ANTIGEN-SPECIFIC T CELL-MEDIATED SUPPRESSION

V. H-2-linked Genetic Control of Distinct Antigen-specific Defects in the Production and Activity of L-Glutamic Acid^{50}-L-Tyrosine^{50} Suppressor Factor*

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The signals which activate suppressor T cells (Ts)[1] are poorly understood. Thus, although a wide array of procedures is known for inducing Ts responses (e.g., the use of deaggregated protein antigens, [1, 2], antigens coupled to syngeneic cells [3–6], low doses of intravenous antigen in vivo [7], or high concentrations in vitro [8–11], with or without macrophage depletion), no common cellular or molecular basis for such Ts induction is apparent from this information. In particular, the issues of antigen presentation on cell membranes and of H-2 restriction of Ts activity are unresolved. This stands in marked contrast to the well-documented H-2K, D, or I influence on antigen recognition and/or T cell activation seen in studies of other T cell subsets (12–14), and the importance of non-T cells bearing these H-2 products for both peripheral triggering and thymic differentiation (12–16). Only in a few cases has H-2 restriction been observed in studies on Ts (17–21), and these findings are by no means generalizable at present, in part because of conflicting results, and also because of the ease of demonstrating Ts binding to antigen in the absence of H-2 products (22–24). This problem is made more intractable because of the difficulty of designing experiments to test H-2 restriction of Ts at the cell level, caused (*a*) by the absence of a clear-cut antigen priming and restimulation test similar to T cell proliferation assays used to explore the role of Ia in macrophage-T cell interaction, (*b*) by the fact that Ts appear to act on other T cells (25–27), thus precluding most cell mixing experiments to test for H-2restriction, because of complicating allogeneic effects, and (*c*) by the paucity of models demonstrating antigen-specific genetic control of Ts function, in contrast with the numerous immune response (Ir) gene models so crucial to development of current theories of helper cell function (28).

This laboratory has focused on this last issue as a potential means of resolving some of the questions concerning Ts activity, by studying the response to the synthetic

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[1] *Abbreviations used in this paper:* GAT, random terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GT, random copolymer of L-glutamic acid^{50}-L-tyrosine^{50}; Ir, immune response; Is, immune suppression; KLH, keyhole limpet hemocyanin; MLR, mixed lymphocyte responses; MBSA, methylated bovine serum albumin; MLR, mixed lymphocyte response(s); PFC, plaque-forming cell(s); SRBC, sheep red blood cells; Ts, suppressor T cell(s); Ts_1, Ts-producing TsF; Ts_2, Ts induced by TsF; TsF, T cell-derived suppressor factor; V_H, variable portion of the Ig heavy chain.

polypeptide antigen, L-glutamic acid^{50}-L-tyrosine^{50} (GT). No inbred mice give a primary plaque-forming cell (PFC) response to GT, but strains fall into two categories, those which show GT-specific suppression (H-2^{d,s,k}) and those which do not (H-2^{a,b,q}) (29, 30). This suppressor response is under the control of two I region genes, termed immune suppression (Is) genes, tentatively mapped to the I-A and I-C subregions (31, 32). GT-induced Ts yield a soluble suppressor factor (TsF) which has an antigen-binding site, bears I-J subregion-coded determinants, has a mol wt of ~50,000, and functions in large measure by inducing, together with antigen, a second set of Ts, termed Ts_2, from unprimed syngeneic or allogeneic T cell populations (33–36).

The discovery of two discrete steps in the suppressor pathway, namely, TsF production, and Ts_2 generation, suggested the possibility of a heterogeneity in the locus of the Is defects in GT nonsuppressor strains. Previous studies have revealed that at least one strain, A/J (H-2^a), can be shown to lack the ability to produce GT-TsF, but to retain the ability to generate Ts_2 upon exposure to active GT-TsF (37). Furthermore, it has been found that F_1 hybrids or intra-H-2 recombinants between H-2^a and H-2^b nonsuppressor strains complement to produce GT-suppressor mice (32). This indicated that H-2^b mice might have a distinct defect from that of A/J, and would be TsF producers unable to be suppressed by this factor. The present report documents that this prediction is correct, and that these complementing Ts defects are under H-2 (Is) control. The implications of these data for understanding initial Ts_1 triggering, and subsequent Ts_1-Ts_2 communication, are discussed.

Materials and Methods

Animals. C57BL/6 (B6, H-2^b), A/J (H-2^a), (B6 × A/J)F_1 [(B6AF_1), H-2$^{b/a}$], C57BL/10 (B10, H-2^b), A.BY (H-2^b), B10.A (H-2^a), and SJL (H-2^s) mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. BALB/c (H-2^d) mice were purchased from Health Research, Inc., West Seneca, N. Y. All mice were maintained on standard laboratory chow and acidified water ad lib., and used at 8–20 wk of age.

