REGULATION OF IMMUNE RESPONSES
BY *I-J* GENE PRODUCTS

II. Presence of Both $I\text{-}J^b$ and $I\text{-}J^k$ Suppressor Factors
in (Nonsuppressor × Nonsuppressor)F_1 Mice*

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I-region genes regulate immune responses by two interrelated processes: (*a*) by controlling the manner in which antigen is presented to antigen-specific helper and suppressor T cells, and (*b*) by the production of antigen-specific T cell factors that help or suppress the immune responses of other immunocompetent cells (1). Responses to the synthetic antigen poly($Glu^{50}\text{-}Tyr^{50}$) (GT)[1] are controlled by genes of the *I*-region of the major histocompatibility complex (*H-2*) of the mouse (2). Most strains of mice have been shown to be GT nonresponders (3). This is not a result of a generalized B cell defect, as all strains make a very good antibody response to GT when it is coupled to the immunogenic carrier molecule methylated bovine serum albumin (GT-MBSA) (3, 4). Nonresponder strains of mice can be classified as either GT-suppressor strains ($H\text{-}2^{d,k,s}$), in which GT-MBSA antibody responses are suppressed by prior injection with GT and GT-nonsuppressor strains ($H\text{-}2^{a,b,q}$), which lack this suppressive effect (3). GT-specific suppression is under the control of at least two immune suppressor (*Is*) genes, which we have tentatively mapped to the *I-A* and *I-C* subregions of the *H-2* complex (4, 5). GT-primed suppressor haplotype mice produce a first-order T cell-derived suppressor factor (TsF) termed $GT\text{-}TsF_1$ which binds antigen, bears antigenic determinants of the *I-J* subregion, and functions largely by inducing, together with antigen, a second distinct subset of suppressor T (Ts_1) cells (6–11).

We have studied two GT-nonsuppressor mouse strains that differ in their genetic defects. Strain A mice ($H\text{-}2^a$) lack the ability to produce $GT\text{-}TsF_1$ after GT priming, however, they can generate Ts_2 upon exposure to the appropriate $GT\text{-}TsF_1$ (6, 8, 12). In contrast, C57BL/6 (B6, $H\text{-}2^b$) mice produce $GT\text{-}TsF_1$ after GT priming, although they cannot be suppressed by this or any other first-order TsF (6, 12). (B6 × A)F_1

hybrid mice, because of complementation which circumvents parental genetic defects, is a suppressor strain and can both produce and accept GT-TsF (12).

In this paper we report that (nonsuppressor × nonsuppressor)F_1 hybrid, (B6 × A)F_1 or (B10.GD × B10.A)F_1, mice produce GT-TsF of *both* parental *I-J* types. These factors were isolated with immunoadsorbent columns constructed with monoclonal anti-I-J antibodies. The isolated factors were tested for their ability to induce Ts_2 in vivo. We found that only one of the GT-specific factors produced by these F_1 hybrid mice is able to stimulate the generation of Ts_2. This not only demonstrates that these two factors are distinct both serologically and functionally, but also suggests, at least in these complementing F_1 hybrids, that distinct H-2-linked genes may control the production of first- and second-order TsF.

Materials and Methods

Animals. BALB/c mice (H-2^d) were purchased from Cumberland View Farms, Clinton, TN. (B6 × A)F_1 and (B10.GD × B10.A)F_1 mice were bred in the animal facilities of Harvard Medical School, Boston, MA. C57BL/6, C57BL/10, and A/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME. Mice used in these studies were 2-4 mo old and maintained on standard laboratory chow and water *ad libitum*.

Antigens. GT (lot 9; originally purchased from Miles Laboratories, Elkhart, IN) was the generous gift of Dr. Baruj Benacerraf, Harvard Medical School. Poly(Glu^{60}-Ala^{30}-Tyr^{10}) (GAT) (lot 1), and MBSA were purchased from Vega Biochemicals, Tucson, AZ. GAT, GT, and their MBSA complexes were prepared as previously described (8).

Antisera. Anti-I-J^k alloantisera were prepared by injecting (DBA/2 × B10.A[3R])F_1 mice every 2 wk with 10^7 B10.A(5R) lymphoid cells. Likewise, anti-I-J^b alloantisera were prepared by injecting (DBA/2 × B10.A[5R])F_1 mice with B10.A(3R) lymphoid cells. Recipient mice were bled after the 14th intraperitoneal injection. Monoclonal anti-I-J^b and anti-I-J^k antibodies were prepared as previously described (11).

GT-TsF Preparation. GT-TsF was prepared as previously described (6, 12). Briefly, mice were primed with 100 μg GT in Maalox (aluminum-magnesium hydroxide gel; Wm. Rorer, Fort Washington, PA) intraperitoneally 3-5 d before killing. Single cell suspensions of their spleens were prepared, adjusted to 6 × 10^8 cells/ml in Hanks' balanced salt solution (HBSS) and sonicated at 50 W for 5 min by a Sonifier Cell Disrupter (Ultrasonic Industries, Westbury, NY) equipped with a microtip. Sonicated material was centrifuged at 40,000 g for 45 min at 4°C, and the GT-TsF-containing supernatants were stored at −85°C until use.

