

A MECHANISM RESPONSIBLE FOR THE INDUCTION OF H-2 RESTRICTED SECOND ORDER SUPPRESSOR T CELLS*

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A specialized antigen-presenting cell (APC)¹ is required for the induction of at least one subset of suppressor T cells (1-3). Thus, we and others have recently provided evidence that such specialized APC are required for the induction of third order (Ts₃) suppressor cells (1, 2). One feature that distinguishes this APC from the cell type that induces conventional helper T cell responses is the expression of I-J-encoded gene products on the APC population involved in suppression (1, 3). These I-J products are critical in imposing the subsequent H-2 (I-J) restrictions on the Ts₃ population (1).

This report describes the nature of the cells involved in the induction of a different suppressor T cell subset, second-order suppressor (Ts₂) cells. In the 4-hydroxy-3-nitrophenyl acetyl (NP) hapten system, Ts₂ cells are generated *in vivo* by administration of soluble factors (TsF₁) from inducer (Ts₁) suppressor cells to nonimmune recipients (4-7). In addition, Ts₂ cells can be generated *in vitro* by incubation of TsF₁ with nonimmune spleen cells (8). TsF₁ bears Igh-V-related idiotypic determinants that appear to be cross-reactive, but not identical with the predominant B cell idiotypes (9, 10). Since Ts₂ cells bind Igh-V related idiotypic determinants, it is likely that the specificity of the Ts₂ cells is at least in part controlled by the idio-type-related determinants present on the TsF₁. Once Ts₂ cells are generated, their ability to suppress is genetically restricted by genes encoded in the H-2 and Igh complexes (5, 6). The Igh genetic restrictions of Ts₂ cells are determined by the Igh genotype of the TsF₁ donor, suggesting that the Ts₂ cells are specific for the Igh-controlled idio-type-related antigen-binding determinants present on the TsF₁, not those expressed by the host (7). In contrast, the H-2 (I-J) restrictions of Ts₂ cells are determined by the genotype of the host, not that of the TsF₁ donor (7). Many of these observations have been independently confirmed in a different suppressor cell system in which strain-restricted idiotypic determinants can be identified (11). In view of these observations it was hypothesized that the H-2 restrictions imposed on Ts₂ cells may result from a process in which host cells present TsF₁. The major histocompatibility complex (MHC) determinants on the factor-presenting cell could then impose the MHC restrictions on the Ts₂ population. The present report presents data that supports this

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¹ *Abbreviations used in this paper:* APC, antigen-presenting cells; B6, C57BL/6 mice; CS, contact sensitivity; DMSO, dimethylsulfoxide; DNFB, dinitrofluorobenzene; FPC, factor-presenting cell; HBSS, Hanks' balanced salt solution; MHC, major histocompatibility complex; NP, 4-hydroxy-3-nitrophenyl acetyl hapten; NP-O-Su, NP-O-succinimide ester; PBS, phosphate-buffered saline; Ts₁, Ts₂, and Ts₃, first-, second-, and third-order suppressor T cells; TsF₁, TsF₂, and TsF₃, suppressor factors derived from Ts₁, Ts₂, and Ts₃ cells.

hypothesis and generalizes the notion that specialized presenting cells are required to induce MHC restrictions on suppressor T cell populations.

Materials and Methods

Mice. All mice were either purchased from The Jackson Laboratory, Bar Harbor, ME, or were bred in the animal facilities at Harvard Medical School, Boston, MA. Mice were used at 3–12 mo of age and were maintained on laboratory chow and acidified, chlorinated water ad lib.

Animals used in this study were maintained 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 (Dept. of Health, Education, and Welfare publication (National Institutes of Health) 78-23, revised 1978).

Antigens. NP-O-Succinimide (NP-O-Su) was purchased from Biosearch Co., San Rafael, CA. Dimethylsulfoxide (DMSO) was purchased from Fisher Scientific Co., Pittsburgh, PA. 2,4-Dinitro-1-fluorobenzene (DNFB) was obtained from Eastman Kodak Co., Rochester, NY.

