REGULATION OF IMMUNE RESPONSES BY I-J GENE PRODUCTS

VI. Recognition of I-E Molecules by I-J-bearing Suppressor Factors

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I-A and I-E gene products of the murine histocompatibility complex (H-2) are expressed by most B cells and to varying degrees by macrophages and T cells (1, 2). I-J products are intimately involved in immune regulation and are expressed by Ts cells and their factors (TsF),[1] as well as by some Th cells and macrophages (3, 4). A great deal is known about the function, serology, and biochemistry of the I-A and I-E molecules, due in part, to a large number of cells expressing these molecules in high density on their cell membranes. The paucity of I-J products has limited their characterization mainly to functional studies (3, 5).

I-J–bearing TsF have been shown in several antigen systems, including KLH (6), Ars (7), GAT (8), and poly($Glu^{50}Tyr^{50}$) (GT) (9). Moreover, in the KLH and Ars systems, I-J identity between the TsF donor and recipient is required for suppression (7, 10). No such restriction pattern has been shown for the GAT- or GT-TsF1 (first-order suppressor factor) (8, 11, 12). Sorensen and Pierce (13), however, reported an I-J–restricted GAT-TsF_2 derived from responder mice. In the GT system, there is not a complete lack of allogenic restriction (11). Injection of H-$2^{b,d,k}$ haplotype mice with GT produces GT-TsF1 that suppress PFC responses of H-$2^{a,d,k}$ mice to the immunogenic form of GT, GTMBSA (GT coupled to methylated bovine serum albumin [MBSA]) (11, 12). H-$2^{b,q,s}$ haplotype mice are not suppressed by GT-TsF1 (reference 11 and this paper).

The present study shows that I-E molecules must be expressed in order for the recipient strain to be suppressed by I-J–bearing GT-TsF1. We show that GT-TsF1 is presented in the context of I-E molecules and that GT-TsF1 presentation is blocked by anti–I-E, but not anti–I-A, antibodies. Our results indicate recognition between I-J and I-E molecules.

Materials and Methods

Mice. AKR/Cum (H-2^k) and BALB/cCum (H-2^d) mice were purchased from Cumberland View Farms, Clinton, TN. C57BL/6J (B6; H-2^b), B10.BR (H-2^k), A.SW (H-2^s), and SJL/J (H-2^s) were obtained from The Jackson Laboratory, Bar Harbor, ME. D2.GD (H-2^{g2}), A.TRF5 (H-2^{ap5}), and (D2.GD × A.TRF5)F1 hybrid mice were the generous gifts of

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[1] *Abbreviations used in this paper:* GT, poly($Glu^{50}Tyr^{50}$); *Ir*, immune response (gene); MBSA, methylated bovine serum albumin; TsF, T cell-derived suppressor factor(s).

Dr. Chella David, Mayo Clinic, Rochester, MN. B10.A(4R) (4R; H-2^{h4}), B10.A(5R) (5R; H-2^{i5}), CBA/JNCr (H-2^{d}), and BALB/cNCr (H-2^{d}) mice were obtained from the Animal Genetics and Production Branch of the National Cancer Institute, Frederick, MD. Female mice, 2–4 mo old, were used throughout and maintained on standard laboratory chow and water ad lib.

Antigens and Immunizations. GT, 39,000 D, (lot No. 51F5054) and GAT 25,000 D, (lot No. 51F5040) were purchased from Sigma Chemical Co., St. Louis, MO. MBSA was prepared by the method of Sueoka and Cheng (14). Antigen solutions and GTMBSA were prepared as previously described (12). For in vivo studies described in Tables II and III, BALB/c and 4R mice were immunized with 20 μg GT as GTMBSA in Maalox (aluminum-magnesium hydroxide gel, Wm. H. Rorer, Inc., Ft. Washington, PA) and *Bordetella pertussis* intraperitoneally as adjuvant.

GT-TsF Preparation. BALB/c (I-J^{d}), B10.BR (I-J^{k}), and B6 (I-J^{b}) GT-TsF1 were prepared as described (11). Briefly, mice were injected intraperitoneally with 100 μg GT in Maalox. 3 d after injection, 6×10^{8} spleen cells/ml in HBSS were sonicated as previously described (11). Sonicated material was centrifuged at $40,000 \times g$ for 45 min at 4°C. The GT-TsF1–containing supernatants were stored at −85°C until use. I-J^{k}–bearing monoclonal GT-TsF1 (WF11.3A1), GT-TsF2 (WF21.M5.A4), and GT-TsF3 (WF21.K3.E9) have been described (15). Factors were used at concentrations indicated in the table legends.

mAbs and Cell Lines. B cell hybridomas secreting anti-I-E^{k} (17.3.3) and anti-I-E^{k} crossreactive with I-E^{d} (14-4-4S) mAb were obtained from the Cell Distribution Center, Salk Institute, La Jolla, CA. An anti-I-E^{k}-containing culture supernatant (Y-17) was the gift of Dr. Charles Janeway, Yale Medical School, New Haven, CT. Anti-I-A^{p} mAb (6.5.2), which crossreacts with I-A^{d}, was the gift of Dr. J. Frelinger, University of North Carolina, Durham. The Ia^{+} BALB/c B cell lymphoma, A20-2J (16, 17), was the kind gift of Dr. A. Abbas, Harvard Medical School, Boston, MA.

