

HAPLOTYPE-SPECIFIC SUPPRESSION OF ANTIBODY RESPONSES IN VITRO

II. Suppressor Factor Produced by T Cells and T Cell Hybridomas from Mice Treated as Neonates with Semiallogeneic Spleen Cells*

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T cells exert both positive (helper) and negative (suppressor) regulatory effects on antibody responses; these regulatory effects are the properties of unique subpopulations of T cells (1). In many experimental systems involving active T cell-mediated suppression, a soluble mediator responsible for observed effects has been demonstrated. These suppressor factors may be antigen-specific or nonspecific and their activity may or may not be restricted by products of the I-region of the major histocompatibility complex (1-4).

We previously described a population of haplotype-specific suppressor T (Ts)¹ cells that were activated by stimulating spleen cells from mice injected as neonates with semiallogeneic F₁ spleen cells in culture with macrophages (Mφ) of the allogeneic haplotype encountered as neonates (5). The Ts cell population was genetically restricted by I-A subregion products in its activation and action, and acted in a non-antigen-specific manner to suppress primary in vitro antibody responses, but not secondary antibody responses or T cell responses to alloantigens by syngeneic spleen cells. This communication demonstrates that these haplotype-specific Ts cells release a soluble factor (TsF-H) after stimulation by the appropriate Mφ that is responsible for the suppressive activity of these Ts cells; moreover, a T cell hybridoma secreting monoclonal TsF-H has been developed. TsF-H from these two sources have been compared by a variety of parameters described here and found to be identical.

Materials and Methods

The strains of mice used and their source, the source and preparation of antigen, the injection of neonatal B10 and BALB/c mice with (B10.D2 × B10)F₁ spleen cells to induce haplotype-

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¹ *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte; GAT, random polymer of L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰; [³H]TdR, tritiated thymidine; Mφ, macrophage; MLR, mixed lymphocyte reaction; PEC, peritoneal exudate cell(s); PFC, plaque forming cell(s); SRBC, sheep red blood cells; Ts cell, suppressor T cell; TsF-H, haplotype-specific Ts cell factor.

specific Ts cells, culture and assay systems for plaque-forming cell (PFC) responses, mixed lymphocyte reactions (MLR), and cytotoxic lymphocyte (CTL) responses have been described in detail in the accompanying manuscript (5).

Preparation of Haplotype-specific TsF-H. Spleen cells from control mice or B10 or BALB/c mice injected as neonates with (B10.D2 × B10)F₁ spleen cells were incubated at 5×10^6 cells/well with 3×10^4 M ϕ of the appropriate allogeneic haplotype in completely supplemented Eagle's minimal essential medium with 10% fetal calf serum (lot 48006; Reheis Chemical Co., Kankakee, Ill.) in a final volume of 0.6 ml in 16-mm wells of a 24-multiwell tissue culture plate (76-033-05; Linbro Division, Flow Laboratories, Inc., Hamden, Conn.) for 48 h under modified Mishell-Dutton conditions (6, 7). Culture supernates were collected, centrifuged at 1,500 rpm for 15 min, filtered through 0.45- μ filters (Millipore Corporation, Bedford, Mass.), and stored at -70°C until use.

Generation of T Cell Hybridomas. Spleen cells (5×10^6 /well) from B10 mice injected with (B10.D2 × B10)F₁ spleen cells as neonates were cultured with 3×10^4 B10.D2-M ϕ for 24 h under modified Mishell-Dutton conditions (6, 7). These activated cells were fused with the hypoxanthine-guanine phosphoribosyl transferase-deficient AKR thymoma, BW5147, using polyethylene glycol (8-10). Cells were cultured in hypoxanthine-aminopterin-thymidine selection medium (8, 10, 11) and scored for growth after 3-6 wk. Cells from primary wells that yielded supernatant fluids that caused >75% suppression of PFC responses by B10 spleen cells were cloned by limiting dilution in soft agar over 3T3 feeder layers (8, 10, 12). Individual colonies were plated and expanded, and supernatant fluids were retested for suppressive activity. The hybridoma to be discussed, clone 267.A4.A1, has been maintained in tissue culture for >6 mo without loss of activity and active cells have been recovered after freezing in liquid nitrogen. Supernatant fluids from BW5147 alone and from several wells of the same fusion were consistently negative for TsF-H activity or nonspecific suppressor activity. Culture supernates from 267.A4.A1 collected from cells grown to confluence were processed in the same way as described for conventional TsF-H containing supernatant fluids. Some culture supernates or 267.A4.A1 supernates were concentrated 10 times by ultra filtration using a YM10 filter (Amicon Corporation, Lexington, Mass.) and applied to a Sephacryl S-200 column equilibrated with phosphate-buffered saline, pH 7.2. Fractions of 5.0 ml each were collected, filtered through 0.45- μ filters, and assayed for suppressive activity in the PFC assay.