Antigens. The random copolymers GT (lot 9, mol wt ~130,000) and L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT, lot 6, mol wt ~32,500) were purchased from Miles Laboratories, Inc., Elkhardt, Ind. Methylated bovine serum albumin (MBSA) was purchased from either Sigma Chemical Co., St. Louis, Mo., or Worthington Biochemical Corp., Freehold, N. J. Sheep blood was purchased from Gibco Diagnostics, Chagrin Falls, Ohio. GAT and GT solutions, MBSA complexes, and GAT-conjugated sheep red blood cells (GAT-SRBC) were prepared as previously described (29).

Preparation of TsF. GAT-TsF and GT-TsF were prepared as previously described (33, 38). In brief, mice serving as donors of cells for suppressor factor preparation were primed with either 10 μg GAT or 100 μg GT in Maalox (aluminum-magnesium hydroxide gel, Wm. Rorer, Inc., Fort Washington, Pa.) intraperitoneally, 3–7 d before use. These mice were then sacrificed, their spleens and thymuses removed, teased in balanced salt solution to provide a single cell suspension, washed, and suspended at 6 × 10^8/ml. This suspension was sonicated (50 W, 5 min) using a Sonifier Cell Disrupter (Ultrasonics Industries, Westbury, N. Y.) with microtip, and the resulting material centrifuged at 40,000 *g* for 1 h at 4°C. The supernatant was recovered and frozen at −60°C until use. This constituted (crude) GAT-TsF or GT-TsF. Similar extracts from mice given Maalox alone served as control factors.

Assay of TsF Activity. The presence or absence of suppressor factor in a given extract was assayed as previously described (36, 39). In short, direct suppression was assessed by addition of GT-TsF to modified Mishell-Dutton cultures, stimulated with GT-MBSA, and assayed on day 5 for IgG PFC using GAT-SRBC. Induction of Ts_2 was assessed by incubating spleen cells with GT-TsF for 2 d, washing the cultured cells, and transferring graded numbers to new Mishell-Dutton cultures, which were then stimulated and assayed as described above. Data are expressed as specific IgG PFC/culture, assayed on GAT-SRBC. Standard errors (usually <10%)

TABLE I

A/J and B6 Nonsuppressor Strains Complement for GT-Suppression in (B6A)F_1 Mice

Strain	Preimmunization*	Challenge‡	PFC/spleen§	Percentage of suppression
				%
A/J	None	50 μg GT-MBSA	5,900 ± 1591	—
	100 μg GT	50 μg GT-MBSA	4,000 ± 902	32‖
B6	None	50 μg GT-MBSA	7,050 ± 2576	—
	100 μg GT	50 μg GT-MBSA	7,325 ± 1359	0‖
(B6A)F_1	None	50 μg GT-MBSA	6,200 ± 2193	—
	100 μg GT	50 μg GT-MBSA	225 ± 14	96¶

* 100 μg GT in Maalox intraperitoneally 4 d before challenge.
‡ 50 μg GT as GT-MBSA in Maalox-pertussis intraperitoneally 7 d before assay.
§ Specific IgG PFC/spleen assayed on GAT-SRBC. Four animals per group.
‖ $P > 0.3$.
¶ $P = 0.03$.

are omitted for clarity. Ts_2 induction was also assayed by transferring the washed cultured cells (4×10^6) into syngeneic recipients, which were then challenged i.p. with either 20 μg of GAT or 50 μg of GT as GT-MBSA in 0.2 ml of saline containing 5% Maalox and 10^9 *B. pertussis* organisms (Eli Lilly, Indianapolis, Ind.) as adjuvant. Specific PFC were determined 7 d later using GAT-SRBC. Data are reported as PFC/spleen ± SEM. All indicated differences are significant at the $P < 0.05$ level, using Student's *t* test.