Immunoadsorbent Columns. Sera and monoclonal antibodies used for the preparation of immunoadsorbent columns were heat-inactivated for 30 min at 56°C. Immunoadsorbent columns were prepared by coupling the immunoglobulin-rich fraction (precipitated by 50% ammonium sulfate, dissolved, and dialyzed against 0.5M NaCl-0.1M NaHCO$_3$ buffer) to cyanogen bromide (CNBr)-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) at 2 mg protein/ml packed gel as previously described (11). In all cases, coupling efficiency of protein to gel was >90%. Coupled gels were stored in phosphate-buffered saline (PBS) containing 0.02% NaN$_3$ at 4°C until use. Immunoadsorbent columns were prepared by packing 1 ml gel into a 3-ml syringe and exhaustively washing the column with PBS. "Crude" GT-TsF extract (0.1 ml, equivalent to 6 × 10^7 lymphoid cells) was incubated on the gel for 20 min at room temperature. The effluent was collected, the column was washed with 10 volumes of PBS, and the bound protein eluted with 0.1 M glycine-HCl buffer, pH 2.5. Eluted material was immediately neutralized to pH 7.2 with a small quantity of 2 M Tris. Concentrations of adsorbed materials are expressed according to original concentrations of factors.

Spleen Cell Cultures. Single cell suspensions of normal BALB/c (H-2^d) spleen cells were aseptically prepared and washed once with HBSS. Cells were resuspended to a final concentration of 16 × 10^6 cells/ml in Mishell-Dutton medium (13) containing 10^{-5} M 2-mercaptoethanol and 10% fetal calf serum (FCS; lot 2851, Pel-Freez Biologicals, Rogers, AR) (11). 2.5 μg GT as GT-MBSA was added to wells of a multiwell culture plate (76-033-05; Linbro Chemical Co., Hamden, CT) each containing 0.5 ml of cell suspension. GT-TsF or column-adsorbed GT-TsF

was added to cultures at final concentrations indicated in the table legends. Cultures were incubated for 5 d in a humidifed atmosphere of 83% N_2, 10% CO_2, and 7% O_2 at 37°C. Cultures were fed daily with a mixture of 50% nutritional cocktail and 50% FCS (11).

Induction of Ts₂. BALB/c mice were injected intravenously with 0.5 ml of immunoadsorbent-purified GT-TsF (3×10^7 cell equivalents) in the presence of a small amount (50 ng) of GT. The amount of GT added in all cases was at least 200 times less than the minimum tolerogenic dose of GT (9). 3 d after the injection of factor, mice were killed, their spleens were aseptically removed, single cell suspensions prepared, and 1.5×10^6 of these "factor-primed" cells were added to fresh Mishell-Dutton cultures containing 6.5×10^6 syngeneic spleen cells. GT-MBSA (2.5 μg) was added to appropriate cultures and cells were incubated for 5 d as described above.

Hemolytic Plaque Assay. Cultures were harvested 5 d after initiation, cells were washed three times in HBSS, and plaque-forming cell (PFC) responses were assayed using sheep erythrocytes (SRBC) coupled with the cross-reacting polymer GAT (14). GT-specific PFC were determined by subtracting the number of PFC detected in the presence of suitable dilution of GAT from the number of PFC detected on GAT-SRBC in the absence of the specific inhibitor. All assays were performed in duplicate and the number of PFC per culture are reported.

Results

Analysis of TsF Produced by Parental and F₁ Hybrid Mice. GT-specific immune suppression is controlled by at least two complementing *Is* genes (4, 5). GT-nonsuppressor strains can be divided into either of two categories depending upon which *Is* gene they lack. Table I summarizes the suppressive characteristics of the parental and F₁ hybrid mice used in the present studies. $H\text{-}2^b$ or $H\text{-}2^{g2}$ haplotype mice cannot be suppressed by GT preimmunization; GT injection results in GT-specific factor production, although these mice are not suppressed by GT-specific factors derived from antigen-primed mice. On the contrary, $H\text{-}2^a$ haplotype mice are unable to make GT-specific suppressor factors after GT injection, although they are readily suppressed by the appropriate GT-TsF. F₁ hybrid mice derived from the mating of the two types of nonsuppressor strains are readily suppressed by GT injection, with the concomitant production of GT-specific Ts and GT-TsF.