Antisera. (B10.A × B10) anti-B10.D2 (anti-KABJ^d), [A.TL × B10.S (9R)] anti-B10.HTT (anti-J^a), B10.A(5R) anti-B10.A(3R) (anti-J^b), and (B10.A × A) anti-B10.A (5R) (anti-KAB^b) antisera were the generous gift of Dr. D. Shreffler (Department of Genetics, Washington University School of Medicine, St. Louis, Mo.). (B10.MBR × A/Sn) anti-B10.A (5R) (anti-A^b) was a gift of Dr. C. David (Mayo Medical School, Rochester, Minn.). A portion of the (B10.A × B10) anti-B10.D2 antisera was absorbed extensively on the H-2^d mastocytoma P815 to remove all anti-K^d activity. Rabbit anti-mouse IgG was prepared as described by Theze et al. (13). The immunoglobulin fractions of the heat-inactivated antisera were used to prepare Sepharose immunoabsorbents.

Results

Demonstration of Haplotype-specific TsF-H. Spleen cells from neonatally treated B10 mice, containing haplotype-specific Ts cells, were cultured with M ϕ of the appropriate and inappropriate haplotype for 48 h; the culture supernates were tested for the ability to suppress primary in vitro PFC responses by syngeneic spleen cells to L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) and sheep erythrocytes (SRBC). Supernatant fluids from spleen cells restimulated with M ϕ of the allogeneic haplotype encountered neonatally (B10.D2) profoundly suppressed primary responses to GAT and SRBC (Fig. 1). Suppression was observed over a substantial range of dilutions; the final dilution of TsF-H producing 50% suppression varied with individual preparations of TsF-H, but was in the range of 1:500 to 1:1,000. TsF-H activity was not observed in culture fluids prepared from normal B10 cells in an identical manner (data not shown) or in supernates of neonatally treated B10 spleen cells stimulated

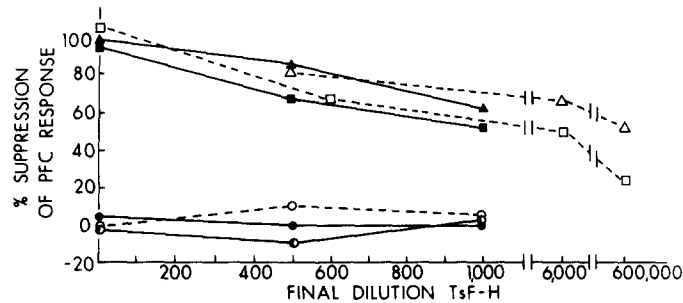


FIG. 1. Effects of haplotype-specific TsF-H on primary PFC responses in vitro. Spleen cells (5×10^6 /culture) from B10 mice injected as neonates with (B10.D2 \times B10) F_1 spleen cells were cultured with 3×10^4 B10.D2 (\blacktriangle , \blacksquare) or C3H (\circ , \bullet) M ϕ for 48 h. Culture supernatant fluids or 267.A4.A1 hybridoma TsF-H fluids (Δ , \square) were harvested, filtered, and tested for suppressor activity at various dilutions in cultures of normal B10 spleen cells (5×10^6 /culture) stimulated with GAT or SRBC. IgG GAT-specific (\blacktriangle , Δ) or IgM SRBC (\blacksquare , \square) PFC/culture were determined on day 5; data are expressed as percent suppression of PFC response. Control supernatant fluids failed to suppress either the GAT (\bullet) or SRBC (\circ) responses as did negative fusion wells (\odot). In the absence of TsF-H, control responses were 630 GAT-specific PFC/culture and 5,094 PFC/culture against SRBC.