GT-TsF and Antigen Presentation. Exponentially growing A20-2J cells were harvested from DMEM supplemented with 10% FCS and washed twice with HBSS. To prohibit A20-2J cell division, 10^{7} cells were treated with 3.7×10^{-5} M mitomycin C in 1 ml DMEM at 37°C for 30 min. The cells were then washed three times in HBSS. Under these conditions, A20-2J cells showed no growth after 7 d of culture. Where indicated, mitomycin C-treated A20-2J cells were incubated with a 1:200 final dilution of anti-I-A^{d} or anti-I-E^{d} mAb for 20 min at 4°C, then washed three times with HBSS. 100 μg of GAT in 1 ml or a 1:200 final dilution of monoclonal GT-TsF1 (WF11.3A1) in 1 ml was incubated with 10^{7} A20-2J cells in DMEM containing 10% FCS for 30 min at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were washed three times with HBSS, counted, and added in numbers indicated to BALB/c spleen cultures.

Spleen Cell Culture and Hemolytic Plaque Assay. Single-cell suspensions of BALB/cCum (H-2^{d}) spleen cells were placed in modified Mishell-Dutton type culture conditions as described (15). mAb, GT-TsF, or treated A20-2J cells were added at culture initiation in concentrations/numbers indicated in table legends. Cultures were harvested 5 d after initiation, cells washed three times in HBSS, and PFC responses were assayed using SRBC coupled with the crossreacting polymer GAT as previously described (18). PFC responses from in vivo primed BALB/c and 4R mice (Table III) were determined 7 d after GTMBSA immunization.

Results

Role of I-E Gene Products on GT-TsF1 Suppression. GT-TsF1 suppresses the GTMBSA PFC responses of several inbred mouse strains, although no correlation between suppression and a particular allele within H-2 has been shown (11). Table I summarizes the suppression patterns for five different H-2 haplotypes. H-$2^{d \text{ and } k}$ mice are suppressed by GT injection, and produce and are suppressed by GT-TsF1. H-2^{b} mice produce GT-TsF1, but are not suppressed by GT preimmunization or GT-TsF1. Conversely, H-2^{a} mice are suppressed by GT-

TABLE I
Strains Suppressed by GT-TsF1

Strain	H-2							Produces GT-TsF1	GTMBSA responses suppressed by:	
	K	A	J	E	C	S	D		GT	GT-TsF1
BALB/c B10.D2 DBA/2	d	d	d	d	d	d	d	Yes	Yes	Yes
B10.BR AKR CBA/J	k	k	k	k	k	k	k	Yes	Yes	Yes
B10 B6, A.BY	b	b	b	(b)	b	b	b	Yes	No	No
A/J B10.A	k	k	k	k	d	d	d	No	No	Yes
(B10 × B10.A)F1	k/b	k/b	k/b	k/(b)	d/b	d/b	d/b	Yes	Yes	Yes
DBA/1	q	q	q	(q)	q	q	q	No	No	No

This table summarizes previously published data (11, 12, 26, 27). ( ), silent alleles.

TsF1, although they lack the ability to produce this factor. H-$2^{a\times b}$ hybrid mice are suppressed by GT preimmunization, and produce and are suppressed by GT-TsF1. GTMBSA responses of H-2^{q} cannot be suppressed. Although this is a limited sampling, it is striking that mice expressing I-E molecules (H-$2^{a,d,k}$ and H-$2^{a\times b}$) are suppressed by GT-TsF1 and those not expressing surface I-E molecules (H-$2^{b,q}$) are not suppressed.

H-2^{s} haplotype mice do not express I-E molecules (19). However, GT injection markedly diminishes the GTMBSA responses of SJL, A.SW, and B10.S mice (20, 21). The protocol of GT injection followed by GTMBSA immunization measures tolerance-type induction and does not address the role of suppressor factors. Are H-2^{s} mice suppressed by GT-TsF1? Table II shows that neither SJL nor A.SW mice are suppressed by BALB/c GT-TsF1 (Exp. 1) and that SJL is not suppressed by B10.BR GT-TsF1 (Exp. 2). Therefore, it appears that H-2^{s} mice are not susceptible to GT-TsF1-mediated suppression. GT injection may induce tolerance rather than suppression in H-2^{s} mice. H-2^{b} mice express I-A^{b} but not I-E, B10.A(3R) and B10.A(5R) mice express both I-A^{b} and I-E^{k} (19). Table II, Exp. 3, shows that 5R mice are readily suppressed by GT-TsF1. Although not shown, 3R mice show a similar result. I-A^{b} has no adverse affect on GT-TsF1 susceptibility and, again, we see concordance of I-E expression and GT-TsF1 suppression.