Cell Preparation. Spleen cell suspensions were made in Hanks' balanced salt solution (HBSS), and the erythrocytes were lysed with 0.83% Tris ammonium chloride. The spleen cells were washed and then used for further separation or for pulsing with TsF₁.

Macrophage-enriched, glass-adherent spleen cells were purified by a 4-h adherence to glass petri dishes, followed by removal with EDTA, as previously described (12). Macrophage-depleted T and B spleen lymphocytes were prepared by passing $1-2 \times 10^8$ splenic leukocytes over a 25-ml column of Sephadex G-10 in a 35-ml syringe barrel and collecting the first 15 ml of eluate as previously described (13). Cells prepared by glass adherence and by filtration through Sephadex G-10 have been extensively characterized in previous reports (13). Briefly, 4-h glass-adherent cells contained 40–70% phagocytic cells; the nonphagocytic cells were comprised of equal numbers of Thy-1⁺, sIg⁺, and Thy-1⁻, sIg⁻ cells. Unfractionated spleen cells were 4–8% phagocytic and G-10 nonadherent cells were 0.1–0.8% phagocytic, while retaining the same T cell to B cell ratios as the input cells. Phagocytosis was assessed by latex ingestion, as previously described (12).

In Vitro Pulsing of Splenic Cells with TsF₁. Generally, ascites containing CKB-Ts₁-39 hybridoma-derived TsF₁ was used for pulsing. In one experiment CKB-Ts₁-17 hybridoma-derived factor was substituted. To produce ascites, the CKB-Ts₁ and the BW5147 cells were grown in (AKR × CKK)F₁ or AKR mice. Spleen cells from normal mice were used as a source of cells for TsF₁ pulsing. $2-4 \times 10^8$ splenic cells were cultured for 2 h in 10 ml RMPI 1640 with 10% fetal calf serum and 0.1 mM Hepes plus 50 μl ascitic fluid. After culture the pulsed splenic cells were washed vigorously with HBSS and resuspended.

Assay for Suppression of Contact Sensitivity (CS). To induce CS responses, mice were primed with NP-O-Su (2 g/100 ml) in DMSO (14). A total of 0.1 ml of antigen was divided equally between two sites on each ventral flank, followed by 0.1 ml of borate-buffered saline at pH 8.6 injected in the midline. In the experiments that did not involve adoptive transfers of Ts₂ cells, the mice received i.v. $3-4 \times 10^7$ syngeneic splenic cells pulsed with TsF₁ or BW5147-derived control factor 1 h before priming with NP-O-Su. 6 d later, the mice were challenged in the left footpad with 0.025 ml PBS containing 30 μg NP-O-Su. Footpad swelling was measured 24 h after challenge. Swelling was determined as the difference, in units of 10^{-3} cm, between the left and right footpad thickness.

Adoptive Transfer System for Assaying Ts₂ Activity. Ts₂ donor mice received $2-4 \times 10^7$ i.v. splenic cells pulsed with TsF₁ or BW5147-derived control factor, 6 d later, the mice were sacrificed and their spleens teased into single cell suspensions that were used as a source of Ts₂ cells. Mice primed 5 d previously with 2 mg NP-O-Su received 4×10^7 i.v. splenic cells from Ts₂ or control donors. Immediately after transfer of control or Ts₂ cells the mice were challenged with NP-O-Su and CS responses were measured 24 h thereafter.

DNFB Contact Sensitivity Responses. Contact sensitivity was induced by two daily paintings on the shaved abdomen with 25 μl of 0.5% DNFB solution (Eastman Kodak Co., Rochester, NY) in acetone/olive oil (4:1). 6 d after the last painting, 20 μl of 0.2% DNFB in the same vehicle was applied to the left ear, and the ear swelling was measured as the difference been

the left and right ear thickness.

Antisera. Both B10.A (3R) anti-B10.A (5R) (anti-I-J^k) and B10.A (5R) anti-B10.A (3R) (anti-I-J^b) alloantisera were produced by immunization with spleen cells (7). Anti-Thy 1.2 and rabbit anti-mouse brain were prepared as described elsewhere (6).