(B6 × A)F₁ Mice Produce Both I-J^k and I-J^b GT-TsF. $H\text{-}2^b$ (B6 and B10) and $H\text{-}2^a$ (A/J and B10.A) mice are GT-nonsuppressor strains, although (B6 × A)F₁ by virtue

TABLE I

Summary of Suppressive Activities of Parental and F₁ Hybrid Mice Used in This Study*

Strain	*H-2*	Sup-pressed by GT pre-immuni-zation	Produce GT-TsF₁	Sup-pressed by GT-TsF₁
C57BL/6 (B6)	*b*	−	+	−
C57BL/10 (B10)	*b*	−	+	−
D2.GD	*g2*	−	+	−
A/J or B10.A	*a*	−	−	+
(B6 × A)F₁	*b × a*	+	+	+
(B10.GD × B10.A)F₁	*g2 × a*	+	+	+

* Data presented in Table I have been published (2–6), or in the case of D2.GD, were previously unpublished. Extracts prepared from GT-primed D2.GD mice suppressed the GT-MBSA PFC response (3,585) of BALB/c spleen cells in vitro at 1:200 (<30), 1:400 (750), and 1:800 (2,580) final concentration.

of complementing *Is* genes derived from the parental strains are suppressed by GT preimmunization (4, 5, 12). We have also shown that $H\text{-}2^a$ mice can be suppressed by, although they cannot produce, GT-TsF$_1$, indicating that they lack or have impaired Ts$_1$ (6, 8, 12). $H\text{-}2^b$ mice, on the other hand, cannot accept GT-TsF$_1$, although they readily produce GT-TsF upon GT injection, indicating that they lack a suitable target (i.e., pre-Ts$_2$) for GT-TsF$_1$ (12). (B6 × A)F$_1$ mice, because the above-mentioned complementation of *Is* genes, can both produce and accept GT-TsF$_1$ and hence are GT-suppressor mice (5, 12). In the present experiments, we confirmed these results using a somewhat different assay scheme. Thus the factors were assayed in vitro using allogeneic BALB/c responding cells (Table II). In addition, we asked the *I-J*-type of the resulting F$_1$ GT-TsF. (B6 × A)F$_1$ mice were injected with 100 μg of GT in Maalox, 3 d later their spleens and thymuses were removed, and GT-TsF was extracted from these tissues. The F$_1$ GT-TsF was sequentially adsorbed on anti-I-J^k and anti-I-J^b alloantisera immunoadsorbent columns. The data shown in Table II show that (B6 × A)F$_1$ GT-TsF suppresses the GT-MBSA PFC responses of BALB/c mice in vitro. A control extract prepared from Maalox-injected (B6 × A)F$_1$ mice is not suppressive, but rather showed an enhancing effect. Adsorption of the F$_1$ GT-TsF on the anti-I-J^k columns shows that both the effluent (material not bound by the column) and the eluate of the column are suppressive. To guard against the possibility that we had merely saturated the column, the effluent from the first (anti-I-J^k) column was passed over an anti-I-J^b immunoadsorbent. It is apparent from Table II that the effluent from the first (anti-I-J^k) column contains no detectable *I-J^k*-bearing material as the effluent from the anti-I-J^b column is totally nonsuppressive. On the other hand, the eluate from the anti-I-J^b column is suppressive, indicating that the bound material bears *I-J^b* determinants. Similarly, the eluate from the first anti-I-J^k column is not bound by the anti-I-J^b column, but is found in the effluent of this second column. These data show that (B6 × A)F$_1$ mice produce both I-J^b and I-J^k GT-TsF.

Sequential Adsorption of (B6 × A)F$_1$ GT-TsF by Monoclonal Anti-I-J^k and Anti-I-J^b Immunoadsorbents. Our laboratory has recently produced monoclonal antibodies to

TABLE II
(B6 × A)F$_1$ Mice Produce Both I-J^b and I-J^k GT-TsF

Factor/fraction	Immunoadsorbent*	GT-specific PFC per culture‡	
—	—	945	
Control (B6 × A)F$_1$ extract	—	1,230	
(B6 × A)F$_1$ GT-TsF	—	548	
		Effluent	Eluate
(B6 × A)F$_1$ GT-TsF	Alloanti-I-J^k	150§	615‖
Effluent alloanti-I-J^k	Alloanti-I-J^b	1,680	<15
Eluate alloanti-I-J^k	Alloanti-I-J^b	<15	1,185

* Immunoadsorbent columns were constructed of anti-I-J^b or anti-I-J^k alloantisera coupled to CNBr-activated Sepharose as detailed in Materials and Methods.
‡ GT-specific PFC responses to 2.5 μg GT as GT-MBSA per culture of BALB/c spleen cells on day 5.
§ Effluent alloanti-I-J^k.
‖ Eluate alloanti-I-J^k.