with C3H M ϕ . TsF-H derived from treated B10 spleen cells had no effect on responses by BALB/c spleen cells to SRBC (data not shown). No difference in cell number or in viability was observed in cultures with control or active TsF-H. The cell responsible for production of TsF-H was determined by stimulating different spleen cell populations with the appropriate M ϕ and testing culture supernates for suppressive activity; only T cells from lymph node or spleen, but not thymus, produced TsF-H (data not shown). These findings were consistent with those for Ts cells (5), and prompted attempts to produce T cell hybridomas producing TsF-H by fusion of Ts cells with the thymoma line BW5147. Supernatant fluids from the 27 wells positive for growth out of a total of 60 wells plated were tested for suppression of primary in vitro PFC responses. The eight wells showing suppressive activity were cloned in soft agar, individual colonies were picked and grown in suspension culture, and supernates were retested for their ability to suppress primary PFC responses. Activity was demonstrated in two wells of the 24 that gave growth, with significant suppression in clone 267.A4.A1 seen at a dilution of 1:600,000 (Fig. 1). Culture supernates from the thymoma fusion partner, BW5147, failed to show suppression over the dilution ranges tested.

Parameters of TsF-H Activity. The ability of conventional and hybridoma TsF-H to suppress primary and secondary in vitro PFC responses MLR and CTL responses were examined. Significant suppression of primary in vitro PFC responses to GAT and SRBC was demonstrated over a wide range of dilutions of TsF-H (Table I); primary TNP-specific PFC responses to TNP-SRBC and TNP-*Brucella abortus* were also suppressed (data not shown). Similar amounts of TsF-H failed to suppress secondary in vitro PFC responses (Table I) or MLR or CTL responses (Table II); this lack of antigen specificity and failure to suppress secondary PFC responses and MLR and CTL responses were observed with the Ts cells (5). Analysis of the kinetics of suppression showed that conventional or hybridoma TsF-H must be present during the first 36–48 h of culture to achieve significant suppression and that TsF-H added at culture initiation blocked development of a significant PFC response (Fig. 2).

TABLE I
Effects of TsF-H on In Vitro PFC Responses

	Responder B10 spleen cells	Final dilution TsF-H	Day 5 IgG PFC/culture	
			GAT	SRBC
Experiment 1	Virgin	None added	655	10,600
	Virgin	1:50	70	4,720
	Virgin	1:500	155	2,680
	GAT-immune	None added	603	5,880
	GAT-immune	1:50	555	2,660
Experiment 2	GAT-immune	1:500	615	1,750
	Virgin	None added	435	2,165
	Virgin	1,000	8	880
	Virgin	10,000	23	720
	GAT-immune	None added	620	ND*
	GAT-immune	1,000	600	ND
	GAT-immune	10,000	610	ND

Spleen cells (5×10^6 /culture) from virgin B10 mice or B10 mice immunized 4 wk earlier with 10 μ g GAT in Maalox-pertussis were cultured with 2 μ g GAT or 10^7 SRBC and the indicated dilution of conventional H-2^b TsF-H in experiment 1 or 267.A4.A1 H-2^b hybridoma TsF-H in experiment 2. Data are expressed as IgG GAT-specific PFC or primary IgM PFC vs. SRBC.

* Not determined.

TABLE II
Effects of TsF-H on CTL and MLR Responses

	Final dilution, TsF-H	CTL re- sponse assay*	MLR assay, [³ H]- TdR incorporation (\bar{x} SEM)‡
		Specific ⁵¹ Cr release	
Experiment 1	None added	77.5	7,621.5 (1.10)
	1:50	74.0	9,665.7 (1.04)
	1:100	76.3	8,466.3 (1.05)
	1:500	70.6	ND§
Experiment 2	None added	69.0	12,667.0 (1.05)
	1:100	70.2	11,881.0 (1.02)
	1:1,000	75.6	11,513.3 (1.05)

* 7×10^6 normal B10 spleen cells were cultured with 3×10^6 irradiated (2,000 rad) B10.D2 stimulator spleen cells and the indicated dilution of TsF-H. In experiment 1, conventional B10-derived TsF-H were used; in experiment 2, H-2^b hybridoma TsF-H were used. After 5 d, percent specific ⁵¹Cr release at an effector to target cell ratio of 20:1 on P815 (H-2^d) target cells was determined. Spontaneous release was 10%.