To treat spleen cells with anti-I-J alloantisera, $8-10 \times 10^7$ spleen cells were pelleted and incubated in 1.0 ml of 1:5 dilution of anti-I-J^k, anti-I-J^b, or normal mouse serum (NMS). After 30 min at room temperature, the cells were centrifuged and resuspended in 1.0 ml of selected rabbit complement diluted 1:5 in HBSS. After an additional 30 min, 37°C incubation, the cells were washed and then pulsed with TsF₁ as described above.

Percent Suppression. The percent suppression in the present study was calculated by the following formula: percent suppression = $100 \times [(\text{swelling of BW5147 tumor supernatant injected group} - \text{swelling of TsF injected group}) / (\text{swelling of BW5147 tumor supernatant injected group} - \text{swelling of unprimed group})]$.

Data Analysis. Statistical analysis of the experimental data with respect to controls was calculated using the two-tailed Student's *t*-test.

Results

TsF₁-Pulsed Cells Induce a Ts₂ Population. The initial series of experiments evaluated whether TsF₁ factor that was pulsed onto spleen cells still retained its ability to induce antigen-specific suppression of NP-O-Su-induced CS responses. To pulse spleen cells with TsF₁, $2-4 \times 10^8$ C57BL/6 spleen cells were cultured for 2 h in media containing hybridoma-derived ascitic fluid. After this 2-h incubation the cells were washed extensively to remove traces of unbound TsF₁. As a control, ascitic fluid derived from the BW5147 tumor was used to pulse spleen cells. Control cells or cells pulsed with TsF₁ derived from CKB-Ts₁-39 hybridoma cells were given intravenously to syngeneic recipients on the day of antigen priming. Additional control groups received the soluble factors directly. In confirmation of previous data, direct intravenous administration of TsF₁ caused significant reduction of the magnitude of the NP-O-Su-induced CS response, but did not affect the CS response to an irrelevant antigen, DNFB in the same mice (Table I). In addition TsF₁ that had been pulsed onto spleen cells also induced significant levels of NP specific nonresponsiveness.

One possible interpretation of these results is that the TsF₁ disassociated from the pulsed cell population and was reprocessed by host cells. To evaluate this possibility, soluble or pulsed TsF₁ was injected intravenously into (B6 × C3H)F₁ (H-2^b/H-2^k)

TABLE I
*TsF₁-Pulsed Spleen Cells Induce Antigen-specific Suppression**

TsF source	Cells used for TsF ₁ pulsing	NP-O-Su and DNFB priming	Swelling response + SE‡	
			NP-O-Su	DNFB
BW5147	None	+	33.5 ± 1.9 (8)	12.5 ± 1.6
CKB-Ts ₁ -39	None	+	17.1 ± 1.5 (7)§	13.7 ± 2.7
BW5147	Spleen	+	31.6 ± 2.0 (9)	10.7 ± 1.1
CKB-Ts ₁ -39	Spleen	+	19.4 ± 1.1 (8)§	12.7 ± 1.1
None	None	-	9.4 ± 1.9 (8)	0.9 ± 0.5

* Groups of C57BL/6 mice were given soluble or pulsed TsF₁ and control BW5147-derived factors 1 h before double priming with NP-O-Su and DNFB. After 5 d the animals were challenged in one footpad with NP-O-Su and on one ear with DNFB.