$I\text{-}J^k$ and $I\text{-}J^b$ gene products (11). Ig fractions of the ascites fluids from anti-I-J hybridoma-bearing mice were coupled to CNBr-activated Sepharose 4B at a final protein concentration of 2 mg/ml. An extract derived from GT-primed (B6 × A)F_1 mice (GT-TsF) caused nearly total suppression of the GT-MBSA PFC response of BALB/c spleen cells in vitro (Table III). When (B6 × A)F_1 GT-TsF is passaged over a monoclonal anti-I-J^k immunoadsorbent column (WF8.C12.8) both the effluent and and eluate are suppressive. Similar to the result seen in Table II, the effluent from this first column can be shown to contain an $I\text{-}J^b$ GT-TsF, as is bound to and can be eluted from a monoclonal anti-I-J^b immunoadsorbent. Likewise, the acid eluate of the first anti-I-J^k column can be shown to contain $I\text{-}J^k$ but not $I\text{-}J^b$ GT-TsF, as none of its suppressive activity is bound by WF9.20.8-Sepharose. In subsequent studies, the monoclonal anti-I-J antibodies were used for the preparation of immunoadsorbent columns, as they showed a much clearer separation of $I\text{-}J^b$ from $I\text{-}J^k$ GT-TsF derived from these F_1 mice.

(B10.GD × B10.A)F_1 Mice Produce both I-J^k and I-J^b GT-TsF. Mice of the $H\text{-}2^{g2}$ (B10.GD) and $H\text{-}2^a$ (B10.A) haplotypes are not suppressed by GT preimmunization, although F_1 mice derived from these haplotype are suppressed by GT (4). As can be seen from Table IV, extracts derived from these GT-primed (B10.GD × B10.A)F_1 mice are able to totally suppress the GT-MBSA responses of BALB/c spleen cells in vitro. Similar to our findings in Tables II and III, (B10.GD × B10.A)F_1 mice produce both an I-J^b and an I-J^k GT-TsF as a consequence of GT priming. Furthermore, Table V indicates that the order of anti-I-J adsorption of the factors does not adversely affect the separation of the I-J^b and I-J^k GT-TsF.

In addition, Table V demonstrates that the $I\text{-}J^b$ and $I\text{-}J^k$ antigenic determinants are not found on the same molecule or molecular complex. The effluent, which is not bound by the WF8.C12.8 immunoadsorbent (anti-I-J^k), is bound to and can be eluted from WF9.40.5-Sepharose (anti-I-J^b). Similarly, when (B10.GD × B10.A)F_1 GT extract is adsorbed by the WF9.40.5 immunoadsorbent, the effluent contains $I\text{-}J^k$-bearing suppressor molecules. From the above findings, we conclude that both (B6 × A)F_1 and (B10.GD × B10.A)F_1 hybrid mice produce both I-J^b and I-J^k GT-TsF after GT priming. Since the (B10.GD × B10.A)F_1 hybrid mice should be homozygous

TABLE III

Adsorption of (B6 × A)F_1 GT Extract by monoclonal Anti-I-J^k and Anti-I-J^b

Factor/fraction	Immunoadsorbent*	GT-specific PFC per culture ‡	
—	—	555	
Control (B6 × A)F_1 extract	—	890	
GT-primed (B6 × A)F_1 extract	—	<10	
		Effluent	Eluate
GT-primed (B6 × A)F_2 Extract	WF8.C12.8 (anti-I-J^k)	<10§	35‖
Effluent WF8.C12.8	WF9.20.8 (anti-I-J^b)	995	<10
Eluate WF8.C12.8	WF9.20.8 (anti-I-J^b)	265	925

* Immunoadsorbent columns were constructed of the Ig-containing fractions of monoclonal anti-I-J^k or anti-I-J^b ascites fluids coupled to CNBr-activated Sepharose as detailed in Materials and Methods.
‡ GT-specific PFC responses to 2.5 μg GT as GT-MBSA per culture of BALB/c spleen cells on day 5.
§ Effluent WF8.C12.8.
‖ Eluate WF8.C12.8.

TABLE IV

Adsorption of (B10.GD × B10.A)F₁ GT-TsF by Monoclonal Anti-I-J^k and Anti-I-J^b

Factor/fraction	Immunoadsorbent*	GT-specific PFC per culture‡	
—	—	580	
Control (B10.GD × B10.A)F₁ extract	—	1,037	
(B10.GD × B10.A)F₁ GT-extract	—	<10	
		Effluent	Eluate
(B10.GD × B10.A)F₁ GT-extract	WF8.C12.8 (anti-I-J^k)	<10§	<10‖
Effluent WF8.C12.8	WF9.40.5 (anti-I-J^b)	1,362	<10
Eluate WF8.C12.8	WF9.40.5 (anti-I-J^b)	<10	1,132

* Immunoadsorbent columns were constructed from the Ig fractions of monoclonal anti-I-J^k- or anti-I-J^b ascites coupled to CNBr-activated Sepharose as detailed in the Materials and Methods.
‡ GT-specific PFC responses to 2.5 μg GT as GT-MBSA per culture on day 5.
§ Effluent WF8.C12.8.
‖ Eluate WF8.C12.8.