Results

(B6A)F_1 and B6, but Not A/J, Mice Produce Detectable GT-TsF. The data in Table I confirm previous studies (31, 32) showing B6 and A/J mice to be GT nonsuppressors, but nonetheless to possess Is genes able to complement for suppression in (B6A)F_1 animals. These findings, together with the information that A/J mice do not produce detectable GT-TsF, suggest that (B6A)F_1 mice can produce and be suppressed by GT-TsF, and that either *both* A/J and B6 contribute genes, which are necessary for *both* TsF generation and activity, or that the B6 mouse can produce GT-TsF, but not be suppressed by it. In the F_1, the two parts of the complete pathway would be brought together, yielding a suppressor animal. To test these hypotheses, extracts were prepared from spleens and thymuses of GT-primed A/J, B6, and (B6A)F_1 mice from the same groups of animals used to check suppressor status, as reported in Table I. The activity of such extracts and control extracts from animals receiving Maalox alone, as assayed by direct suppression of GT-MBSA responses in vitro, is shown in Figs. 1 and 2. Fig. 1 demonstrates that, as predicted, (B6A)F_1 and B6 mice produce GT-TsF active on the GT-MBSA response of the suppressor strain BALB/c. However, as already demonstrated (37), A/J mice fail to yield active GT-TsF, as assayed on BALB/c cells. Although these cells should be appropriate targets for any GT-TsF produced by the A/J cells because no H-2 barriers to GT-TsF activity in suppressor strains are known to exist, and in particular, BALB/c GT-TsF is active in A/J mice, to verify the absence of GT-TsF in sonicates of GT-primed A/J cells, the F_1 and A/J extracts were also retested on (B6A)F_1 cells. Such F_1 cells are the most likely candidate for an appropriate target for any cryptic A/J GT-TsF. As shown in Fig. 2, the F_1 GT-TsF had the expected activity on F_1 cells, but the A/J material was still devoid of suppressive potential. Although not shown, all factors presented in these figures and

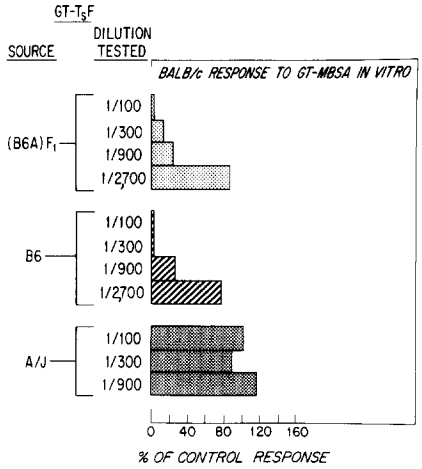

FIG. 1. B6 and (B6A)F_1, but not A/J, mice produce GT-TsF active on allogeneic BALB/c mice. The indicated factors were added at the given final dilutions to cultures of 7.5×10^6 BALB/c spleen cells stimulated with 2.5 μg GT as GT-MBSA. Specific IgG PFC were assayed on GAT-SRBC 5 d later, and are expressed as % control response

$$= \frac{\text{PFC with GT-TsF}}{\text{PFC with control extract}} \times 100\%.$$

the experiments reported below were tested for nonspecific suppression of SRBC and/or GAT responses, and shown to be specific for GT-MBSA responses.

A/J, but Not B6, Mice Can Be Suppressed by GT-TsF, and Generate Active Ts_2 upon TsF Exposure. Earlier studies also demonstrated that A/J mice could be directly suppressed by GT-TsF from another strain, and yield Ts_2 in vivo or in vitro when exposed to such GT-TsF (35, 36). Table II shows that B6 mice, in contrast, cannot be suppressed directly in vitro by (B6A)F_1 TsF. This defect is expected since in mice which produce GT-TsF, nonsuppressor status must result from a functional failure of TsF activity.

The ability of the nonsuppressor strain B6 to produce GT-TsF active in direct suppression of other strains raised the question of whether (*a*) this active extract also could cause Ts_2 induction, and (*b*) if so, whether the defect in B6 mice responsible for their nonsuppressor status was the inability to generate functional suppression at the Ts_2 or later stages in the pathway. It has already been shown that GAT-TsF fails to suppress directly GAT responder mice, but nonetheless can induce Ts_2 which are active in such responder animals (40). To explore these issues, in vitro generation of Ts_2 was attempted using (B6A)F_1 and B6 GT-TsF as well as extracts from GT-primed A/J mice. As shown in Fig. 3, B6 GT-TsF, but not the A/J extract, could induce active Ts_2 using A/J cells in vitro, which were able to suppress the GT-MBSA response of syngeneic A/J mice. Fig. 4 reveals, however, that although both B6 and (B6A)F_1 GT-TsF induce Ts_2 using A/J spleen cells, neither suppressor factor preparation can cause B6 spleen cells to become active suppressors. These data indicate either a failure of GT-TsF to produce Ts_2, or a failure of such Ts_2 to be able to mediate suppression, in B6 mice.

Specificity of the B6 Defect in TsF Activity. Because I region genetic control of

