IMMUNOSUPPRESSIVE FACTORS FROM LYMPHOID CELLS OF NONRESPONDER MICE PRIMED WITH L-GLUTAMIC ACID^{60}-L-ALANINE^{30}-L-TYROSINE^{10}

IV. Lack of Strain Restrictions Among Allogeneic, Nonresponder Donors and Recipients*

By JUDITH A. KAPP

(From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and Department of Pathology and of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110)

Antibody responses to the synthetic terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT)[1] in mice are controlled by H-2-linked-immune response (Ir) genes (1). Injection of nonresponder mice, H-$2^{p,q,s}$, with GAT fails to stimulate GAT-specific plaque-forming cell (PFC) responses but does activate suppressor T cells (Ts cells) specific for GAT (GAT-Ts cells) (1, 2). GAT-Ts cells inhibit the development of GAT-specific IgG PFC responses by syngeneic mice immunized with GAT complexed to the carrier methylated bovine serum albumin (GAT-MBSA) in vivo and in vitro.

Extracts prepared from lymphoid cells of GAT-primed nonresponder, but not responder, mice specifically inhibit the development of antibody responses to GAT-MBSA by normal syngeneic nonresponder mice in vivo and in vitro (3). The suppressive factor in these extracts is a protein of approximately 45,000 mol wt (4) which can be extracted from purified T cells but not B cells, and is therefore referred to as GAT-TsF (5). GAT-TsF specifically binds to insolubilized GAT and can be eluted with 3.0 M KCl. Despite this affinity GAT-TsF does not bear μ, γ_1, γ_{2a}, γ_{2b}, or α-heavy chain or κ-light chain constant region determinants (4). However, GAT-TsF is absorbed by insolubilized antisera specific for determinants encoded by the I region of the H-2 gene complex (4), and therefore belongs to the same class of immunosuppressive molecules described by Tada et al. (6). In the crude lymphoid cell extracts, GAT-TsF is associated with an immunologically detectable fragment of GAT (4). However, the amount of GAT associated with GAT-TsF is, by itself, insufficient to cause suppression (4). Furthermore, GAT-TsF purified by absorption to and elution from insolubilized

[1] *Abbreviations used in this paper:* GAT, random terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GAT-MBSA, GAT complexed to methylated bovine serum albumin; GAT-SRBC, GAT complexed to sheep erythrocytes; GAT-Ts cells, GAT-specific suppressor T cells; GAT-TsF, GAT-specific suppressor T-cell factor; H-2, murine major histocompatibility complex; Ir gene, immune response gene(s); Mø, macrophage(s); PFC, plaque-forming cell(s); Ts cells, suppressor T cell(s).

GAT no longer contains detectable GAT; presumably, the fragment of GAT complexed to GAT-TsF is displaced by insolubilized GAT. Nevertheless, purified GAT-TsF bears Ia antigen and is suppressive, suggesting that the original GAT fragment associated with GAT-TsF is not essential for suppression. However, this observation does not establish that antigen is unnecessary for specific suppressive activity since the assay for activity requires the presence of GAT-MBSA.

GAT-TsF has been detected in extracts from two nonresponder strains of mice (DBA/1 and A.SW) (3). In addition, an extract from GAT-primed DBA/1 mice inhibits the response to GAT-MBSA by spleen cells from a histoincompatible nonresponder strain of mice (A.SW) but not a histoincompatible responder strain (C57BL/6) (5).

The observations that GAT-TsF is associated with a fragment of GAT in crude extracts and that GAT-TsF from one strain of nonresponder mice could suppress another histoincompatible nonresponder strain of mice distinguish this molecule from the carrier-specific TsF described by Tada et al. (6, 7). Carrier-specific TsF is not associated with antigen and suppresses immune responses only when donors and recipients are syngeneic at I-J.

To determine whether the suppression by GAT-TsF across histocompatibility barriers was peculiar to the two strains of mice tested or was a general characteristic of the GAT system, the following experiments were performed. GAT-TsF from mice bearing the three nonresponder H-$2^{p,q,s}$ haplotypes on a variety of non-H-2 backgrounds was prepared and analyzed for specific suppressor activity on in vitro PFC responses to GAT-MBSA by spleen cells from mice that were syngeneic or allogeneic with the GAT-TsF donor.