TABLE V

I-J^b and I-J^k Antigenic Determinants Are Not Found on the Same Molecule

Factor/fraction	Immunoadsorbent*	GT-specific PFC per culture‡	
Control (B10.GD × B10.A)F₁ extract	—	570	
(B10.GD × B10.A)F₁ GT-extract	—	155	
		Effluent	Eluate
(B10.GD × B10.A)F₁ GT-extract	WF8.C12.8 (anti-I-J^k)	95§	170
Effluent WF8.C12.8	WF9.40.5 (anti-I-J^b)	640	60
(B10.GD × B10.A)F₁ GT-Extract	WF9.40.5 (anti-I-J^b)	20‖	170
Effluent WF9.40.5	WF8.C12.8 (anti-I-J^k)	650	130

* Immunoadsorbent columns were constructed of monoclonal anti-I-J^k or anti-I-J^b ascites coupled to CNBr-activated Sepharose as detailed in the Materials and Methods.
‡ GT-specific PFC response to 2.5 μg GT as GT-MBSA per culture of BALB/c spleen cells on day 5.
§ Effluent WF8.C12.8.
‖ Effluent WF9.40.5.

at non-*H-2*-linked loci, we can conclude that the genes responsible for Ts₂ factor production are controlled by the *H-2* complex.

Ts₂ Induction by I-J^b but Not by I-J^k GT-TsF Derived from F₁ Hybrid Mice. We have previously demonstrated that B6 GT-TsF acts by inducing a second distinct population of suppressor T cells (Ts₂) (12). The question then arises whether the I-J^b and the I-J^k GT-TsFs derived from (B6 × A)F₁ or (B10.GD × B10.A)F₁ GT-primed mice can both induce Ts₂. Sequentially purified I-J^b or I-J^k GT-TsF from (B6 × A)F₁ or (B10.GD × B10.A)F₁ mice were injected along with 50 ng GT into BALB/c mice. 3 d later, 1.5 × 10⁶ of these spleen cells were added to 6.5 × 10⁶ fresh BALB/c spleen cells and cultured for 5 d in the presence of GT-MBSA. The results in Table VI clearly show that the I-J^b and I-J^k F₁ GT-TsFs from both hybrid mice cause >80% suppression of the GT-MBSA PFC response of BALB/c spleen cells when added directly to Mishell-Dutton cultures. However, when we assayed the ability of these TsF to generate second-order Ts, only the *I-J^b*-bearing GT-TsF is able to induce Ts₂;

TABLE VI

I-J^b but Not I-J^k GT-TsF from F$_1$ Hybrid Mice Induces Ts$_2$ in BALB/c Mice

TsF added to culture or used to generate Ts$_2$ cells*	GT-specific PFC‡ when following materials were added to culture	
	TsF§	Ts$_2$ cells‖
Experiment I		
None	1,345	1,033
I-J^b-bearing (B6 × A)F$_1$ GT-TsF	270 (80)¶	<10 (99)
I-J^k-bearing (B6 × A)F$_2$ GT-TsF	60 (96)	1,272 (0)
Experiment II		
None	1,345	585
I-J^b-bearing (B10.GD × B10.A)F$_1$ GT-TsF	195 (86)	195 (67)
I-J^k-bearing (B10.GD × B10.A)F$_1$ GT-TsF	535 (60)	1,358 (0)

* GT-TsF was prepared from either (B6 × A)F$_1$ or (B10.GD × B10.A)F$_1$ mice as indicated. *I-J^b*-bearing GT-TsF was prepared from the effluent anti-I-J^k (WF8.C12.8) then bound and eluted from anti-I-J^b (WF9.40.5) immunoadsorbents. Likewise, *I-J^k*-bearing GT-TsF was prepared from the effluent of WF9.40.5 then bound and eluted from WF8.C12.8 immunoadsorbents.

‡ GT-specific PFC responses to 2.5 μg GT as GT-MBSA per culture of BALB/c spleen cells on day 5.

§ Indicated GT-TsF added at 1:200 final dilution.

‖ BALB/c mice were injected 3 d previously with 0.5 ml HBSS containing 3 × 10^7 cell equivalents of indicated factor. 1.5 × 10^6 "factor-primed" spleen cells were added to 6.5 × 10^6 fresh BALB/c spleen cells as indicated.

¶ Percent suppression is in parentheses.

the *I-J^k*-bearing GT-TsF does not induce Ts$_2$. From these data we must conclude that not only are the I-J^b and I-J^k GT-TsF distinct antigenically, but they have distinct biological activities as well.