Is the I-E requirement qualitative or quantitative? A.TFR5 (H-2^{ap5}, E^{f}_{β}, E^{k}_{α})

TABLE II

GT-TsF1 Suppresses B10.A(5R) (H-2^{i5}) But Not H-2^{s} Mice

Strain	Number of mice per group	Factor*	GT-specific PFC per spleen‡	P value§
Experiment I				
BALB/c (H-2^{d})	6	BALB/c Maalox	7,995 ± 1,531	
	6	BALB/c GT-TsF1	470 ± 201	<0.001
SJL (H-2^{s})	11	BALB/c Maalox	8,342 ± 819	
	11	BALB/c GT-TsF1	8,286 ± 1,066	0.96
A.SW (H-2^{s})	8	BALB/c Maalox	7,800 ± 1,748	
	7	BALB/c GT-TsF1	4,910 ± 1,261	0.12
Experiment II				
B10.BR (H-2^{k})	3	B10.BR Maalox	6,412 ± 1,537	
	2	B10.BR GT-TsF1	175 ± 35	0.04
SJL (H-2^{s})	5	B10.BR Maalox	3,420 ± 1,168	
	5	B10.BR GT-TsF1	3,760 ± 1,720	0.87
Experiment III				
AKR/Cum (H-2^{k})	4	None	9,425 ± 1,096	
	4	Monoclonal GT-TsF1	5,475 ± 825	0.029
B10.A(5R) (H-2^{i5})	4	None	10,350 ± 676	
	4	Monoclonal GT-TsF1	5,075 ± 863	0.003

* Mice were injected intravenously with 0.5 ml of a control (Maalox) or GT-TsF1 containing cell-free extract (1.5×10^7 cell equivalents) or with a culture supernatant (WF11.3A1) containing monoclonal GT-TsF1 (1:20 final dilution). Mice were immunized intraperitoneally immediately thereafter with 10 μg GT as GTMBSA in Maalox-pertussis as adjuvant.

‡ 7 d after GTMBSA immunization, GT-specific PFC per spleen were counted. Numbers represent arithmetic mean ± standard error of the mean. Underlining indicates suppression.

§ The *F*-distribution statistic was used to test significance in analysis of variance (ANOVA) in comparing GT-TsF1 injected animals with appropriate control mice.

mice express membrane I-E at low density levels, 80+% less than "normal" mice (22). This is most likely due to the fact that E^{f}_{β} is not expressed, although E^{k}_{α} is expressed (22). (A.TRF5 × D2.GD)F1 hybrid mice express I-E surface molecules. Does this correlate with GT-specific suppression? The GTMBSA PFC responses of (A.TRF5 × D2.GD)F1 hybrids are suppressed by GT preimmunization, by B6 (I-J^{b}) GT-TsF1, BALB/c (I-J^{d}) GT-TsF1, and B10.BR (I-J^{k}) GT-TsF1 (Table III). In contrast, parental strains D2.GD and A.TFR5 are not suppressed by GT or GT-TsF1 injection. These data suggest that GT-TsF1 suppression requires expression of an E_{β} gene product and/or that the density of I-E expression is important.

Suppressor Defect in B10.A(4R) Mice. Araneo and Kapp (23) showed suppression of T cell proliferative responses to GTMBSA by GT-TsF. With one notable exception, their proliferation data are concordant with our PFC data. Araneo and Kapp (23) suppressed proliferative responses of B10.A(4R) to GTMBSA using GT-TsF1. T cell proliferative, delayed hypersensitivity, and PFC responses are not always concordant, indicating the assay of different cell populations (24, 25). Can GTMBSA PFC responses of 4R mice be suppressed? Table IV indicates that neither GT nor BALB/c (I-J^{d}) GT-TsFs injection is inhibitory to subsequent

TABLE III

I-E Gene Complementation and GT-TsF1 Suppression

Strain	I-Region*				GT-specific PFC per culture‡				
	A_β	A_α	E_β	E_α	Control GTMBSA	GT + GTMBSA	B6 GT-TsF1	BALB/c GT-TsF1	B10.BR GT-TsF1
D2.GD	d	d	d/b	(b)	1,760	1,900	1,760	1,560	1,490
A.TFR5	f	f	(f)	k	1,890	2,200	2,540	1,740	1,490
(A.TFR5 × D2.GD)F1	f/d	f/d	(f)/d	k/(b)	950	60§	<15	260	60

* Assigned alleles for I-A and I-E loci are indicated. A recombinant event occurred in D2.GD strain within the E_β locus, hence this locus is partially composed of both E_β^b and E_β^d genetic material. ( ), silent alleles.

‡ D2.GD, A.TRF5, or (A.TRF5 × D2.GD)F1 spleen cells were placed in 5-d Mishell-Dutton cultures containing 2.5 μg GT as GTMBSA. The cultures contained no additions (control), 10 μg GT, or B6 ($I\text{-}J^b$), BALB/c ($I\text{-}J^d$), or B10.BR ($I\text{-}J^k$) GT-TsF1-containing suppressor extracts at 1:400 final concentration (1.5×10^6 cell equivalents).