Materials and Methods

Mice. C57BL/10 (H-2^b), P/J (H-2^p), DBA/1 (H-2^q), SWR (H-2^q), and SJL (H-2^s) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. A.SW (H-2^s) and B10.S (H-2^s) mice were bred in the animal facilities at The Jewish Hospital by brother-sister mating of the offspring of breeder pairs obtained from The Jackson Laboratory. B10.P (H-2^p) mice were kindly provided by Dr. Donald Shreffler, Washington University School of Medicine. Mice used were 2- to 8-mo-old and were maintained on laboratory chow and water ad lib.

Antigens. GAT, mol wt 45,000, was purchased from Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind. MBSA (Worthington Biochemical Corp., Freehold, N. J.), sheep erythrocytes (SRBC), GAT and GAT-MBSA were prepared as described previously (1).

Preparation of Cell-Free Extracts. Cell-free extracts of lymphoid cells from GAT-primed mice were prepared as previously described (3). Briefly, mice were injected i.p. with 10 μg GAT in Maalox (Wm. H. Roher, Inc., Fort Washington, Pa.). These mice were sacrificed 3–7 days later, and single cell suspensions of spleens and thymuses were prepared, pooled, washed twice in Hanks' balanced salt solution, and resuspended to 6 × 10^8 cells/ml in Eagle's minimum essential medium supplemented with 4 mM Hepes, 2 mM L-glutamine and 50 U each penicillin and streptomycin (Microbiological Associates, Walkersville, Md.). The cells were sonicated at 7°C in a Sonifier Cell Disruptor model W-140-E (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.). The lysate was centrifuged at 40,000 g for 30 min and the supernate collected and stored at −80°C.

Antisera. Alloantisera specific for the (I-B, -J, -E, -C; S; G)^b (anti-I^b) and (I, S, G)^s (anti-I^s) regions of the *H-2* complex were donated by Dr. Shreffler. Anti-I^b was prepared by immunizing [B10.A (2R) × C3H.Q]F$_1$ with B10.A (4R) lymphoid cells. Anti-I^s was prepared by immunizing (A.TL × A.TFR3)F$_1$ with A.TH lymphoid cells. Anti-GAT sera was obtained from rabbits 7 days after the second subcutaneous immunization with 1 mg GAT in complete Freund's adjuvant.

Preparation of Immunoadsorbents. GAT-Sepharose 4B: GAT was coupled to amino hexyl-Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.) as previously described (4). Briefly,

GAT was dissolved in water and coupled to the beads with 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide (Story Chemical Corp., Muskegon, Mich.). The coupling conditions were empirically adjusted such that 1-2 mg GAT was coupled to 1 ml of packed Sepharose.

Anti-GAT, anti-I^b, and anti-I^s: all sera were heat inactivated at 56°C for 30 min and the globulin fractions were prepared by precipitation with 50% ammonium sulfate. The precipitates were dissolved in and extensively dialyzed against 0.5 M NaCl containing 0.1 M NaHCO$_3$. The globulin fractions of these sera were coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals) according to the manufacturers directions. Coupling conditions were adjusted to obtain 2 mg of protein per ml packed Sepharose.

Use of Immunoadsorbents. Extracts were diluted 1:10, applied to immunoadsorbent columns, and reacted at 4°C for 1-2 h. The unbound material was collected, the columns extensively washed with Hanks' balanced salt solution, and bound material was eluted with 3.0 M KCl. KCl was removed from the eluates by filtration through Sephadex G-25 (Pharmacia Fine Chemicals) (4).

Cell Cultures, Assay of GAT-TsF and PFC Assay. Replicate 1-ml cultures containing 8-10 × 10^6 spleen cells were established according to the modifications of the Mishell-Dutton system used in our laboratory (8) and dilutions of GAT-TsF and 5 μg GAT as GAT-MBSA or SRBC was added at culture initiation. PFC responses were assayed 5 days later using SRBC or GAT coupled to SRBC (GAT-SRBC) as indicator cells. The activity of GAT-TsF is expressed at S$_{50}$ U/ml which is defined as the inverse of the final dilution of extracts that causes 50% suppression of the PFC response. If no suppressive activity is observed at the lowest dilution tested, the titer is expressed as less than the inverse of the dilution. Conversely, if the 50% end point is not obtained at the highest dilution tested, the titer is recorded as greater than that dilution.