B6 GT-TsF Generates GT-TsF$_2$ in A/J Mice. The above data suggest that I-J^b and I-J^k GT-TsF produced by F$_1$ hybrid mice may act at different cellular levels. Since the I-J^b F$_1$ GT-TsF can readily generate Ts$_2$, whereas I-J^k F$_1$ GT-TsF cannot, we asked whether I-J^b F$_1$ GT-TsF acts by generating Ts$_2$ (i.e., it is a GT-TsF$_1$) and if the I-J^k F$_1$ GT-TsF is produced by Ts$_2$ (and hence a GT-TsF$_2$). Previous experiments have shown that GT priming of A/J (*H-2^a*) mice results neither in the generation of Ts nor in the production of GT-TsF. Therefore, B6 GT-TsF$_1$ (extract from GT-primed B6 mice) was injected intravenously into A/J mice in the absence of additional antigen. 5 d later an extract was prepared from the spleens and thymuses of these "factor-primed" mice. Table VII shows that the factor derived from B6 GT-TsF-primed A/J mice (provisionally called GT-TsF$_2$) can readily suppress the GT-MBSA response of BALB/c mice in vitro. That GT-TsF$_2$ is produced by the A/J mouse is demonstrated by the fact that it is bound by a monoclonal anti-I-J^k immunoasorbent column (WF8.C12.8) and not by a anti-I-J^b immunoadsorbent WF9.40.5), which rules out contamination by B6 GT-TsF$_1$. From these data we conclude that A/J mice, when primed with B6 I-J^b GT-TsF, have the ability to produce a second-order suppressor factor (I-J^k GT-TsF$_2$).

Specificity of GT-TsF$_2$. Table VIII shows that I-J^k GT-TsF$_2$ produced either in (B6 × A)F$_1$ or (B10.GD × B10.A)F$_1$ mice does not suppress the anti-SRBC PFC responses of BALB/c spleen cells in vitro. Likewise, the I-J^k GT-TsF$_2$ produced in A/J mice as

TABLE VII

B6 GT-TsF₁ Generates GT-TsF₂ in A/J Mice

Factor*	Treatment‡	GT-specific PFC per culture§	
		Experiment I	Experiment II
None	—	788	510
A/J GT-TsF₂	—	<15	160
A/J GT-TsF₂	Adsorbed WF8.C12.8 (anti-I-J^k)	1,624	1,020
A/J GT-TsF₂	Acid eluate WF8.C12.8	<15	120
A/J GT-TsF₂	Adsorbed WF9.40.5 (anti-I-J^b)	<15	NT‖
A/J GT-TsF₂	Acid eluate WF9.40.5	1,766	NT

* A/J (I-J^k) mice were injected 3 d previously with 0.5 ml of B6 (I-J^b) GT-TsF (3 × 10^7) cell equivalents. 3 d later extracts were prepared from spleens and thymuses of these "factor-primed" mice. Extract (A/J GT-TsF₂) equivalent to 1.5 × 10^6 lymphoid cells was added to indicated cultures.

‡ Extracts from B6 GT-TsF₁-primed A/J mice were adsorbed onto and eluted from indicated immunoad-sorbent columns as detailed in Materials and Methods.

§ GT-specific PFC responses to 2.5 μg GT as GT-MBSA per culture of BALB/c spleen cells on day 5.

‖ Not tested.

TABLE VIII

Antigen Specificity of I-J^k and I-J^b GT-TsF Derived from F₁ Hybrid and A/J Mice

Factor/fraction*	GT-specific PFC per culture‡	SRBC-specific PFC per culture§
Experiment I		
Control GT-MBSA/SRBC response	1,010	2,100
I-J^b-bearing (B6 × A)F₁ GT-TsF₁	350	1,900
I-J^k-bearing (B6 × A)F₁ GT-TsF₂	<20	1,900
Experiment II		
Control GT-MBSA/SRBC response	1,875	1,650
A/J I-J^k-bearing GT-TsF₂	<25	2,300

* I-J^b- and I-J^k-bearing (B6 × A)F₁ GT-TsF₁ and GT-TsF₂ were prepared by sequential adsorption as indicated in legend of Table VI and in Materials and Methods. A/J GT-TsF₂ was extracted from the spleens and thymuses of B6 GT-TsF₁-primed A/J mice as outlined in legend of Table VII.

‡ GT-specific PFC responses to 2.5 μg GT as GT-MBSA per culture of BALB/c spleen cells on day 5.

§ SRBC-specific PFC responses to 2 × 10^6 SRBC per culture of BALB/c spleen cells on day 5.

the result of B6 GT-TsF₁ priming does not suppress the anti-SRBC response in a nonspecific manner.

Discussion

Genetic defects are useful in dissecting T cell subsets involved in antigen-specific immune suppression. Previous studies have shown a heterogeneity in *Is* gene defects in GT-nonsuppressor strains of mice (2, 8, 12). Strain A (*H-2^a*, I-J^k) mice lack the ability to produce GT-TsF₁, but can produce Ts₂ upon exposure to the appropriate GT-TsF₁. In contrast, B6 or B10 (*H-2^b*, I-J^b) mice readily produce GT-TsF₁ after GT injection, although they lack a suitable target for this factor (i.e., pre-Ts₂). Gene