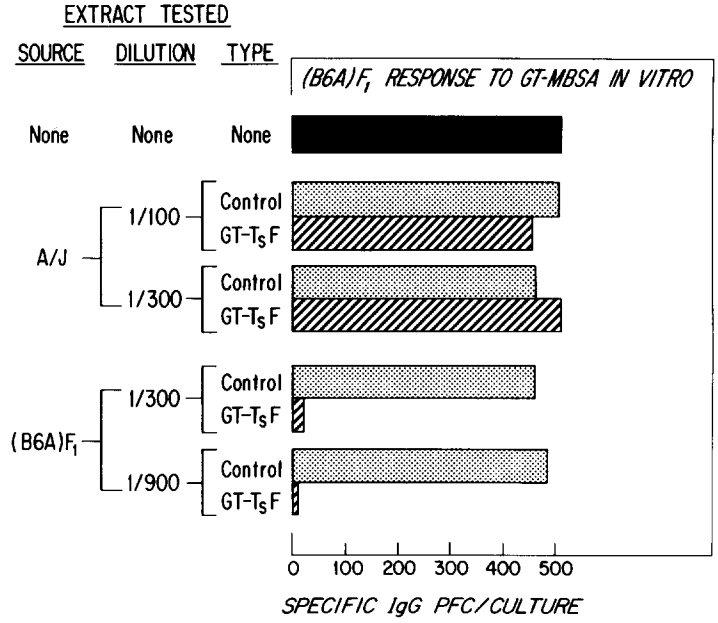

FIG. 2. Extracts from GT-immunized A/J mice fail to suppress (B6A)F_1-responding spleen cells. The protocol was the same as for Fig. 1, except data are expressed as PFC/culture of 7.5×10^6 (B6A)F_1 cells.

TABLE II

Failure of (B6A)F_1 GT-TsF to Suppress Directly B6 GT-MBSA PFC Responses in Vitro

Responding spleen cells*	Antigen‡	(B6A)F_1 GT-TsF§	Specific PFC/culture‖	Percentage of suppression¶
				%
(B6A)F_1	5 μg GT-MBSA	−	215	—
(B6A)F_1	5 μg GT-MBSA	+	<10	>95
A/J	5 μg GT-MBSA	−	220	—
A/J	5 μg GT-MBSA	+	<10	>95
B6	5 μg GT-MBSA	−	275	—
B6	5 μg GT-MBSA	+	370	0

* 7.5×10^6 cells/1 ml modified Mishell-Dutton culture.
‡ 5 μg GT as GT-MBSA added per 1 ml culture.
§ −, no factor added; +, 1/900 final dilution of (B6A)F_1 GT-TsF in the culture.
‖ Specific IgG PFC/culture on day 5, assayed on GAT-SRBC.
¶ Compared with group without TsF.

immunity is typically a highly antigen-specific phenomenon (28), the specificity of the defect in TsF activity in B6 mice was investigated. As noted above, GAT responder mice can be suppressed by nonresponder-derived GAT-TsF if such TsF is first permitted to induce GAT-Ts_2, either in vivo or in vitro (40). Thus, the ability of B6 (GAT responder) mice to yield Ts_2 active in suppressing GAT responses upon co-culture with GAT-TsF was tested. SJL mice, which are suppressors for both GAT and GT, were used to prepare GAT-TsF and GT-TsF, to avoid any complicating effects caused by use of TsF derived from different strains of mice. B6 spleen cells were

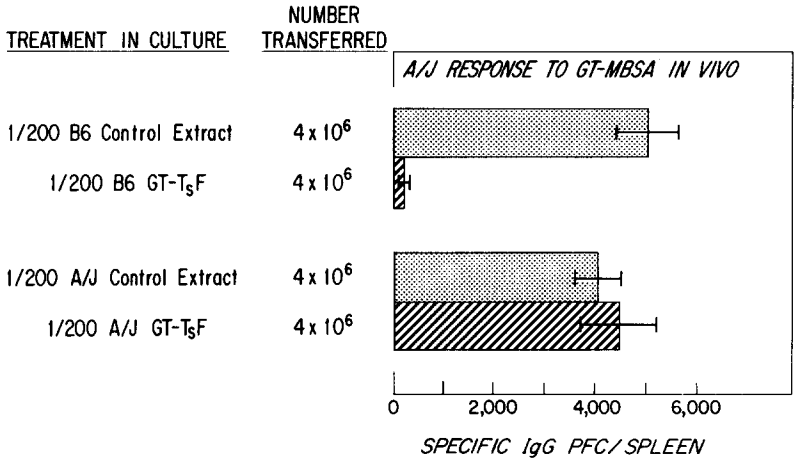

FIG. 3. Extracts from GT-primed B6, but not A/J, mice induce GT-Ts_2. A/J normal spleen cells were incubated for 2 d in vitro with 1/200 final dilutions of the indicated extracts. The recovered cells were washed and 4×10^6 viable cells transferred intravenously into each syngeneic A/J recipient, which was then challenged with 50 μg GT as GT-MBSA in Maalox-pertussis adjuvant. Specific IgG PFC were assayed on GAT-SRBC 7 d later, and the data are expressed as PFC/spleen ± SEM.