‡ The swelling responses were recorded 24 h after challenge. The results of two experiments were pooled. The number of mice tested is indicated in parentheses. Significant suppression ($P < 0.001$) is indicated by §.

mice to generate Ts₂ cells. 6 d after administration of TsF₁ these F₁ donors were sacrificed and their spleen cells, which contained the Ts₂ population, were adoptively transferred to NP-primed C57BL/6 (H-2^b), B10.BR (H-2^k), or B10.RIII (H-2^r) recipients. 1 h after adoptive transfer of Ts₂ cells the recipients were challenged with NP-O-Su. Under these conditions we could observe the H-2-restricted suppressive activity mediated by Ts₂ cells. (B6 × C3H)F₁-derived Ts₂ cells generated by intravenous administration of soluble CKB-derived TsF₁ would suppress NP-O-Su-induced CS responses of C57BL/6 or B10.BR mice that share one of the Ts₂ donor H-2 haplotypes, while the same population of cells failed to suppress H-2 incompatible B10.RIII congenic mice (Table II). The failure to suppress H-2 mismatched B10.RIII recipients was probably not due to the rejection of the Ts₂ cell population since the cells only remained in these recipients for 24 h before termination of the assay. Furthermore, the same population of F₁ cells suppressed the CS responses of either C57BL/6 or B10.BR recipients that could also recognize allogeneic H-2 determinants on the F₁ cells. These results verify the H-2 restrictions of Ts₂ cells and demonstrate that Ts₂ cells derived from F₁ mice have the potential of suppressing recipients of either parental H-2 haplotype. When Ts₂ cells were induced by injecting hybridoma-derived TsF₁ pulsed onto (B6 × C3H)F₁ (H-2^b/H-2^k) spleen cells the resulting Ts₂ population also suppressed H-2 haploidentical C57BL/6 and B10.BR recipients (Table II). The critical observation that suggested that the H-2 phenotype of the TsF₁-presenting cells controlled the H-2 restrictions of the F₁-derived Ts₂ cells, was the specificity of the F₁-derived Ts₂ population that was induced by TsF₁ pulsed onto

TABLE II
*H-2 Genotype of TsF₁-Pulsed Spleen Cells Controls Ts₂ Cell Restrictions**

Ts ₂ Induction in F ₁ mice		Recipient of F ₁ - derived Ts ₂ cells	Normalized per- cent suppression ± SE‡
TsF source	Cells for TsF ₁ pulsing		
BW5147	None	C57BL/6	0 ± 6 (16)
CKB-Ts ₁ -39	None	(H-2 ^b)	77 ± 19 (9)*
	(B6 × C3H)F ₁		61 ± 6 (13)*
	C57BL/6		58 ± 3 (14)*
	B10.BR		18 ± 5 (15)
BW5147	None	B10.BR	0 ± 4 (13)
CKB-Ts ₁ -39	None	(H-2 ^k)	80 ± 9 (10)*
	(B6 × C3H)F ₁		50 ± 6 (10)*
	C57BL/6		1 ± 9 (10)
	B10.BR		63 ± 6 (10)*
BW5147	None	B10.RIII	0 ± 13 (6)
CKB-Ts ₁ -39	None	(H-2 ^r)	8 ± 10 (11)

* Ts₂ cells were generated in H-2^b × H-2^k heterozygous mice by administration of either soluble TsF₁ or by intravenous injection of TsF₁-pulsed spleen cells. After 5 d, the F₁ spleen cells were adoptively transferred to previously NP-O-Su-primed C57BL/6 or B10.BR recipients. The recipients were challenged with NP-O-Su 1 h after transfer of Ts₂ cells.

‡ The results of four independent experiments were normalized and pooled using the response with BW5147 or BW5147-pulsed cells (data not shown) as the control. The control responses ranged from 28 to 43 × 10³ mm with backgrounds in unprimed control groups yielding <10 × 10³ U of swelling. The number of mice tested is indicated in parentheses. Significant suppression (*P* < 0.01) is indicated by §.

parental C57BL/6 (H-2^b) spleen cells. The data demonstrated that the latter Ts₂ cells only functioned when transferred to C57BL/6 recipients (Table II). The same population of (B6 × C3H)F₁Ts₂ cells failed to suppress B10.BR (H-2^k) recipients. In reciprocal experiments (B6 × C3H)F₁-derived Ts₂ cells, which were induced by intravenous administration of TsF₁ pulsed onto B10.BR spleen cells, functioned in B10.BR but not C57BL/6 recipients (Table II). These experiments have been repeated on three separate occasions using various combinations of (H-2^b × H-2^k)F₁ mice; in each experiment we obtained similar results. Thus, the H-2 restrictions of the F₁-derived Ts₂ cells were controlled by the genotype of the cells presenting TsF₁, not by the genotype of the cells that produced TsF₁.