§ Underlining indicates suppression.

TABLE IV

Effect of GT-TsF1 or GT on the GTMBSA PFC Responses of BALB/c and 4R Mice

Strain	No. Mice per Group	Treatment* given on day 0	Antigen‡ immunized on day 3	GT-specific PFC/spleen§ measured on day 10	P value‖
BALB/c	7	None	GTMBSA	9,200 ± 910	—
	8	BALB/c GT-TsF1	GTMBSA	2,470 ± 720	<0.001
	4	GT	GTMBSA	2,480 ± 2,130	0.007
B10.A(4R)	11	None	GTMBSA	15,470 ± 2,890	—
	12	BALB/c GT-TsF1	GTMBSA	16,120 ± 2,410	0.898
	7	GT	GTMBSA	10,540 ± 1,400	0.579

* BALB/c and 4R mice were either injected with 0.5 ml BALB/c GT-TsF1 intravenously diluted 1:5 in HBSS (1.2×10^7 cell equivalents), injected intraperitoneally with 100 μg GT in Maalox, or were uninjected.

‡ 3 d after GT or BALB/c GT-TsF1 injection, the mice were immunized intraperitoneally with 20 μg GT as GTMBSA in Maalox-pertussis as adjuvant.

§ 7 d after GTMBSA immunization (day 10) GT-specific PFC per spleen were enumerated. Numbers represent the arithmetic mean ± standard error of the mean. Underlining indicates suppression.

‖ The *F*-distribution statistic was used to test significance in analysis of variance (ANOVA) between experimental groups.

GTMBSA immunization. As control, the GTMBSA PFC responses of GT or GT-TsF1 injected mice are suppressed by >70%.

Previously, we showed that $H\text{-}2^b$ mice produce GT-TsF1 upon GT injection, however they are unable to make second-order Ts (Ts2). Do 4R mice display a similar defect? GTMBSA PFC responses of $H\text{-}2^b$ mice are suppressed by factors that circumvent Ts2 (26, 27). Hence, $H\text{-}2^b$ mice are suppressed by monoclonal $I\text{-}J^k$ GT-TsF2 and $I\text{-}J^k$ GT-TsF3, but not by monoclonal $I\text{-}J^k$ GT-TsF1. To ascertain the cellular defect, monoclonal TsF were injected into 4R mice. Neither GT-TsF1 nor GT-TsF2 suppresses GTMBSA PFC responses of 4R spleen cell cultures, although these factors specifically suppress BALB/c spleen cell cultures (Table V). Monoclonal GT-TsF3 suppresses the 4R GTMBSA PFC response, which suggests that 4R mice have a Ts3 defect.

TABLE V

Effect of GT-TsF1,2,3 on the GTMBSA PFC Responses of B10.A(4R) and BALB/c Spleen Cells in Vitro

Strain	Hybridoma factor source*	Type of factor‡	GT-specific PFC per culture§	Percent GTMBSA response‖
B10.A(4R)	None	—	775	—
	WF11.3A1	TsF1	820	106
	WF21.M5.A4	TsF2	1,160	150
	WF21.K3.E9	TsF3	60	8
BALB/c	None	—	850	—
	WF11.3A1	TsF1	<20	2
	WF21.M5.A4	TsF2	<20	2
	WF21.K3.E9	TsF3	<20	2

* Supernatants, each containing a different I-J^k GT-specific suppressor factor, were added at 1:400 final dilution at culture initiation. All Mishell-Dutton cultures contained 2.5 μg GT as GTMBSA.
‡ Nominal factor designation based upon kinetics of suppression (15).
§ 5 d after culture initiation, GT-specific responses were assayed. Underlining indicates suppression.
‖ Relationship of control GTMBSA culture (not receiving factor) to factor containing culture.

Demonstration of the Requirement for I-E Molecules Using mAbs. Strains of mice not expressing I-E are not suppressed by GT-TsF1 (Tables I–III and references 11, 26, 27), implying, but not proving, that I-E expression is essential for factor-mediated suppression. This suggests that GT-TsF1 is presented by MHC class II-bearing cells and/or that GT-TsF1 recognizes (or is recognized in the context of) I-E molecules. To resolve these possibilities, we asked whether GT-TsF1 suppressive activity can be absorbed by normal spleen cells. Table VI shows that the suppressive activity of monoclonal GT-TsF1 (WF11.3A1) is absorbed by AKR/Cum spleen cells at 4°C. If AKR/Cum (H-2^k) spleen cells are incubated with monoclonal anti–I-E^k (17-3-3) at 4°C, before incubation with GT-TsF1, then they no longer absorb suppressor activity (Table VI). Incubation of AKR/Cum spleen cells with a supernatant derived from the secretory P3X63Ag8 (P3) myeloma does not inhibit the ability of these cells to absorb GT-TsF1 activity. Although not shown, an immunoadsorbent column constructed with this same anti–I-E^k mAb (17-3-3) does not bind I-J^k GT-TsF1; therefore, the effect of 17-3-3 is directed not against the factor, but toward the target cell of the factor. Direct addition of anti–I-E^k mAb (17-3-3) to AKR/Cum spleen cell cultures blocks GT-TsF1–mediated suppression (Table VII). Addition of either 17-3-3 or Y-17 anti–I-E^k mAb blocks suppression mediated by either I-J^k GT-TsF1 (WF11.3A1) or I-J^d BALB/c GT-TsF1, while neither antibody adversely affects the GTMBSA PFC response of CBA/J (H-2^k) spleen cell cultures. These data lend support for the notion that anti–I-E^k mAb blocks factor presentation and that the antibody is not directed against the factor.