‡ 200- μ l portions were removed at 72 h, pulsed with 1 μ Ci [³H]TdR for 18 h, and harvested for [³H]TdR incorporation.

§ Not determined.

Genetic Constraints on TsF-H Production and Activity. Activation of Ts cells and their interaction with appropriate target cells were highly genetically restricted by products of the I-A subregion of the H-2 complex (5). A similar analysis was made with TsF-H. Spleen cells from B10.D2 mice neonatally treated with (B10.D2 \times B10)F₁ cells were

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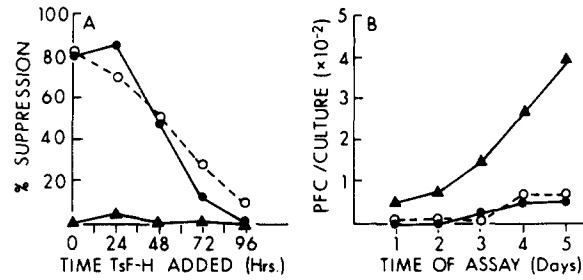


FIG. 2. Kinetics of TsF-H-mediated suppression. B10 spleen cells (5×10^6) were cultured with 10^7 SRBC under Mishell-Dutton conditions. Control fluid (▲) conventional TsF-H (●) (1:100 final dilution) or H-2^b hybridoma TsF-H, 267.A4.A1 (○) (1:10,000 final dilution) was added at the indicated time; IgM PFC/culture against SRBC were determined on day 5 (panel A). IgM PFC/culture against SRBC were determined on the indicated days in control (▲) cultures or cultures receiving conventional TsF-H (1:100 final dilution) (●) or H-2^b hybridoma TsF-H, 267.A4.A1 (○) on day 0 (panel B).

TABLE III
Genetic Requirements for Production of TsF-H

	Source of TsF-H*	Stimulating M ϕ	Shared H-2 regions‡	Day 5 GAT-specific PFC/culture§
Experiment 1	B10.D2	None	None	633
		B10.A (3R)	KABJ	95
		B10.A (5R)	KAB	13
		B10.A (4R)	BJE	603
Experiment 2	B10	None	None	570
		B10.D2	KABJECSD	70
		B10.GD	KA	100
		B10.LG	K	603

* Spleen cells (5×10^6) from B10.D2 mice (experiment 1) or from B10 mice (experiment 2) neonatally treated with (B10.D2 \times B10)F₁ cells were incubated 48 h with 3×10^4 M ϕ of the indicated haplotype. Supernatant fluids were harvested and tested for TsF-H activity.

‡ H-2 regions shared between the stimulating M ϕ and the allogeneic haplotype encountered neonatally.

§ Normal B10.D2 spleen cells (experiment 1) or B10 spleen cells (experiment 2) (5×10^6) were cultured with 2 μ g GAT and TsF-H (1:500 final dilution) under Mishell-Dutton conditions; IgG GAT-specific PFC/culture were determined on day 5.

stimulated with a panel of M ϕ and TsF-H activity was determined; M ϕ from B10, B10.A(3R), and B10.A(5R) stimulated production of TsF-H, whereas B10.A(4R) M ϕ failed to stimulate TsF-H production (Table III). Using spleen cells from B10 mice neonatally treated with (B10.D2 \times B10)F₁ cells, B10.D2 and B10.GD M ϕ stimulate TsF-H production, whereas B10.LG M ϕ failed to stimulate TsF-H production, demonstrating that syngenicity at the I-A subregion of the H-2 complex between the stimulator M ϕ and the allogeneic haplotype used to treat neonates was necessary and sufficient.