The I-J Genotype of the TsF₁-Presenting Cells Determines the Genetic Restrictions of the Ts₂ Population. We have previously demonstrated that the activity of Ts₂ cells or factors are restricted by genes in the I-J region of the H-2 complex (5, 6). To determine whether the I-J region encoded determinants were also involved in the induction of Ts₂ restrictions, we compared the abilities of I-J congenic 3R and 5R cells to present TsF₁. Ts₂ cells were generated in B6AF₁ (H-2^b × H-2^a) mice by injection with CKB-derived TsF₁ pulsed onto either B6AF₁, 3R, or 5R spleen cells. After 6 d spleen cells from these B6AF₁ donors were adoptively transferred to NP-O-Su-primed C57BL/6 or B10.BR recipients. In agreement with the data in Table II, Ts₂ cells induced by administration of TsF₁ pulsed onto B6AF₁ spleen cells could suppress either C57BL/6 or B10.BR recipients (Table III). Although the B10.BR recipients differed at the I-C, S, and D regions of the H-2 complex, with the B6AF₁ donors suppression was still observed. This result is consistent with those obtained elsewhere, which demonstrate that homology at the I-J region between the Ts₂ donor and the recipient is sufficient for transfer of suppressive activity (5, 6). When 3R (I-J^b) cells were pulsed with TsF₁ and used to induce suppressor cells in B6AF₁ mice the resulting TsF₂ population only functioned when transferred to C57BL/6 (I-J^b) recipients (Table III). There was no detectable level of suppression when the B6AF₁-derived Ts₂ population was transferred to B10.BR (I-J^k) recipients. In reciprocal experiments the B6AF₁ Ts₂ donors were injected with TsF₁ pulsed onto 5R cells. Under these conditions the activation of B6AF₁ Ts₂ cells was restricted to B10.BR not C57BL/6 recipients (Table III). Thus,

TABLE III
*The I-J Region of the Antigen-presenting Cells Determines the H-2
Restrictions of Ts₂ Cells**

Ts ₂ Induction in F ₁ mice		Recipient of F ₁ - derived Ts ₂ cells	Normalized per- cent suppression ± SE
TsF source	Cells for TsF ₁ pulsing		
BW5147 CKB-Ts ₁ -39	(B6A)F ₁	C57BL/6	0 ± 4 (16)
	(B6A)F ₁		70 ± 5 (15)*
	3R		50 ± 6 (16)*
	5R		17 ± 9 (15)
BW5147 CKB-Ts ₁ -39	(B6A)F ₁	B10.BR	0 ± 4 (12)
	(B6A)F ₁		63 ± 11 (11)*
	3R		3 ± 9 (15)
	5R		57 ± 6 (13)*

* Ts₂ cells were generated in (B6A)F₁ mice; refer to legend of Table II for additional details.

the I-J phenotype of the cells used to present TsF₁ determines the genetic restrictions of the Ts₂ population.

Phenotype of the TsF₁-Presenting Cells. To directly demonstrate the presence of I-J gene products on the TsF₁-presenting cells, treatment of the TsF₁-presenting cells with anti-I-J alloantisera plus complement was used to lyse the critical cell population. In a series of seven experiments, we observed some evidence of specific lysis on four occasions (data not shown). It is not clear why the remaining experiments failed, but we assume that technical factors (antibody titer, complement source, etc.) prevented more consistent success.