Cellular Presentation of GT-TsF1. Our data implicate I-E, and hence APCs, in GT suppression. We would predict that I-E–bearing cells should function in a factor-presenting capacity. To test this hypothesis, we used the Ia-bearing BALB/c lymphoma cell line A20-2J. Between 10^2 to 10^4 mitomycin C-treated A20-2J cells were added to BALB/c spleen cell Mishell-Dutton cultures. Mito-

TABLE VI
Absorption and Blocking of GT-TSF1 by 17-3-3 in BALB/c and AKR/Cum Spleen Cells Cultures

Strain	Monoclonal factor*	Treatment‡	Antigen§	GT-specific PFC per culture‖
BALB/c	None	None	GTMBSA	915
	WF11.3A1	None	GTMBSA	<30
	WF11.3A1	Factor absorbed on AKR/Cum spleen cells	GTMBSA	830
	WF11.3A1	Factor absorbed with 17-3-3-blocked AKR spleen cells	GTMBSA	<30
AKR/Cum	None	None	GTMBSA	1,010
	WF11.3A1	None	GTMBSA	<20
	WF11.3A1	Factor absorbed with sham-blocked AKR spleen cells	GTMBSA	1,155
	WF11.3A1	Factor absorbed with 17-3-3-blocked AKR spleen cells	GTMBSA	<20

* (Un)absorbed culture supernatant containing I-J^k monoclonal GT-TsF1 (WF11.3A1) was added at 1:500 final dilution at culture initiation.

‡ Where indicated above, normal AKR/Cum (10^7) spleen cells were incubated with 1.0 ml of supernatant from the P3X63Ag8 myeloma (sham) or with 1.0 ml anti-I-E^k-containing supernatant from the 17-3-3 hybridoma for 20 min at 4°C. The (un)absorbed cells were washed three times in HBSS and the pellet resuspended to 1.0 ml with a culture supernatant containing monoclonal GT-TsF1 (WF11.3A1) diluted 1:50 and incubated for 30 min at 4°C. The AKR/Cum spleen cells were removed by centrifugation and the WF11.3A1 supernatants were added to Mishell-Dutton cultures at 1:500 final dilution (10% by vol).

§ At initiation all cultures received 1.25 μg GT as GTMBSA as immunogen.

‖ 5 d after culture initiation, GT-specific responses were assayed. Underlining indicates suppression.

mycin C blocked cellular division of the A20-2J cells. Addition of unpulsed A20-2J cells along with GTMBSA had no inhibitory effect upon the GT-specific PFC response of BALB/c spleen cells (Table VIII). A20-2J cells were incubated with monoclonal GT-TsF1 (WF11.3A1) for 15 min at 4°C, washed four times, then added to BALB/c spleen cell cultures. Table VIII shows that 10^4 or 10^3 factor-pulsed spleen cells suppress the GTMBSA response. 10^2 to 10^4 GAT-pulsed A20-2J present GAT to BALB/c spleen cell cultures.

Blocking of Factor Presentation by mAbs. The involvement of I-E molecules in factor presentation is demonstrated by the blocking of A20-2J factor presentation by mAb. Direct addition of GT-TsF1 (WF11.3A1) to BALB/c spleen cells inhibits the GTMBSA response (Table IX). Unpulsed A20-2J cells are not inhibitory. WF11.3A1-pulsed A20-2J cells inhibit the GT-specific PFC response. Incubation with anti-I-E^d mAb before factor pulsing blocks the ability of A20-2J cells to present GT-TsF1. The blocking of A20-2J factor presentation by anti-I-E^d mAb is most likely not due to steric hinderance, as anti-I-A^d does not block factor presentation. Conversely, anti-I-A^d, but not anti-I-E^d, mAb blocks GAT presentation by A20-2J. This serves as a negative control for anti-I-E^d mAb and a positive control for anti-I-A^d. Although not shown, mAb treatment of unpulsed A20-2J cells has no inhibitory effect upon the GTMBSA responses of BALB/c

TABLE VII

Effect of Anti-I-E^k mAbs on GT-TsF1 Activity in AKR/Cum and CBA/J Spleen Cell Cultures