Results

GAT Stimulates Production of GAT-TsF in Nonresponder Strains of Mice Bearing H-2^{p,q,s} Haplotypes. Extracts prepared from lymphoid cells of GAT-primed B10.P (H-2^p), DBA/1 (H-2^q) and A.SW (H-2^s) mice were diluted and added to cultures containing spleen cells syngeneic with the GAT-TsF donors stimulated with SRBC or GAT-MBSA (Fig. 1). Control cultures containing B10.P, DBA/1, or A.SW spleen cells stimulated with GAT-MBSA in the absence of any extracts developed 340, 1,375, and 800 GAT-specific IgG PFC/culture, respectively. Although not shown, none of these extracts suppressed the IgM or IgG PFC responses to SRBC at dilutions of 1/400 or greater. Clearly, GAT-TsF can be produced by GAT-primed mice bearing any one of the three nonresponder haplotypes. Repeated titrations of a given extract have been remarkably consistent although some variation in suppressive activity from one batch to another has been observed (3).

Suppression of Immune Responses of Nonresponder Mice by Allogeneic GAT-TsF. Suppression of GAT-MBSA responses by spleen cells from five nonresponder strains of mice by an H-2^q GAT-TsF is shown in Table I. PFC responses to GAT-MBSA by nonresponder mice bearing H-2^p (P/J) and H-2^s (SJL) haplotypes but not responder mice bearing H-2^b (C57BL/10) haplotype were specifically suppressed by H-2^q (DBA/1 or SWR) GAT-TsF. Furthermore, responses by spleen cells from congenic strains (A.SW, B10.S and B10.P) bearing nonresponder *H-2* haplotypes were also suppressed by DBA/1 GAT-TsF regardless of the origin of non-*H-2* genes.

Extracts from GAT-primed B10.P and A.SW mice specifically suppressed PFC responses by B10.P, DBA/1, and A.SW spleen cells (Table II). The data in Tables I and II demonstrate that GAT-TsF from donors bearing any one of the three nonresponder haplotypes reciprocally suppressed recipients bearing any one of the three nonresponder haplotypes. In this experiment the activity of
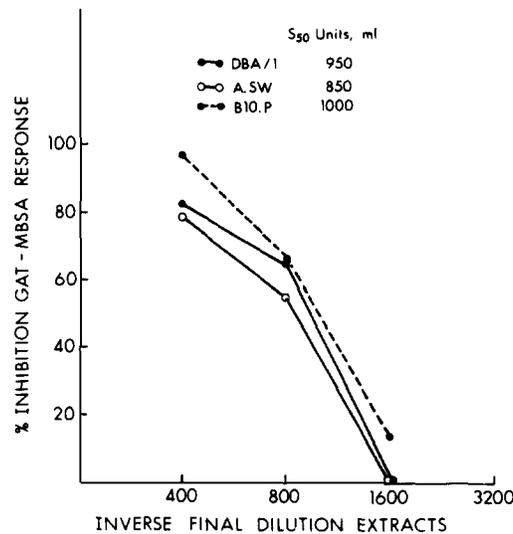
FIG. 1.   In vitro analysis of lymphoid cell extracts from GAT-primed DBA/1, A.SW, and B10.P mice. All extracts were prepared from pooled thymus and spleen cells (6 × 10^8 cells/ml) from mice primed i.p. 3 days earlier with 10 μg GAT in Maalox. Extracts were diluted and added to spleen cells from normal syngeneic mice at culture initiation. IgG PFC responses stimulated by GAT-MBSA and SRBC (not shown) were assayed after 5 days. Suppression is expressed as S$_{50}$ U/ml which is the inverse of the dilution of extract that causes 50% inhibition of the PFC response by control cultures.

GAT-TsF from A.SW mice was somewhat lower than that from B10.P or DBA/1 mice (Table I). Multiple extracts prepared from A.SW mice demonstrate fluctuations in levels of activity and frequently contain less activity than H-2^p or H-2^q extracts (data not shown).

The data in Table I demonstrated that non-H-2 genes did not detectably alter the susceptibility of recipient nonresponder spleen cells to a given GAT-TsF. Furthermore, nonresponder mice bearing the H-2^p or H-2^s haplotypes on a background of responder (C57BL/10 or A) genes were capable of producing GAT-TsF (Fig. 1 and Table II), suggesting that non-H-2 genes did not control production of GAT-TsF. The possibility that non-H-2 genes might subtly regulate production of GAT-TsF was examined in a greater detail by comparing suppressive activities of extracts from GAT-primed mice bearing the H-2^s haplotype and various non-H-2 genes on the immune response by DBA/1 spleen cells. In the experiment shown in Table III, no detectable differences were found in the level or specificity of suppression mediated by extracts of lymphocytes prepared simultaneously from age matched SJL, A.SW, or B10.S mice 3 days after priming with GAT, suggesting that responder non-H-2 genes do not regulate production of GAT-TsF.