Finally, we examined the adherence properties of the cells involved in Ts₂ induction. Spleen cells were separated on G10 or by adherence for 4 h on glass petri dishes. The unfractionated, nonadherent, and adherent fractions were pulsed with TsF₁ and injected in graded doses into syngeneic mice immediately before antigen priming (Table IV). The minimum number of TsF₁ pulsed unfractionated spleen cells required to cause suppression varied among experiments; generally $2.5\text{--}10 \times 10^6$ cells were sufficient, while $<2.5 \times 10^6$ cells consistently failed to induce significant levels of suppressive activity (data not shown). Administration of $2\text{--}4 \times 10^7$ nonadherent splenic cells pulsed with TsF₁ could not induce suppression while as few as $2.5\text{--}5 \times 10^5$ splenic adherent cells were capable of presenting TsF₁ (Table IV). Thus, an adherent cell population is required to induce Ts₂ cells.

Discussion

Several groups have demonstrated that in vivo administration of TsF could induce a population of second-order suppressor T cells (4, 9, 11, 15, 16). However, the mechanism of Ts₂ induction had not been well characterized. The present report analyzed the process by which TsF₁ induced Ts₂ cells. Previous experiments have demonstrated that the H-2 restriction of the Ts₂ population was controlled by the host H-2 genotype, not that of the TsF₁ donor (7, 11, 17). The present experiments

TABLE IV
*Splenic Adherent Cells Present TsF₁**

Spleen cells for TsF ₁ pulsing	TsF source	Normalized percent suppression \pm SE \ddagger
4×10^7 unfractionated	BW5147	0 ± 8 (13)
4×10^7 unfractionated	CKB-Ts ₁ -39	35 ± 14 (13)§
1×10^7 unfractionated	CKB-Ts ₁ -39	41 ± 8 (10)§
2.5×10^6 unfractionated	CKB-Ts ₁ -39	32 ± 9 (11)§
$2\text{--}4 \times 10^7$ G10 nonadherent	BW5147	0 ± 6 (7)
$1\text{--}4 \times 10^7$ G10 nonadherent	CKB-Ts ₁ -39	12 ± 8 (11)
2.5×10^6 G10 nonadherent	CKB-Ts ₁ -39	4 ± 7 (7)
$1\text{--}2.5 \times 10^6$ adherent	BW5147	0 ± 6 (13)
$1\text{--}2.5 \times 10^6$ adherent	CKB-Ts ₁ -39	46 ± 4 (12)§
$2.5\text{--}5 \times 10^6$ adherent	CKB-Ts ₁ -39	33 ± 7 (11)§

* Groups of C57BL/6 mice were given TsF₁ pulsed onto graded phases of either unfractionated, nonadherent, or adherent syngeneic spleen cells, intravenously. Following administration of TsF₁-pulsed cells the mice were primed with NP-O-Su.

‡ The results of three experiments were normalized and pooled; refer to legend of Table II.

§ Significant suppression.

evaluated how such restrictions were imposed. The data indicate that TsF₁ is presented by a specialized population of I-J-bearing, factor-presenting cells present in the host. Thus, when Ts₂ cells are generated with either soluble TsF₁ or with TsF₁ pulsed onto syngeneic F₁ spleen cells, the Ts₂ population acquires a restriction for host H-2 determinants. However, when the Ts₂ population is induced in F₁ mice by administration of TsF₁ pulsed onto parental spleen cells, the Ts₂ cells can only suppress recipients that share an H-2 haplotype with the cells used to present TsF₁. These experiments suggest that the TsF₁ is directly presented on the splenic cell population and is not reprocessed or represented by host cells. Secondly, the data demonstrate that the H-2 genotype of the TsF₁ source does not influence the genetic restrictions. Thus, TsF₁ derived from H-2^k-bearing CKB mice could induce populations of Ts₂ cells whose activity was restricted to mice bearing the H-2^b haplotype. In addition, the data demonstrate that H-2 heterozygous F₁ hybrid mice have the potential of generating two distinct populations of Ts₂ cells. Thus, as shown in Tables II and III, when soluble TsF₁ or Ts₁-pulsed F₁-presenting cells are used to induce Ts₂ cells in H-2 heterozygous F₁ mice, both types of Ts₂ populations are generated, but only one Ts₂ population is generated when TsF₁ is pulsed onto parental TsF₁-presenting cells.