complementation between $H\text{-}2^a$ and $H\text{-}2^b$ or $H\text{-}2^{g2}$ nonsuppressor parental strains results in GT-suppressor F_1 hybrid mice. In this paper we report that (nonsuppressor × nonsuppressor)F_1 hybrid mice produce GT-TsFs of both parental haplotypes. Using monoclonal anti-I-J antibodies that display little or no cross-reactivity between the $I\text{-}J^b$ and $I\text{-}J^k$ haplotypes (11), we have demonstrated that the $I\text{-}J^b$ and $I\text{-}J^k$ antigenic determinants appear on different molecules or molecular complexes. The present studies show that only the I-J^b GT-TsF, derived from (nonsuppressor × nonsuppressor)F_1 mice, is able to induce Ts$_2$. This is consistent with the observation that injection of B6 I-J^b GT-TsF$_1$ plus 50 ng GT into A/J mice results in the production of an antigen-specific $I\text{-}J^k$-bearing GT-TsF$_2$ (12). The genetic control of GT-specific suppression has been shown to be under the control of two (or more) Is genes, tentatively mapped in the $I\text{-}A$ and $I\text{-}C$ subregions of the $H\text{-}2$ complex (4, 5). Our present data demonstrate that GT-specific suppression is a result of the generation of two subsets of Ts and suggest that the genetic defects found in the $H\text{-}2^a$ and $H\text{-}2^b$ mice are at the level of Ts$_1$ and Ts$_2$ (or Ts$_3$) cells, respectively.

The failure to detect $I\text{-}J^k$-bearing TsF$_1$ in A/J, B10.A, or F_1 mice derived from crosses involving these strains (6, 12) suggests that there is a obligate defect in the ability of these mice to produce GT-TsF$_1$-inducing factor with $I\text{-}J^k$ determinants. This implies that one site of Is gene action is at the Ts$_1$ cell or its factor. Furthermore, the present results suggest that the Is gene phenotype is dominant in F_1 animals, thus (B6 × A)F_1 or (B10.A × B10.GD)F_1 hybrid mice, like the parental A/J and B10.A strains, fail to produce an $I\text{-}J^k$-bearing, GT-specific TsF$_1$-inducing factor. Is gene complementation may occur through a different cellular mechanism than was previously observed for Ir gene complementation (15–17). In the present system, complementation does not restore the ability to generate $I\text{-}J^k$-bearing TsF$_1$-inducing factor, but seems to represent complementation of two distinct genetic defects, i.e., the ability to produce or accept TsF$_1$. This is in sharp contrast to complementation for $H\text{-}2$-linked Ir genes in which one observes restoration of a common genetic defect in antigen-presenting cells (17). Furthermore, in accord with previous suggestions (2, 5, 12), Is gene complementation would require pairing of two distinct $H\text{-}2$-controlled defects involving different cells required for immune suppression. Because it is postulated that both of these cellular defects are controlled by genes of the I-region, one must also postulate that GT-TsF$_1$ which function in these F_1 hybrids is not $H\text{-}2$ restricted. The ability of $I\text{-}J^b$-derived factors to suppress allogeneic BALB/c ($H\text{-}2^d$) GT-MBSA PFC responses supports this contention.

Analysis of T cell function has revealed that antigen-specific immunosuppression is mediated through the interaction of various subsets of suppressor T cells (Ts$_1$, Ts$_2$, Ts$_3$) and their factors (TsF$_1$, TsF$_2$, TsF$_3$). First- and second-order suppressor factors have been reported for the nitrophenyl (NP), azobenzene arsonate (ABA), and keyhole limpet hemocyanin (KLH) suppressor systems (18–20). In addition, Ts$_3$ cells and factors have been described in the NP system. The $I\text{-}J^b$-bearing GT-specific factor derived from F_1 hybrid mice, in the present study, resembles the GT-TsF$_1$ TsF$_1$ as originally defined (8), the $I\text{-}J^k$-bearing GT-TsF appears to differ from the above reported factors in several important respects. The KLH-TsF$_3$, ABA-TsF$_2$, NP-TsF$_2$ and NP-TsF$_3$ are $H\text{-}2$ restricted in their action. The $I\text{-}J^k$-bearing GT-TsF reported in the present study failed to show $H\text{-}2$ restriction as the suppressive activity was assayed in third-party BALB/c ($H\text{-}2^d$, $I\text{-}J^d$) mice. The present data also indicate that $Igh\text{-}V$

gene restriction is not seen with either GT-TsF factor; in contrast, Igh restriction has been reported for the SRBC-, ABA-, KLH-, and NP-antigen systems (18–21). It is important to note that the lack of V_h and *H-2* restriction seen in the GT-antigen system may be a result of how the factors are assayed. By assaying TsF activity in allogeneic mice we would not detect *H-2*- or *Igh*-restricted factors. Presently, experiments are underway to detect additional GT-TsF which display such genetic restrictions. However, in the GT and GAT antigen systems, all strains of mice appear to produce a similar idiotype (22, 23) and, consequently, one may not be able to detect *Igh* restriction if the idiotype is nonpolymorphic. An additional reason for lack of *H-2* restriction in the GT system, may result from how the factors are prepared. In the present studies, mice were injected with GT 3–5 d before factor preparation. In contrast, the ABA-factor is prepared 7 d after antigen injection (19), NP-TsF 7 d (18), and the KLH-TsF is prepared 2 wk after secondary injection with KLH (20). Differences in the interval between injection of antigen and extraction of factor may result in the preparation of TsF from animals in different immunological states. Different kinetic activities of Ts subsets would help explain why each antigen system would show different restrictions. It may be that the physiological state as well as genotype of the animal may influence which Ts subset(s) predominate after antigen injection, i.e., the longer the interval between antigen injection and factor preparation, the more restricted the suppression.