Genetic restrictions on the activity of TsF-H were investigated using responder spleen cells from mice sharing various regions of the H-2 complex with the TsF-H-producing cells. Significant suppression was observed only when the TsF-H-producing cell and the responder target spleen cells shared an identity at the I-A subregion of

the H-2 complex (Table IV). This was demonstrated by the ability of B10 TsF-H to suppress primary responses by B10, B10.A(3R), and B10.A(5R) spleen cells, but not B10.A(4R) spleen cells (this does not exclude the requirement for identity at K) and BALB/c TsF-H to suppress PFC responses by B10.GD but not B10.LG spleen cells. An interesting point was the lack of a requirement for syngenicity at the I-J subregion between the TsF-H-producing cell and its target population identity, which is required in some other suppressor systems (3, 4).

Characterization of Conventional and Hybridoma TsF-H

MOLECULAR WEIGHT DETERMINATION. The relative molecular weight of TsF-H was determined by gel filtration through a Sephacryl S-200 column. TsF-H activity was recovered in fractions collected between the bovine serum albumin (BSA) and ovalbumin (OVA) markers, placing the apparent molecular weight in the range of 45,000 to 68,000 (Fig. 3).

SENSITIVITY TO HEAT, pH, AND TRYPSIN. The stability of TsF-H under different temperatures, pH, and treatment with trypsin was examined (Table V). Biological activity was destroyed at 60°C (partially at 40°C) for 30 min, by pH 3.5, and by treatment with trypsin, which suggests that TsF-H is a protein and that some degree of tertiary structure was required for activity.

PRESENCE OF Ig OR H-2 COMPLEX DETERMINANTS. TsF-H was analyzed for the presence of serologically detectable Ig or H-2 complex determinants by the ability of immunoabsorbents of alloantisera specific for subregions of the H-2 complex to absorb out suppressor activity. BALB/c TsF-H could be absorbed by and eluted from a (B10.A × B10) anti-B10.D2, anti-(KABJE)^d immunoabsorbent, but failed to be

TABLE IV
Genetic Restrictions of TsF-H Activity on Target Cells

Responder spleen cells	Source of TsF-H	Shared H-2 regions*	Day 5 PFC/culture‡	
			GAT	SRBC
B10	None	None	413	5,350
B10	B10	KABJECSGD	15	200
B10.A (3R)	B10	KABJ	<10	428
B10.A (3R)	267.A4.A1	KABJ	<10	380
B10.A (5R)	B10	KAB	10	325
B10.A (5R)	267.A4.A1	KAB	<10	375
B10.A (4R)	B10	BJECSGD	653	5,296
B10.A (4R)	267.A4.A1	BJECSGD	450	5,150
B10.D2	None	None	461	ND§
B10.D2	BALB/c	KABJECSGD	<10	ND
B10.GD	BALB/c	KA	<10	ND
B10.LG	BALB/c	K	405	ND

* H-2 regions shared between the cells producing TsF-H and the responder spleen cells.

‡ 5×10^6 responder spleen cells of the indicated strains were cultured with 2 μ g GAT or SRBC (10^7), conventional TsF-H (1:500 final dilution), and H-2^b hybridoma TsF-H, and 267.A4.A1 (1:60,000 final dilution) under Mishell-Dutton conditions; IgG-GAT-specific or IgM vs. SRBC PFC/culture were determined on day 5.

§ Not determined.

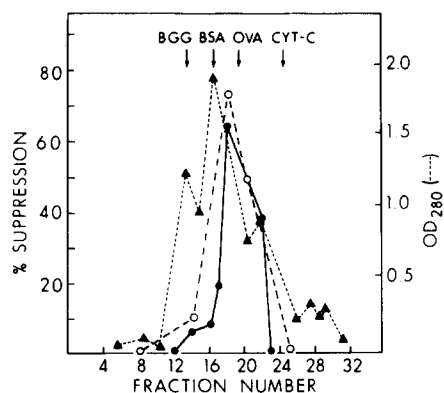


FIG. 3. Apparent molecular weight determination of TsF-H by gel filtration. BALB/c conventional TsF-H (●) or hybridoma TsF-H, 267.A4.A1, (○) were concentrated 10 times by ultra-filtration on an Amicon YM-10 filter before chromatography on a Sephacryl S-200 column. Approximately 5.0-ml fractions were collected and assayed for activity by their ability to suppress a primary *in vitro* response to GAT by BALB/c spleen cells cultured under Mishell-Dutton conditions. Arrows indicate the fractions where the marker proteins eluted. OD at 280 nm (▲).