Strain	GT-TsF1 added*	Anti-I-E^k added to culture‡	Antigen§	GT-specific PFC per culture‖
AKR/Cum	None	None	GTMBSA	1,170
	None	17-3-3	GTMBSA	1,460
	WF11.3A1	None	GTMBSA	<u>≤25</u>
	WF11.3A1	17-3-3	GTMBSA	<u>1,290</u>
CBA/J	None	None	GTMBSA	1,560
	None	17-3-3	GTMBSA	1,200
	None	Y-17	GTMBSA	2,110
	WF11.3A1	None	GTMBSA	<u>≤25</u>
	WF11.3A1	17-3-3	GTMBSA	<u>1,510</u>
	WF11.3A1	Y-17	GTMBSA	1,575
	BALB/c GT-TsF1	None	GTMBSA	<u>≤25</u>
	BALB/c GT-TsF1	Y-17	GTMBSA	<u>1,425</u>

* Monoclonal I-J^k GT-TsF1 (WF11.3A1) containing culture supernatants or a BALB/c spleen cell extract containing I-J^d GT-TsF1 (6×10^8 cell equivalents/ml) were added at 1:400 final concentration at culture initiation.

‡ 10 μl undiluted culture supernatants containing anti–I-E^k mAb were added at culture initiation.

§ At initiation, cultures received 1.25 μg GT as GTMBSA.

‖ After 5 d of culture, GT-specific responses were assayed. Underlining indicates suppression.

TABLE VIII

Factor and GAT Presentation by A20-2J Cells in BALB/c Spleen Cell Cultures

Mitomycin C-treated cells*	Additions directly to culture‡		GT-specific PFC per culture§
	Factor	Antigen	
None	None	GTMBSA	1,155
None	WF11.3A1	GTMBSA	<u>≤20</u>
10^4 A20-2J + —	None	GTMBSA	1,060
10^3 A20-2J + —	None	GTMBSA	1,580
10^2 A20-2J + —	None	GTMBSA	1,240
10^4 A20-2J + WF11.3A1	None	GTMBSA	<u>350</u>
10^3 A20-2J + WF11.3A1	None	GTMBSA	<u>≤20</u>
10^2 A20-2J + WF11.3A1	None	GTMBSA	1,030
10^4 A20-2J + GAT	None	None	1,330
10^3 A20-2J + GAT	None	None	1,325
10^2 A20-2J + GAT	None	None	1,060

* Mitomycin C-treated A20-2J B lymphoma cells were either pulsed with monoclonal GT-TsF1 (WF11.3A1), GAT, or not pulsed (as control) and added at numbers indicated at initiation of Mishell-Dutton culture.

‡ GTMBSA (1.25 μg/culture) was added at culture initiation to indicated cultures. Some cultures were immunized by GAT-pulsed A20-2J cells and received no additional antigen. Monoclonal GT-TsF1 (WF11.3A1) was added (1:400 final concentration) at culture initiation to one culture.

§ GT-specific responses were assayed 5 d after culture initiation. Underlining indicates suppression.

spleen cells. Our data strongly implicate a role for the association of GT-TsF1 and the I-E surface molecules in the presentation of suppressor activity.

TABLE IX
Blocking of A20-2J Factor Presentation by Anti-I-E^k mAb

Mitomycin C, A20-2J cells*		Direct addition to culture		Antigen-specific PFC per culture‖
Antibody blocking	Factor or antigen pulsing	Factor‡	Antigen§	
—	—	—	GTMBSA	1,020
—	—	WF11.3A1	GTMBSA	<20
None	Unpulsed	—	GTMBSA	1,250
None	WF11.3A1	—	GTMBSA	225
Anti-I-E^d	WF11.3A1	—	GTMBSA	990
Anti-I-A^d	WF11.3A1	—	GTMBSA	180
—	—	—	GAT	340
None	GAT	—	—	240
Anti-I-E^d	GAT	—	—	330
Anti-I-A^d	GAT	—	—	<20

* 10^5 mitomycin C-treated A20-2J cells were added to indicated cultures. Surface *I*-region molecules were blocked with anti-I-E^{k,d} (14-4-4S) or anti-I-A^{p,d} (6.5.2) mAb as indicated above and described in the Materials and Methods. (Un)blocked A20-2J cells were pulsed with either monoclonal I-J^k GT-TsF1 (WF11.3A1), GAT, or unpulsed as indicated and added at culture initiation.

‡ As indicated, monoclonal I-J^k GT-TsF1 (WF11.3A1) was added directly to culture at 1:400 final concentration at culture initiation.

§ At initiation, 1.25 μg GT as GTMBSA, 5 μg GAT, or no antigen additions were made to cultures.

‖ After 5 d culture, GT- or GAT-specific responses were assayed. Underlining indicates suppression.