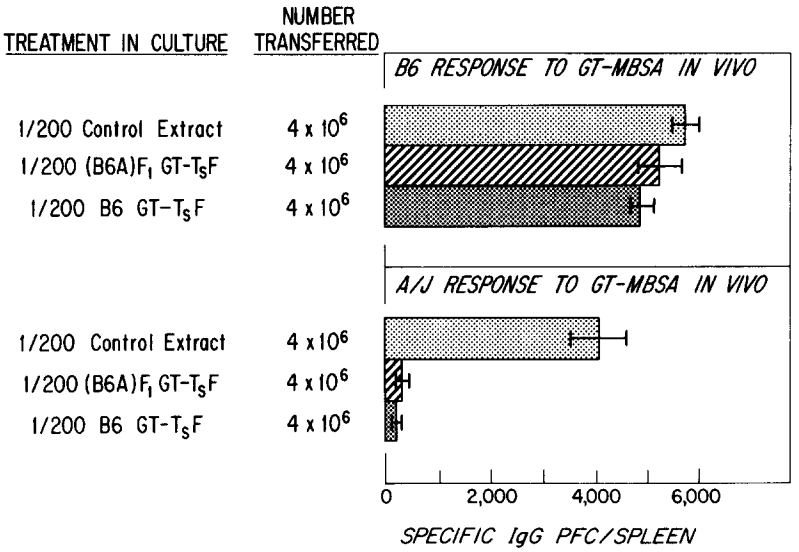

FIG. 4. A/J, but not B6, spleen cells show functional Ts_2 activity after exposure to GT-TsF. Same basic protocol as in Fig. 3.

incubated for 2 d in either factor, washed, and transferred in vivo into separate groups of B6 mice which were challenged either with GAT or GT-MBSA. As a positive control, A/J cells were cultured in SJL GT-TsF and transferred to A/J mice which were challenged with GT-MBSA. The results of one of several such experiments, shown in Fig. 5, clearly demonstrate that the defect in B6 mice is exquisitely antigen specific. Thus, only GT-TsF fails to induce active Ts_2 in B6 mice; GAT-TsF yields Ts_2 which efficiently suppress the GAT, but *not* GT-MBSA response of the B6 mice. The

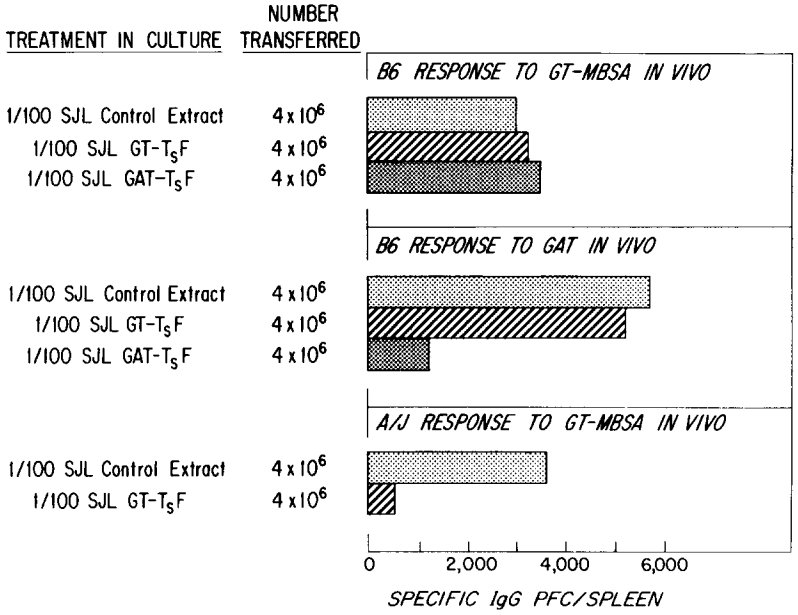

FIG. 5. The defect in Ts_2 activity in B6 mice is antigen specific. Same basic protocol as in Fig. 3, except 20 μg of GAT was used to challenge one set of B6 mice.

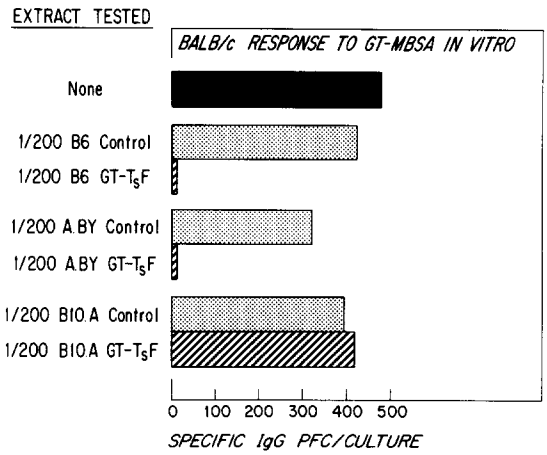

FIG. 6. H-2-linked control of GT-TsF production. Same basic protocol as in Fig. 1.

GT-TsF used was active, since it induced potent GT-Ts_2 in A/J mice.