TABLE V
Partial Characterization of TsF-H

Treatment of TsF-H	Day 5 GAT-specific PFC/culture	
	Conventional TsF-H	Hybridoma TsF-H 267.A4.A1
None	420	310
None	55	25
pH 3.5	403	290
60°C, 30 min	513	326
Trypsin	345	300
None	840	330
None	43	<10
40°C, 30 min	40	13
50°C, 30 min	345	168
60°C, 30 min	813	326

5×10^6 B10 spleen cells were cultured with 2 μ g GAT and conventional TsF-H (1:100 final dilution) or hybridoma TsF-H 267.A4.A1 (1:6,000 final dilution) treated as shown under Mishell-Dutton conditions; IgG GAT-specific PFC/culture were determined on day 5.

absorbed by a (B10.A \times A) anti-B10.A (5R), anti-(KAB)^b immunoabsorbent (Table VI), suggesting the presence of H-2 complex determinants on TsF-H. The (B10.A \times B10) anti-B10.D2 antisera extensively absorbed with the H-2^d mastocytoma P815 to remove all anti-K^d activity was still able to remove suppressor activity, which suggests the presence of I-region determinants on the factor. The identification of the subregion involved was made using B10-derived TsF-H. The H-2^b factor could be absorbed by and eluted from a (B10.A \times A) anti-B10.A (5R), anti-(KAB)^b, or a (B10.MBR \times A/Sn) anti-B10.A (5R), anti-(A)^b immunoabsorbent, but failed to be absorbed by a B10.A (5R), anti-B10.A(3R), anti-(J)^b, or the (B10.A \times B10) anti-B10.D2 immunoabsorbent previously shown to remove the H-2^d TsF-H (Table VI). The anti-J^b immunoabsorbent was shown to be functional by the ability to remove a GT-specific

TABLE VI
*Partial Immunochemical Characterization of Conventional and Monoclonal
 TsF-H*

TsF-H source*	Immunoabsorbent	Suppression of day 5 GAT-specific PFC response	
		Effluent‡	Eluate‡
BALB/c	None		90
BALB/c	α Ig	88	8
BALB/c	α (KABJE) ^d	0	92
BALB/c	α (KABJ) ^d abs P815§	8	86
BALB/c	α (KAB) ^b	70	12
BALB/c	α A ^b	90	0
B10	None		92
B10	α Ig	80	10
B10	α (KAB) ^b	12	98
B10	α A ^b	0	86
B10	α J ^b	94	6
B10	α J ^a	93	7
B10	α (KABJE) ^d	88	2
B10	α (KABJ) ^d abs P815	86	5
267.A4.A1	None		84
267.A4.A1	α Ig	90	11
267.A4.A1	α (KAB) ^b	0	85
267.A4.A1	α A ^b	0	95
267.A4.A1	α J ^b	90	12
267.A4.A1	α (KABJE) ^d	91	10

* Normal BALB/c or B10 spleen cells (5×10^6) were cultured with 2 μ g GAT and the conventional TsF-H (1:100 final dilution) or hybridoma TsF-H 267.A4.A1 (1:600 final dilution) under Mishell-Dutton conditions; IgG GAT-specific PFC/culture were determined on day 5. Data are presented as percent suppression of control responses.

‡ Effluents from the immunoabsorbents were tested directly; eluates were recovered with 2.0 M KCl and dialyzed before assay.

§ Antiserum was extensively absorbed on P815 to remove anti-K^d activity. No further activity was demonstrated by microcytotoxicity analysis on P815 cells.

suppressor factor of the appropriate specificity (J. Kapp, personal communication). Rabbit anti-mouse Ig or rabbit anti-mouse Fab reagents failed to absorb either B10 or BALB/c TsF-H. These results strongly suggest that TsF-H does not bear classical immunoglobulin constant-region determinants, but does bear determinants encoded by the I-A subregion of the H-2 complex.

