts cells were then added to these cultures (Table VIII). Responses of B10 (B plus accessory) cells mediated by Th clone 8-

5 were suppressed by $H\text{-}2^b$-restricted Ts clone 8-4 but not by $H\text{-}2^k$-restricted Ts clone 9-5 or 9-9. In contrast, responses of B10.BR (B plus accessory) cells mediated by Th clone 9-16 were suppressed by $H\text{-}2^k$-restricted Ts clone 9-5 or 9-9 but not by $H\text{-}2^b$-restricted Ts clone 8-4 (Table VIII). These results demonstrate that these cloned Ts cells are able to function as effector cells in the absence of any apparent source of additional Ts populations.

Discussion

In the present studies, nonhelper as well as helper clones were identified among antigen-specific and MHC-restricted proliferating T cell populations that had been derived by limiting dilution. It was then demonstrated that certain of the nonhelper T cell clones functioned as active Ts cells in suppressing the in vitro antibody responses of conventional carrier-primed Th cells and hapten-primed B cells. These Ts clones were Lyt-1^+2^- in phenotype and were MHC restricted and antigen specific in their effector function. Thus, these cloned Ts cells suppressed the responses mediated by MHC-restricted Th cells only when Th cells and cloned Ts cells shared the same antigen specificity and MHC restriction. In experiments using cloned Th and cloned Ts populations, it was demonstrated that these Lyt-1^+2^- cloned Ts cells function not simply as inducers, but rather as effectors of suppression.

The precise relationship between the cloned Ts cells characterized here and the recently characterized heterogeneous populations of MHC-restricted Ts cells (6) is uncertain. Immunofluorescence studies of the antigen-specific clones described here revealed that they were Thy-1^+ and Lyt-1^+2^-. In addition, it appears that these cloned Ts cells are able to function as final effector cells since they efficiently suppress responses mediated by cloned Th cells without requiring the participation of any additional T cell populations. In previous studies (5), however, it was found that heterogeneous antigen-primed Lyt-1^+2^- T cells mediated antigen-nonspecific suppression through a mechanism requiring unprimed Lyt-1^-2^+ T cells, whereas antigen-primed Lyt-1^-2^+ T cells mediated antigen-specific suppression without any apparent requirement for additional T cells. It is therefore not possible to identify any concordance in functional specificity and Lyt phenotype between the Ts clones derived and characterized here and the heterogeneous Ts cell populations previously characterized (5, 6). The possibility remains that cells corresponding to the cloned Ts cells reported here are present in active heterogeneous Ts populations at undetectably low frequency and were selected by the cloning procedures used. Alternatively, the process of repeated stimulation and cloning may have led to the generation of a differentiated Ts cell phenotype distinct from that found in conventionally primed T cell populations.

Both the helper and suppressor T cell clones characterized in the present report express a Lyt-1^+2^- phenotype. Previous studies of conventional T cell populations have similarly identified that Th cells are Lyt-1^+2^-; and recent studies have demonstrated that Lyt-1^+2^- T cells can also function as the inducers of active suppressor effector cells (1, 12, 13). For these heterogeneous populations, Th cells have been distinguished from suppressor inducers on the basis of Qa-1 phenotype (Qa-1^- Th cells and Qa-1^+ Ts inducers) (1). The Th and Ts clones characterized here have not yet been analyzed with respect to additional

cell surface markers such as Qa-1 or Ia. It should be noted that the Lyt-1^+2^- Ts clones used in the present studies appear to be capable of functioning as suppressor effector populations, and not simply as inducers, and that these clones thus express a Lyt phenotype not generally associated with the suppressor effector activity that they demonstrate (1–3, 12, 14, 15). The finding that these cloned Ts cells have the same Lyt phenotype as cloned Th cells raises the specific possibility that these Ts cells are of the same lineage as Th cells and represent cells that have lost their helper function during in vitro culture. However, the finding that only a subset of nonhelper T cell clones functions as Ts cells suggests that this may not be the case. Moreover, both Th and Ts clones have been generated relatively early during in vitro culture, and have been maintained for prolonged periods as stable functional phenotypes. The requirement shown in the present studies that Th and Ts cells recognize determinants on the same antigen also appears to distinguish the Ts cell function characterized here from that of the cloned Lyt-1^+2^- Ts population recently described by Bottomly et al. (16).