H-2 Linkage of A/J and B6 Prototype Defects. Over-all suppression induced by GT priming is clearly under H-2-linked Is gene control (29–32). It was therefore expected that the selective defects in factor production and activity seen in A/J and B6 mice, respectively, would also be H-2 linked. A limited series of experiments has been performed to test this prediction. Extracts of spleen and thymus have been prepared from GT-primed B6 ($H\text{-}2^b$), A.BY ($H\text{-}2^b$), and B10.A ($H\text{-}2^a$) mice, and previously tested GT-TsF assayed on several of these same strains. As shown in Fig. 6, both $H\text{-}2^b$ strains B6 and A.BY produce active GT-TsF, while B10.A, an $H\text{-}2^a$ strain, fails to

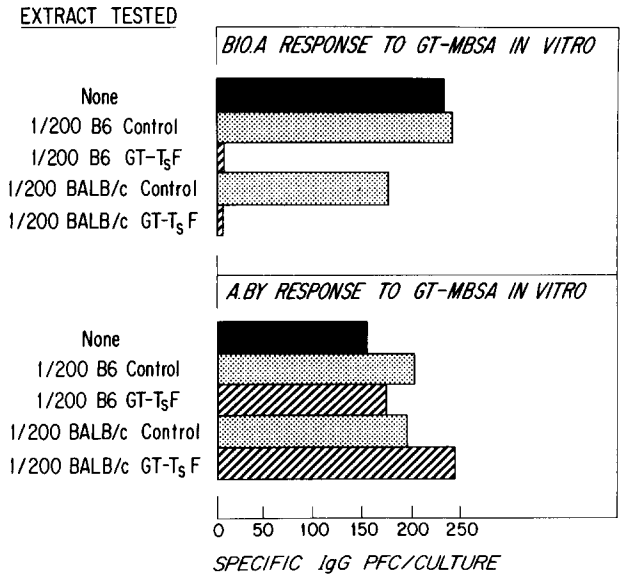

FIG. 7. H-2-linked control of GT-TsF activity. Same basic protocol as in Fig. 1.

TABLE III

H-2 Control of Direct Suppression Mediated by GT-TsF

Responding spleen cells*	Antigen‡	A.BY extract tested§	Specific PFC/culture‖	Percentage of suppression¶
B10 (H-2^b)	5 μg GT-MBSA	Control	270	—
B10 (H-2^b)	5 μg GT-MBSA	GT-TsF	833	0
B10.A (H-2^a)	5 μg GT-MBSA	Control	705	—
B10.A (H-2^a)	5 μg GT-MBSA	GT-TsF	15	98
B10.BR (H-2^k)	5 μg GT-MBSA	Control	758	—
B10.BR (H-2^k)	5 μg GT-MBSA	GT-TsF	<15	>98
A.BY (H-2^b)	5 μg GT-MBSA	Control	893	—
A.BY (H-2^b)	5 μg GT-MBSA	GT-TsF	750	16
A/J (H-2^a)	5 μg GT-MBSA	Control	502	—
A/J (H-2^a)	5 μg GT-MBSA	GT-TsF	127	75

* 7.5 × 10^6/1 ml modified Mishell-Dutton culture.
‡ 5 μg GT as GT-MBSA/1 ml culture.
§ 1/250 final dilution of control or GT-TsF.
‖ Specific IgG PFC/culture on day 5, assayed on GAT-SRBC.
¶ Compared with group receiving control extract.

yield active GT-TsF, as was the case for A/J. Thus, the ability to produce GT-TsF correlates with H-2 status (made by H-2^b, not by H-2^a), irrespective of background genes. Fig. 7 illustrates the converse situation: B10.A (H-2^a) cells can be suppressed by active GT-TsF, as already shown for A/J (H-2^a), while A.BY (H-2^b), like B6 (H-2^b), fails to be suppressed by the same GT-TsF preparations. Table III shows the same pattern with the pairs B10 (H-2^b)-B10.A (H-2^a) and A.BY (H-2^b)-A/J (H-2^a) in a single experiment. Again, H-2^b responses are not suppressed by GT-TsF, while H-2^a responses are markedly reduced by the same material. It should be noted that increased responses in H-2^b mice to extracts from GT suppressor strains have been

seen in several, though not all, experiments, and may reflect the presence of additional stimulatory factors in such preparations which are masked in those strains able to be suppressed by the predominant GT-TsF component.

Discussion

The data presented above document that independent antigen-specific H-2-linked genetic lesions can affect two previously defined steps in suppressor T cell pathways. Thus, H-2^a animals fail to produce detectable GT-TsF when challenged with GT. Nevertheless, such H-2^a animals possess cells able to mature into active Ts_2, when exposed to active GT-TsF from any of several allogeneic sources. Conversely, H-2^b mice produce GT-TsF after GT priming, but neither this factor nor that from other strains, can cause suppression of H-2^b responses to GT-MBSA. Either defect (lack of TsF production or failure of TsF action) is sufficient to render the animal phenotypically a GT nonsuppressor. Combination of H-2^a and H-2^b genomes in the form of F_1 mice such as (B6A)F_1 is sufficient to restore the ability to demonstrate GT suppression, involving GT-TsF generation and activity.