Discussion

Suppressive activity has been demonstrated in supernatant fluids of spleen cells from mice treated as neonates with semiallogeneic F₁ spleen cells cultured with M ϕ of the allogeneic H-2 haplotype encountered as neonates. Production of TsF-H required T cells (lymph node or spleen, but not thymus) from mice treated as neonates and M ϕ syngeneic at the I-A subregion of H-2 with the allogeneic partner used in the neonatal treatment. TsF-H suppressed only primary in vitro antibody responses by spleen cells from mice sharing identity at the I-A subregion with the mice producing

TsF-H. *In vivo* activity of TsF-H is currently being tested. TsF-H, although restricted genetically with respect to production and interaction with target cells, was non-antigen-specific and suppressed responses to both T cell-dependent and T cell-independent antigens. TsF-H failed to suppress secondary antibody responses and MLR or CTL responses. TsF-H is a protein with a molecular weight in the range of 45,000–68,000 that bears determinants of the I-A subregion of the H-2 complex but not demonstrable Ig constant-region determinants. A T cell hybridoma producing monoclonal TsF-H with identical activities and properties has been produced by fusion of the AKR thymoma BW5147 with T cells from B10 mice treated as neonates with (B10.D2 × B10)F₁ cells (8).

Numerous examples of Ts cells and soluble suppressor molecules that mediate their effects have been reported (3, 4, 14–18); these diverse systems have been compared, contrasted, and integrated by Germain and Benacerraf (14). The suppressor pathway is initiated by the interaction of antigen with antigen-specific Ly-1⁺ or Ly-1,2,3⁺ (frequently I-J⁺) Ts cells that produce suppressor factors (TsF₁) that act across H-2 and V_H differences. These factors are proteins with molecular weights of ~50,000 that bind antigen, bear I-J determinants, and, where tested, bear the appropriate idiotype of the system. These TsF₁ appear to induce together with antigen a second set of Ts cells (Ts₂ cells) that are Ly-1,2,3⁺, I-J⁺, and either antigen-specific or specific for idiotype (i.e., anti-idiotypic). These cells or a succeeding set of Ly-2,3⁺, I-J⁺ effector Ts cells produce suppressor factors (TsF₂) whose activity is restricted by H-2 (I-J subregion) and, for anti-idiotypic Ts₂, V_H-linked genes. These TsF₂ are also proteins with a molecular weight of ~50,000 that are I-J⁺ and bear receptors for antigen or idiotype in cases where activity is restricted by V_H-linked genes. Finally, antigen-primed Ly-1,2,3⁺, I-J⁺, idiotype-positive Ts₃ cells after appropriate activation produce nonspecific suppressor factors that appear ultimately to mediate suppression. Important distinctions exist between these various antigen-specific suppressor factors and the haplotype-specific suppressor factor described here apart from the lack of antigen specificity. The antigen-specific suppressor factors described to date bear determinants encoded by the I-J (in one case I-C) subregion of the H-2 gene complex (3, 4, 14). Tada (3, 19) reports a strict requirement for syngenicity at I-J between a TsF₂ and its target cell, whereas Kapp (20) demonstrated the presence of I region determinants (presumably I-J determinants) on the GAT-TsF, most likely a TsF₁, but showed that GAT-TsF from nonresponders can cross-suppress responses by other nonresponder cells (3, 14). Other suppressor factors in the GT system (4, 14), the feedback suppressor pathway in responses to SRBC (14, 15), and delayed hypersensitivity to the azobenzenearsonate and 4-hydroxy-3-nitrophenyl acetate systems (4, 14, 17), are presumably in the TsF₁ class, and are I-J⁺ and not H-2-restricted. A suppressor factor involved in contact hypersensitivity responses bears I-C subregion products and is H-2-restricted and is therefore in the TsF₂ class (14, 16). In contrast, we have been unable to demonstrate I-J determinants on TsF-H or a restriction in TsF-H activity by the I-J subregion. Instead, TsF-H activity is restricted by the I-A subregion and I-A determinants are present on TsF-H. This same lack of involvement of I-J, and restriction by products of the I-A subregion was observed with the haplotype-specific Ts cell itself (5). These findings and others bring into question the actual role, if any, of I-J determinants in suppression. One possibility is that the I-J subregion encodes more than one product, and that distinct I-J products are expressed by the various