In a previous report (6), it was speculated that the mode of action of heterogenous MHC-restricted Ts cell populations on heterogeneous Th cells might involve (*a*) Ts cell recognition of both the specific antigen and the appropriate MHC product (e.g., Ia) bound to the appropriate clonally expressed Th cell receptors, leading to the functional inactivation of only those Th cells expressing specificity for the appropriate antigen plus Ia; (*b*) Ts cell recognition of antigen presented by the Th cell receptor and the B cell Ia products that were being presented to the same Th cell; or (*c*) Ts cell recognition of and binding to antigen and Ia products on B cells, thus directly competing with Th cells of the same specificity. These alternatives were each constructed to account for the apparent requirement that active Ts cells be matched in both MHC restriction and antigen specificity with the Th cells that function in a given response. However, the finding that monoclonal Ts cell populations can completely inhibit responses involving heterogeneous Th cells adds a new level of complexity to such speculation. It might be expected that heterogeneous Th cell populations specific for an antigen such as KLH would be heterogeneous in their MHC-restriction specificities, as well as in their antigen fine specificities. In particular, *I-A*-restricted, as well as *I-E*-restricted, KLH-specific Th cells might be present in such populations (17). Since any given KLH-specific Ts clone might be restricted to either *I-A* or *I-E*, but not both, it might therefore have been anticipated that no single *H-2*^{k}-restricted Ts clone would suppress the activity of both *I-A*^{k}-restricted and *I-E*^{k}-restricted Th cells. However, it was observed that H-2^k-restricted Ts clones, while unable to suppress the activity of *H-2*^{b}-restricted Th cells, completely suppressed the activity of *H-2*^{k}-restricted Th cells. It is of course possible that the "heterogeneous" KLH-specific Th cells generated in these studies are overwhelmingly *I-A*-restricted (18), and that the Ts clones used are similarly restricted. Alternatively, these Ts clones may recognize cross-reactive determinants on *I-A* and *I-E* products. Studies are currently in progress to subregion map the MHC restrictions of these Ts clones and to further evaluate the effects of these cells on the function of cloned Th cells with known MHC restrictions. Such studies may clarify the mechanism that mediates MHC restriction in Ts cell function.

The present studies have identified cloned Lyt-1^+2^- Ts cells with suppressor effector function that is both antigen specific and MHC restricted. These cloned Ts cells appear to act by specifically inhibiting the MHC-restricted interaction between Th cells and the B and/or accessory cells with which they interact. Further studies using monoclonal Th and Ts populations may provide additional insight into the mechanism of these regulatory events.

Summary

The present studies have identified cloned Lyt-1^+2^- T suppressor (Ts) cells that are both antigen specific and major histocompatibility complex (MHC) restricted in their activation requirements and that function to regulate the MHC-restricted activation of B cells by T helper (Th) cells. Parent$_A$-restricted Ts clones suppressed, in antigen-specific fashion, the responses generated by (A × B)F_1 Th cells cooperating with parent$_A$ (B plus accessory) cells, but did not suppress responses by the same (A × B)F_1 Th cell population cooperating with parent$_B$ (B plus accessory) cells. Moreover, responses of (A × B)F_1 → parent$_A$ Th cells and (A × B)F_1 (B plus accessory) cells were suppressed by parent$_A$-restricted Ts clones but not by parent$_B$-restricted Ts clones. Thus, these findings suggest that the cloned Ts cells that have been characterized here function by specifically inhibiting the MHC-restricted interaction between Th cells and B and/or accessory cells. It was further demonstrated in experiments using cloned Th and Ts populations that these Lyt-1^+2^- Ts cells act not simply as inducers of suppression but rather function in a restricted fashion as effector cells in the suppressor pathway.

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