Several other groups have reported isolated genetic defects in either T suppressor factor production or activity, but these observations appear to differ from those described above. Thus, Taniguchi et al. (41) have shown that A/J mice fail to produce keyhole limpet hemocyanin (KLH)-TsF, but can be suppressed by KLH-TsF from other I-J^k mice. In the same model all B10 congenic mice produce KLH-TsF but fail to be suppressed by any I-J matched KLH-TsF. Although these defects resemble those reported here, Taniguchi et al. found that these KLH-related defects are *not* linked to H-2, but rather to background genes. In addition, B6 mice can be suppressed by KLH-TsF, in contrast to GT-TsF. It should also be noted that A/J mice have been shown to produce TsF active in some humoral (42) and cell-mediated (43) immune responses, indicating that the defects for KLH and GT do not reflect a general lesion at this level in these mice. Rich et al. (44) have demonstrated a failure of B6 mice to produce a suppressor factor active in decreasing mixed lymphocyte responses (MLR). These mice possess the structural gene for MLR-TsF, however, because F_1 mice between B6 and BALB/c yield TsF showing an I-C restriction for activity in the nonproducer strain. It remains to be seen how these similar but genetically distinct defects relate to one another.

The findings reported in this paper have particular significance for two aspects of suppressor cell function. First, by documenting a genetic basis for the previously proposed $Ts_1 \rightarrow Ts_2$ model of Ts function, they strengthen the concept of suppression as the end result of a series of T-T interactions involving distinct T cell subsets. Such T cell circuits have been described by several groups. In the GT and GAT models, it was first reported that TsF acted by inducing Ts_2, in vivo or in vitro, even in animals phenotypically nonsuppressor by virtue of a defect in TsF production (35, 36, 39, 40, 45, 46). This further agreed with the finding that suppressor mice, treated with cyclophosphamide, failed to produce GT-TsF but nonetheless could be suppressed by such suppressor material, suggesting two distinct T cell populations, differing in cyclophosphamide sensitivity, involved in suppression (47). Tada has reported almost identical findings for KLH-TsF (48). The target of this factor is a KLH-primed Ly 123^+ T cell which adheres to nylon wool, in contrast to the Ly 23^+ nylon nonadherent TsF producer. In both models, Ts_2 induction requires both I-J determinant-bearing

factor and specific antigen. The ability of TsF to induce new (Ts_2) suppressors has also been demonstrated for tumor-specific antigens (49), and for haptens using delayed type hypersensitivity models (50). Finally, the feedback suppression loop described by Eardley et al. (51) in the response to SRBC also involves T-T interactions which are apparently mediated by TsF (52). Thus, the GT model serves as a prototype of a generalizable model for T cell suppression, in which mice with distinct lesions in each step can serve as unique tools for studying selectively the origin of the defect and thus, the normal mechanism of Ts activation.

This last point relates directly to the issue of I region gene involvement in Ts activity. Previous studies have revealed that GT suppression is controlled by at least two complementing genes located in the I-A and I-C subregions of H-2 (32). These subregions do not appear to contain structural genes for GT-TsF, a function ascribed to a third gene located in the I-J subregion (34). It is therefore necessary to consider other possible ways in which I-A and I-C genes might control Ts activity in an antigen-specific manner. To us, the most likely hypothesis is that, in analogy with other T cell subsets, these genes produce cell surface glycoproteins involved in "antigen presentation." BALB/c ($I\text{-}A^d$, $I\text{-}C^d$) mice and B10.BR ($I\text{-}A^k$, $I\text{-}C^k$) mice each can produce and be suppressed by GT-TsF (33). Furthermore, B10.BR GT-TsF is active in BALB/c mice, and BALB/c GT-TsF is active in both B10.BR and A/J mice (37). Therefore, it appears that the defect in A/J ($I\text{-}A^k$, $I\text{-}C^d$) mice for production of GT-TsF cannot be ascribed to a problem with either one or the other Is gene, but must be due to a failure of the two genes to interact appropriately for the postulated presentation function (i.e., this system shows coupled complementation [53]). Thus, in a suppressor system Is control of GT-triggering of Ts_1 for TsF production would be analogous to Ir gene control of T_H activation for the terpolymer L-glutamic acid-L-lysine-L-phenylalanine (53, 54). Such a model leads to several testable predictions. First, in the complementing F_1 (B6 × A), successful GT presentation should elicit both $I\text{-}J^b$ and $I\text{-}J^k$ determinant-bearing TsF, since the postulated defect in A/J is not in a locus affecting TsF structure per se. For the same reason, the TsF produced by the suppressor B10.A(5R) strain which has its I-J subregion derived from the $H\text{-}2^a$ haplotype, should also be bound by anti-$I\text{-}J^k$ alloantibodies. Second, in the same $(B6A)F_1$, presentation of GT on parental cells should fail to evoke Ts_1 activity, whereas GT on B6 cells should trigger TsF formation. The identity of the hypothetical "presenting" cell is totally unknown, but is likely to be a subpopulation of either macrophages or T cells. Furthermore, the relevant Ia molecule involved in this presentation activity need not be the same as that found on conventional antigen presenting cells or B cells. In fact, such a notion is given support by the occurrence of Ts_1 activity in $H\text{-}2^b$ and $H\text{-}2^s$ mice, which lack serologically detectable E/C products (54), as well as the fact that macrophage and B cell Ia appears to be a product of I-A and I-E, not I-C, genes.