The administration of microliter quantities of anti-I-J antibodies intravenously to mice has been shown to augment both humoral (24) and cellular (25) immunity, apparently through the inactivation of Ts. We have recently shown that some anti-I-J monoclonal antibodies can augment a humoral immune response although they are unable to bind TsF (11). *I-J* gene products have been shown to be expressed on helper T (Th) cells and macrophage subsets as well as Ts (7, 26). Tada et al. (27) have shown that the *I-J* determinant found on Th is distinct from that found on Ts. Collectively, these results suggest a heterogeneity of *I-J* gene products. Data presented in the present study suggest that there may be even more heterogeneity within the *I-J* subregion than has been heretofore reported with regard to *I-J* expression on Ts (7). We have shown that the (nonsuppressor × nonsuppressor)F_1 hybrid mice produce an I-J^b (and not an I-J^k) GT-TsF$_1$ and an I-J^k (not an I-J^b) GT-TsF$_2$. In addition, we have looked for I-J^k GT-TsF$_1$ in concentrations 10-fold greater than those used in the above studies and have seen no suppressive activity. There are several possibilities for this finding; first, the F_1 hybrid animal may make GT-TsFs of both parental haplotypes in quantitatively different amounts; our assay may only detect the predominant one in the cell extract. We have produced T cell hybridomas from (nonsuppressor × nonsuppressor)F_1 GT-primed mice and are currently exploring this possibility. The second possibility is that I-J^b and I-J^k gene products are allelically excluded on F_1 cells and only Ts$_1$ and Ts$_2$ cells of the appropriate *I-J* phenotype are induced. As an alternative, only I-J^b Ts$_1$ and I-J^k Ts$_2$ are detected functionally, although genotypically both I-J^b and I-J^k Ts$_1$ and Ts$_2$ may be present. The third possibility is that the *I-J* subregion may be comprised of multiple loci. Therefore, I-J^b Ts$_1$ and I-J^k Ts$_2$ would represent separate gene products from different gene loci located within the *I-J* subregion, each contributed the respective parental haplotype. This last possibility is most intriguing and would help explain the regulatory role for

the *I-J* subregion and the selective expression *I-J* gene products on different subpopulations of regulatory T cells and macrophages (20, 26, 27).

Summary

Antigen-specific suppression to poly(Glu^{50}-Tyr^{50}) (GT) is under the control of two complementary immune suppressor (*Is*) genes located in the major histocompatibility (*H-2*) complex of the mouse. Suppressor strains of mice produce both suppressor T (Ts) cells and Ts-derived suppressor factors (TsF) that bear antigenic determinants of the *I-J* subregion of the *H-2* complex. Nonsuppressor strains of mice, on the other hand, are not suppressed by GT preimmunization. These nonsuppressor mice, however, can be classified according to those that lack the ability to make GT-specific T cell-derived suppressor factor (GT-TsF) after GT injection (i.e., $H-2^a$, $I-J^k$ mice) and those that lack the ability to be suppressed by the appropriate GT-TsF (i.e., $H-2^{b, g2}$, $I-J^b$ mice). In the present study, we demonstrate that $(H-2^a \times H-2^{b, g2})F_1$ hybrid mice produce distinct GT-specific suppressor factors of both parental *I-J* haplotypes. Moreover, only the $I-J^b$-bearing GT-TsF derived from these F_1 hybrid mice is able to induce second-order suppressor cells (Ts_2). This is consistent with the observation that injection of GT-TsF_1 derived from C57BL/6 ($I-J^b$) mice into A/J ($I-J^k$) mice leads to the production of an antigen-specific I-J^k GT-TsF_2. Our results suggest that *Is* gene complementation occurs through a different cellular mechanism than was previously observed for *Ir* gene complementation. Further, we show that complementing (non-suppressor × nonsuppressor)F_1 hybrid mice produce an $I-J^b$ (and not an $I-J^k$) GT-TsF_1 and an I-J^k (not an I-J^b) GT-TsF_2, thus suggesting a heterogeneity of *Ia* loci within the *I-J* subregion. Data presented in the present study suggest that there may be even more heterogeneity within the *I-J* subregion than has been heretofore reported with regard to *I-J* expression on Ts.

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