Additional characterization of these TsF₁-presenting cells demonstrated that they lack Thy-1 antigenic determinants (data not shown), and are adherent to glass (Table IV). Thus, these specialized factor-presenting cells are probably of a macrophage or dendritic cell lineage.

Further experiments demonstrated that genes of the I-J subregion expressed on the TsF₁-presenting cells controlled the H-2 restrictions of the Ts₂ population. Thus, TsF₁-presenting cells from I-J congenic 3R and 5R mice induced distinct populations of Ts₂ cells in F₁ mice (Table III). Considering previous data demonstrating that the activity of Ts₂ cells and factors is restricted by genes of the I-J region (6, 9, 17); we propose that the I-J determinants present on the TsF₁-presenting cells, determine the subsequent I-J restrictions of the Ts₂ cells and factors. This implies that the Ts₂ cells (and factors) have anti-self I-J receptors. If as Schrader (18) first postulated, the alloantisera used to detect I-J determinants also contained antiidiotypic antibodies to specificities present on anti-I-J antibodies, one may mistake cells that carried I-J determinants with those that express idiotypic anti-self receptors for I-J. The implications of this hypothesis are important. If suppressor T cells bear anti-self I-J receptors plus specific receptors for antigen or TsF (either as distinct entities or as a single combined receptor), one can draw numerous functional homologies among the receptors of the various T cell lineages (helper, killer, and suppressor). In addition, this hypothesis would predict that molecular genetic evidence (19) for I-J-encoded determinants may be obtained by analysis of the specialized APC required for induction of suppressor cells. Additional data also support the contention that I-J determinants exist on at least some macrophage like cells. Niederhuber et al. (20) have reported that some antigen-presenting cells involved in the induction of burro erythrocyte responses also possess the I-J marker. In addition, Murphy et al. (21) described a non-T, non-B cell population that carried I-J determinants.

To date, our laboratory has identified two T cell populations in the suppressor cell cascade that recognize I-J determinants on specialized presenting cells. In addition to the three distinct suppressor T cell populations, two accessory or presenting cell populations are required to manifest suppression. A summary of our present view of

the cellular elements of the suppressor cascade is illustrated in Fig. 1. The figure depicts the role of the I-J-bearing TsF_1 -presenting cells (FPC) and the I-J-bearing APC that are involved in Ts_3 induction. It will be important to determine whether the accessory cells of the suppressor cascade represent a single cell type or whether different accessory cells are required in each stage of the suppressor pathway. In this regard, additional studies are presently underway to re-evaluate the nature of the signals required for Ts_1 induction. Previous data in another experimental system (22) suggested that Ia positive adherent cells are required to initiate the events leading to the suppressor cell pathway, however, the I-J phenotype of these cells was not determined. Other portions of the suppressor cascade in which an I-J-bearing