As concerns Is regulation of TsF function, exemplified by the defect in $H\text{-}2^b$ mice, a similar model involving I-A:C control of TsF presentation may be postulated. Recent studies have indicated that TsF molecules bear idiotypic determinants shared with antibody of the same antigen specificity, particularly in systems with common cross-reactive idiotypes (55, 56). Although T cell-mediated suppression is antigen specific, in these cases, idiotype-specific effects will appear antigen specific. Thus, triggering Ts_2 by idiotype-bearing TsF would involve the activation of suppressor cells

with anti-idiotypic specificity, as proposed previously by Singer and Williams (57). We have recently found that Ts_2 induced by azobenzenearsonate-specific TsF in A/J mice can be bound selectively to plastic plates coated with antiarsonate antibodies bearing idiotypic determinants shared with the TsF used to induce the Ts_2, thus confirming this prediction in at least one experimental system (50). Similarly, Eardley et al. have shown variable portion of the Ig heavy chain (V_H) restriction of T-T interactions in the feedback suppression loop (58). Ts_2 triggering in many cases may therefore involve the activation of anti-idiotypic cells recognizing the idiotype of TsF, perhaps in the form of idiotype-antigen complexes, since antigen is critical for Ts_2 triggering (39). Such recognition may require TsF presentation in the context of H-2 products, and therefore, the potential for Ir gene regulation. Thus, the failure of GT-TsF to cause Ts_2 activation may derive from failure of certain I-A:C gene products to mediate effective GT-TsF idiotype presentation to Ts_2 cells. An alternative explanation for the data is that the control of TsF activity resides in I-A:C-coded acceptor molecules on precursors of Ts_2. In this case, to account for the antigen specificity of the defect in $H\text{-}2^b$ mice, one would have to postulate that the I-A:C-coded acceptors are restricted with respect to the specificity of the TsF they can recognize due to selective V-C (V_H:I-J) association in factor formation (see Munro and Taussig [59] for details).

Finally, it should be noted that the accumulating data in the GT model, together with information derived from studies of many other investigators, strongly suggest that over-all immune regulation cannot be neatly divided into major histocompatibility complex vs. idiotype directed pathways. In particular, H-2-linked Ir genes may play a major role in controlling auto-anti-idiotypic responses. Thus, the products and activities of these two multigene clusters seem to be intimately interrelated in the activities of the complex networks that constitute the immune system.

Summary

The occurrence of distinct genetic defects affecting the generation of T cell-derived suppressor factor (TsF) or the suppressive activity of such TsF was investigated. For the synthetic polypeptide L-glutamic $acid^{50}$-L-$tyrosine^{50}$ (GT), it could be shown that the nonsuppressor strain A/J fails to produce suppressor T cells (Ts_1) capable of GT-TsF generation upon challenge with GT. Conversely, B6, another nonsuppressor strain, produces GT-TsF active on other allogeneic strains such as A/J, but itself fails to be suppressed by this material. $(B6A)F_1$ mice both make GT-TsF, and are suppressed by it. Further experiments revealed that the production of GT-TsF and the ability to be suppressed by GT-TsF are under the control of H-2-linked genes. Finally, the defect in GT-TsF activity in B6 mice was shown to be exquisitely antigen specific, in that this strain can be suppressed by a closely related TsF specific for L-glutamic $acid^{60}$-L-$alanine^{30}$-L-$tyrosine^{10}$. It is suggested that H-2 (I) control of suppressor T cell (Ts) activity may reflect the involvement of I-A and I-C gene products in antigen presentation to Ts in analogy with other T cell subsets, and that TsF function might also involve such presentation, in this case of the idiotypic structures of the TsF-combining site. Predictions deriving from this hypothesis are discussed, including the possibility that H-2 linked immune response genes regulate auto-anti-idiotypic responses in immune networks.

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