precursor, inducer, and effector cells in the helper-suppressor T cell circuits (3, 14, 21, 22; D. Murphy, personal communication). The existence of distinct I-J products on these cells could be the basis for their interrelationship and communication. The presence of I-J determinants on the haplotype-specific Ts cell has not been ruled out; studies to date have only shown that the I-J subregion does not restrict either the suppressor cells or TsF-H. Studies are in progress to determine the surface phenotype of these suppressor cells and to examine the possibility that I-J determinants other than those classically expressed on Ts cells (3, 4, 14, 19, 22; D. Murphy, personal communication) are expressed on the cells or the factor.

The haplotype-specific Ts cell-TsF-H system has more similarities to the MLR suppressor system of Rich and Rich (23-25) than to the antigen-specific systems. This system is activated after exposure to alloantigens *in vivo* and restimulation *in vitro*; activated Ts cells are short-lived cells that act via a factor MLR-TsF that suppresses the MLR in a genetically restricted but non-antigen-specific manner (24, 25). Syngenicity at I-J between factor and target cell for expression of MLR-TsF activity is not required; instead, syngenicity at the I-E/C subregion of H-2 is necessary and sufficient. It is interesting to speculate about why suppressor systems in response to I-subregion-encoded determinants appear to have regulatory or genetic constraints that differ from Ts cells that are stimulated by conventional antigens. Suppressor systems involving conventional antigens are intimately associated with I-J subregion regulation, whereas the haplotype-specific and MLR Ts systems do not appear to involve the I-J subregion, but are regulated by I-A and/or I-E/C subregions. The major exception, the contact hypersensitivity suppressor system (16) is regulated by the I-C subregion. However, this system and the suppressor systems involving delayed hypersensitivity to azobenzenearsonate and 4-hydroxy-3-nitrophenyl acetate are activated by presenting the relevant determinant to the immune system on lymphoid cells whose I-region products may be modified by these determinants (14). Thus, these hypersensitivity suppressor systems and the MLR and haplotype-specific suppressor systems are examples of Ts systems that, because of the critical involvement of I-region products in their activation, may not be regulated by the I-J subregion. Regulation of suppressor systems involving conventional antigens, which may or may not be presented in the context of I-region products, but which do not involve responses to I-region products or modified I-region products, may be regulated exclusively by I-J subregion products. Further data that should be forthcoming from several laboratories should answer these questions.

Further, studies on the biochemical nature, structure, and mechanism of the action of TsF-H will be facilitated by the availability of T cell hybridomas producing monoclonal TsF-H. Preliminary studies indicate that TsF-H induces another population of T cells to produce a second factor that acts in a non-antigen-specific, non-MHC-restricted fashion to inhibit antibody responses. Characterization of these cells and their factor is in progress.

Summary

Culture supernatant fluids from spleen cells from C57BL/10 or BALB/c mice neonatally treated with semiallogeneic (B10.D2 × B10)F₁ cells to induce haplotype-specific suppressor T cells and restimulated with macrophages syngeneic at I-A with the allogeneic haplotype encountered as neonates contain a soluble factor capable of

suppressing primary in vitro antibody responses of normal syngeneic spleen cells in a non-antigen-specific manner. This haplotype-specific suppressor factor, TsF-H, has also been recovered in culture fluids of a T cell hybridoma produced by fusion of the AKR thymoma BW5147 and the haplotype-specific suppressor T cells. TsF-H is inactivated by low pH (3.5) trypsin, for 30 min at 50°C, and has a molecular weight in the range of 45,000 to 68,000. Studies with specific immunoabsorbents demonstrate the presence of determinants encoded by the I-A subregion of the haplotype of the T cell producing TsF-H but not I-J subregion or immunoglobulin constant-region determinants on the TsF-H. Suppression is restricted to primary in vitro antibody responses, and not secondary antibody, mixed lymphocyte, or cytotoxic lymphocyte responses by spleen cells syngeneic at the I-A subregion of H-2 with the T cell producing the factor. The properties and activities of TsF-H and the haplotype-specific suppressor T cell are compared and contrasted with antigen-specific and genetically restricted suppressor T cells and their factors.

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