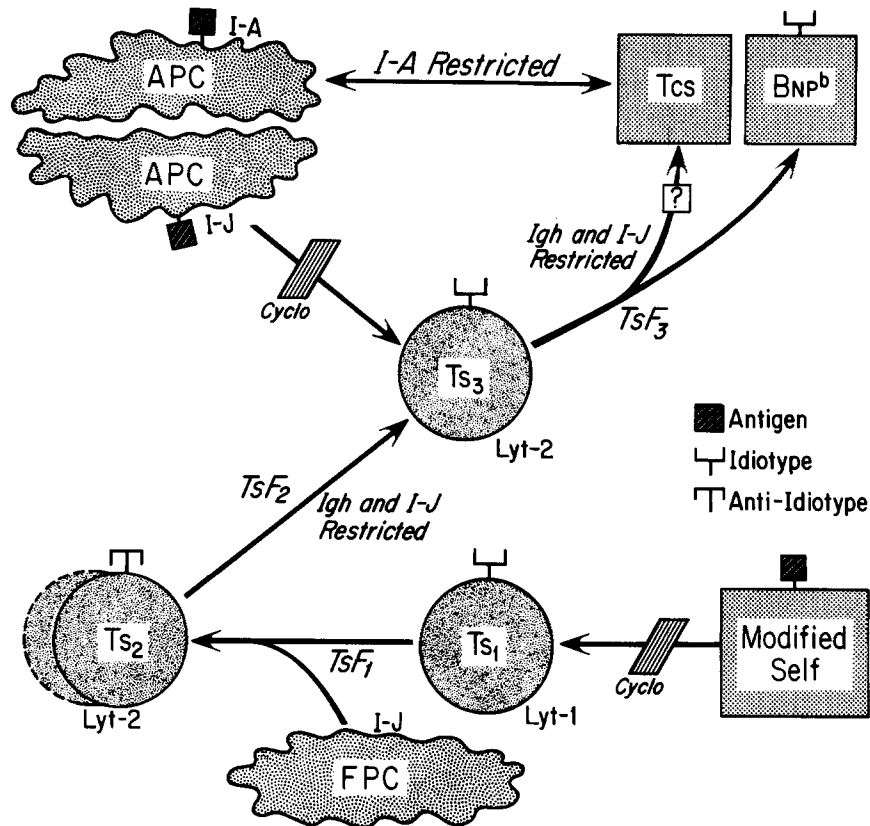


FIG. 1. A summary of the NP suppressor cell cascade. Intravenous administration of hapten-modified syngeneic cells induces a cyclophosphamide-sensitive Lyt-1-bearing Ts_1 population. Soluble factors (TsF_1) released by Ts_1 cells are then presented by specific factor-presenting cells (FPC) to induce Lyt-2-bearing Ts_2 cells. In turn, Ts_2 cells mediate their activity via another suppressor factor (TsF_2). The ability of TsF_2 to suppress NP responses is genetically restricted by genes in the Igh and I-J regions. The TsF_2 activates a third-order suppressor cell (Ts_3) that is initially induced following conventional antigen priming. The antigen-presenting cells (APC) required for Ts_3 induction also bear I-J determinants. Once the Lyt-2-bearing Ts_3 cells are activated by TsF_2 they release another factor (TsF_3) that directly suppresses B cells and either directly or indirectly suppresses T cell-mediated CS responses.

The receptors on the suppressor cells have not been fully defined. However, the data indicate that these receptors have either idiotypic or anti-idiotypic properties. Suppressor T cells may also have anti-self I-J receptors to account for the observed MHC restrictions.

accessory cell may be involved include the presentation of TsF₂ and the final effector stage of the pathway.

In spite of deficiencies in our overall understanding of this complex cascade, it is clear that the suppressor cell cascade represents a system of cellular interactions that are dependent on I-J products for induction and activation of effector T cells. The process of inducing genetic restrictions on suppressor cells uses the same mechanism that is responsible for imposing genetic restrictions on helper cells, i.e., antigen (or factor) is presented on a specialized adherent cell population. The differences between cells that present antigen to elements of the suppressor pathway and the cells that present antigen to cells of the helper pathway require further investigation. However, the analogies between the strategy of recognizing antigen in the context of self MHC products employed by both suppressor and helper cells (although the latter involve I-A and/or I-E gene products) emphasize the common mechanisms used by distinct T lymphocyte subsets.

Summary

The mechanism by which I-J restrictions were imposed on second-order suppressor cells (Ts₂) was analyzed. The induction of Ts₂ cells requires presentation of an inducer suppressor factor by a specialized population of factor-presenting cells. The I-J phenotype of this factor-presenting population controls the H-2 restriction of the Ts₂ cells. The splenic cells responsible for presenting inducer factor appear to be of macrophage or dendritic cell lineage.

Several homologies exist between the mechanism responsible for the induction of H-2-restricted suppressor and helper T cells. Thus, the I region products on specialized presenting cells determine the specificity and genetic restrictions of the T cell. In an H-2 heterozygous F₁ animal, two distinct populations of cells can be induced, one specific for each parental H-2 heplotype. Furthermore, the data suggest that the suppressor cells also bear receptors for self H-2 products. The ramifications of these observations for the suppressor cell cascade are discussed.

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