Discussion

GT is unique in that it fails to induce humoral or cell-mediated immune responses in most inbred strains of mice (24, 25). GT injection of H-2^{b,d,k} haplotype mice results in the production of an I-J–bearing TsF (GT-TsF1) that suppresses GTMBSA responses of H-2^{a,d,k} haplotype mice. In the present study, we show that GT-TsF1 function is restricted by I-E molecule expression in the recipient strain. In contrast, I-E expression does not appear to influence GT-TsF1 production; I-E–negative mice (e.g., H-2^b) can make GT-TsF1 (26, 27). GT-TsF1 suppresses susceptible strains regardless of haplotype (e.g., I-J^k, GT-TsF1 suppresses H-2^a, I-J^k, H-2^d, I-J^d; and H-2^k, I-J^k mice) so long as the recipient strain expresses I-E molecules. Those strains unable to express I-E molecules are not suppressed by GT-TsF1. Therefore, I-E expression by the recipient is required for factor-mediated suppression. This observation suggests a role for MHC class II molecules (e.g., I-E) and implies the role of class II–bearing cells or APCs in the presentation of factor. Indeed, we find an association between I-E molecules and GT-TsF1 presentation. Normal spleen cells or the Ia$^+$ cell line, A20-2J, present GT-TsF1 to normal syngeneic spleen cells in vitro. Factor presentation is specifically blocked by anti-I-E mAb. In contrast, anti-I-A, but not anti-I-E, mAb blocks presentation of the I-A–restricted antigen GAT by A20-2J cells. This reciprocal experiment serves as a control for both GT-TsF1 and GAT presentation, and rules out nonspecific steric hindrance by mAb as the reason for lack of factor presentation by I-E–blocked A20-2J cells. Although at this juncture it is impossible to establish that I-J$^+$ GT-TsF1 recognizes (or is recognized by) I-E molecules, our data point in this direction.

Araneo and Kapp (23) found no MHC restriction between donors and recipi-

ents of GT-TsF in the suppression of T cell proliferative responses to GTMBSA. Our data are concordant with theirs with one notable exception and, therefore, we come to a different conclusion. Araneo and Kapp (23) showed the GT-specific T cell proliferative responses of GTMBSA-primed B10.A(4R) mice were suppressed by GT-TsF derived from H-2^d mice. We are unable to demonstrate suppression of the PFC responses of 4R mice either in vivo with H-2^d (I-J^d) GT-TsF1 (Table IV) or in vitro with I-J^k GT-TsF1 (Table V). This disparity between T cell proliferation and PFC data is probably best explained by the fact that each assay measures different cell populations. Recently, we have shown (24, 25) that T cell proliferative, delayed hypersensitivity and PFC responses are not always concordant, when assigning immune response (*Ir*) gene responder status in the GAT and GT copolymer system. 4R mice do not express I-E surface molecules and GT-TsF1 does not suppress their GTMBSA PFC response. This is not to say that 4R mice cannot be suppressed by any GT-specific factor. GT suppression results from a cascade of suppressive events involving several distinct suppressor cells and their factors (12). Neither monoclonal GT-TsF1 nor GT-TsF2 suppress 4R mice, indicating a lack of suitable cellular target for these factors. On the other hand, monoclonal GT-TsF3 suppresses the GTMBSA PFC responses of 4R mice (Table V) showing a functional target cell for TsF3. It is possible that the factor-containing extract used by Araneo and Kapp (23) may have contained sufficient GT-TsF3 to suppress the T cell proliferative responses of 4R mice.

In the present study, we find that GT-TsF1 is restricted to recipients expressing I-E, and A.SW and SJL mice that do not express surface I-E (19), are not suppressed by GT-TsF1 (Table II). 5R mice, unlike parental B10 mice, express I-E, and are suppressed by GT-TsF1. Possible interference in the suppressive process by I-A^b is ruled out, because B6, B10, A.BY (nonsuppressible), and 5R (suppressible) mice all express I-A^b. A.TFR5 mice, which express low levels of E^k_α, are not suppressed by GT-TsF1 (Table III). This raises the exciting possibility that either the surface I-E requirement for GT-TsF1 susceptibility is quantitative and A.TFR5 expresses too little, or that the I-E restriction requires expression of the E_β gene. Our laboratory is currently addressing this question. Indeed, ours is not the first laboratory to show the role of MHC class II molecules in suppressor systems. Dorf and co-workers (28, 29) showed the involvement of I-A–bearing APCs in Ts1 and Ts3 induction. They suggest that the presence of I-A on cells responsible to Ts induction does not necessarily imply a direct involvement of I-A in the Ts induction process (30). Further, they suggest that Ia antigens are somehow involved in the induction of I-J determinants. Nagy and colleagues (31) were the first to report a role for I-E in suppression of the T cell response, and that mouse strains expressing I-E^k are nonresponders to lactate dehydrogenase B. Nonresponsiveness is mediated by E_β-specific Ts (31) and an E_β-specific TsF (32). Our present data using GT-TsF1 are consistent with the E_β restriction.

Originally, the I-J subregion was defined and mapped by Murphy et al. (5) to a segment of the 17th chromosome of the murine MHC between the I-A and I-E subregions. Steinmetz and co-workers (33, 34) found that this portion of the 17th chromosome contained <3.4 kb DNA available for the I-J subregion, which is generally considered insufficient to encode for a single molecule ≥20 kDa.

Nevertheless, several laboratories have reported I-J heterogeneity and their selective expression on different cellular (sub)sets. Anti-I-J^k mAb detects I-J epitopes which are selectively expressed by different monoclonal TsF and have been used to phenotypically distinguish Ts subsets (15, 35, 36). Evidence for the expression of I-J expression by T cells other Ts has been reported. Tada and Hayakawa (10) showed that some Th cells express I-J determinants distinct from those found on Ts. Gershon et al. (37) described contrasuppressor T (Tcs) cells that are suppressor antagonists and express unique I-J determinants. Niederhuber et al. (38), using alloantisera, found I-J determinants on APCs. Murphy et al. (4), using alloantisera, showed I-J (J_2) determinants expressed by macrophages to be distinct Ts I-J (J_1) determinants. Likewise, Nakamura et al. (39) and Dorf and co-workers (40) have reported the requirement for I-J–bearing APCs in suppressor systems.

What is "I-J"? Tada (41) proposed that I-J molecules are self-recognition structures on T cells that recognize self MHC class II (i.e., I-E) molecules. Our data agree with this hypothesis. To a large extent much of the confusion surrounding I-J appears to be a matter of nomenclature. We propose that I-J structures are expressed *only* by T cells and they recognize self class II determinants on B cells and macrophages. During ontogeny, T cells develop a self-recognition repertoire (I-J molecules) for self class II molecules. Tada (41) calls these self-recognition structures antetopes; Murphy et al. (4) called these determinants expressed on Ts, J_1. Antibodies directed toward T cell self-recognition structures are correctly called anti–I-J antibodies. Alloantisera can contain both anti-antetope (or anti-J_1) antibodies (i.e., anti–I-J) *and* antibodies reactive with the Ia epitopes (Tada [41] calls these prototopes; Murphy et al. [4] calls these J_2 determinants) seen by the antetope. These anti-prototope antibodies should be reactive with class II MHC molecules found on B cells and macrophages. Moreover, we would predict that anti-idiotype antibodies directed against anti–I-J (anti-antetope) mAb should react with class II epitopes. Anti-idiotype antisera directed against either anti-I-J^k (WF8.C12.8) or anti-I-J^d (WF18.2B15) mAb bind B cells and macrophages (42). This same anti-idiotype blocks GT-TsF1–mediated suppression, in a manner similar to that of the present report. Therefore, expression of I-J determinants on cells other than T cells would appear to be artifactual.

We conclude that I-J is not H-2 encoded, but H-2 (e.g., I-E) influenced. Lack of sufficient DNA in the interval between E_β to E_α to account for a separate definable I-J locus (33, 34) would not be a problem in that I-J is not H-2 encoded. In fact, the MHC influences expression of several non-MHC genes (43). The MHC may influence the selective expression of non-MHC I-J genes, thus accounting for the apparent allelic nature of I-J. Still, several problems remain. First, how do B10.A(3R) and B10.A(5R) differ? Both appear to express identical I-E molecules in two-dimensional gels (44). Small conformational differences of the translated E_β polypeptide chain could account for the difference in self-recognition repertoire between 3R and 5R, or alternatively there is strain-specific posttranslational modification of I-E molecules. Second, if I-J recognizes I-E, then how are I-J^b molecules produced in H-2^b haplotypes, since H-2^b mice do not appear to express E_β surface molecules (44)? Possibly I-J^b is complementary

to I-A^b, or alternatively, a few, but sufficient, E_β cytoplasmic molecules are expressed on the surfaces of B cells and macrophages to allow the development of a I-J^b T cell repertoire. This can be addressed with the B6.C^{bm12} (*bm12*) mutant mouse. This mutation affects the A^b_β locus and arose by gene conversion from the E^b_β locus (45). If I-J^b structures are complementary to I-A^b molecules, then one would predict that the I-J^b repertoire of H-2^{bm12} *bm12* would be different from parental H-2^b B6. Finally, why haven't *I-J* genes been identified? The mechanism for generating diversity of the antigen-recognition repertoire of immunoglobulins and T cell receptors results from gene rearrangement. It is this very rearrangement together with the expression on T cells, but not B cells, that allowed the identification of T cell receptor genes (46). If, however, I-J molecules are genomically encoded and not rearranged and/or expressed by a very small subset of T cells, then our current technology may be unable to detect these genes.

Summary

Poly($Glu^{50}Tyr^{50}$) (GT) is not immunogenic in most inbred mouse strains. GT injection produces an I-J–bearing, GT-specific T-cell–derived suppressor factor (GT-TsF1) in H-$2^{b,d,k}$ haplotype mice. GT-TsF1 generates second-order suppressor T cells (Ts2) in H-$2^{a,d,k}$ haplotype mice. Here, we show that in order for GT-TsF1 to act, the recipient strain must express I-E molecules. This suggests that T cells are not the primary target of GT-TsF1. GT-TsF1 can be presented by Ia^+ A20-2J B lymphoma cells. GT-TsF1 presentation is blocked by anti–I-E, but not by anti–I-A, mAb, whereas GAT presentation is blocked by anti–I-A, but not by anti–I-E, mAbs. These data suggest that I-J recognizes (or is recognized by) I-E. The existence and role of I-J molecules in immune regulation are discussed in light of these data.

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