Is Suppression of Responses by Syngeneic and Allogeneic Spleen Cells Mediated by the Same Molecule?   Although GAT-TsF mediated suppression of PFC responses by syngeneic and allogeneic recipient spleen cells which was antigen-specific, it is still possible that suppression of responses by allogeneic spleen cells is mediated by a moiety distinct from that active in syngeneic recipient spleen cells. We have previously demonstrated that suppression of

TABLE I

Suppression of Responses by Nonresponder Mice by GAT-TsF from H-2�q Mice

Spleen cells*	H-2	SRBC	GAT-MBSA
		S_{50} U/ml‡	
DBA/1	q	<400	1,600§
P/J‖	p	<400	1,600
SJL	s	<400	1,200
C57BL/10¶	b	<400	400
A.SW	s	<400	1,500
B10.S	s	<400	1,500
B10.P	p	<400	1,500

* Spleen cells cultured at 8–10 × 10^6 cells/ml.

‡ Inverse of the final dilution GAT-TsF causing 50% suppression of the PFC response.

§ Data in this Table are compiled from several experiments in which an H-2�q GAT-TsF was titrated on the immune response by spleen cells from DBA/1 and other nonresponder strains. The activity of GAT-TsF in individual experiments was normalized to the average activity on DBA/1 responses of 1,600 S_{50} U/ml.

‖ Spleen cells from P/J mice were treated with GAT-TsF from SWR (H-2�q) mice.

¶ C57BL/10 mice are responders to GAT.

TABLE II

Suppression of Responses by Nonresponder Mice of Different Haplotypes by GAT-TsF from H-2ᵖ or H-2ˢ Mice in Vitro

Spleen cells‡		H-2ᵖ GAT-TsF		H-2ˢ GAT-TsF	
Strain	H-2	SRBC	GAT-MBSA	SRBC	GAT-MBSA
		s_{50} U/ml*			
B10.P	p	<400	1,000	<400	1,000
DBA/1	q	<400	2,100	<200	930
A.SW	s	<400	1,600	<400	850

* Inverse final dilution of extracts from B10.P (H-2ᵖ) or A.SW (H-2ˢ) mice causing 50% suppression of the immune response.

‡ Spleen cells cultured aᵥ 8–10 × 10^6 cells/ml.

responses in DBA/1 spleen cells by DBA/1 GAT-TsF is mediated by a factor that bears determinants encoded by the *I* region of the H-2�q complex (4). Therefore, a crude extract from GAT-primed A.SW mice and the extract after absorption with insolubilized anti-Iᵇ or anti-Iˢ alloantisera were tested for suppression of responses by spleen cells from DBA/1 and A.SW mice (Table IV). Titration of these materials on syngeneic spleen cell responses indicated that H-2ˢ GAT-TsF was absorbed by an alloantisera reactive against Iˢ but not Iᵇ. Similarly, GAT-specific suppression of the PFC response by DBA/1 spleen cells occurred when these cells were incubated with the untreated H-2ˢ GAT-TsF and the extract which was passed over an anti-Iᵇ immunoadsorbent. Unbound material from an anti-Iˢ immunoadsorbent failed to suppress the DBA/1 PFC response.

Suppression of PFC Responses in Syngeneic and Allogeneic Recipient Spleen Cells by Crude and Purified GAT-TsF. In addition to suppressing responses

TABLE III

Immune Responses by DBA/1 Spleen Cells are Suppressed by Extracts from Mice Bearing the H-2ˢ Haplotype and Various Non-H-2 Genes

Source GAT-TsF	H-2 Haplotype of donor	SRBC	GAT-MBSA
		S_{50} U/ml*	
DBA/1	q	<400	1,450
SJL	s	<400	1,150
A.SW	s	<400	1,350
B10.S	s	<400	1,500

* See Table I.

TABLE IV

Suppressive Moiety in GAT-TsF from A.SW Mice Bears Antigens Encoded by the H-2 Complex

A.SW GAT-TsF Treatment	A.SW		DBA/1	
	SRBC	GAT-MBSA	SRBC	GAT-MBSA
	S_{50} U/ml*			
None	<400	1,150	<200	930
Absorption with anti-Iᵇ	480	2,100	<200	1,600
Absorption with anti-Iˢ	410	<400	<200	<200

* See Table I.

TABLE V

Suppression of Responses by Syngeneic and Allogeneic Spleen Cells with Crude and Purified GAT-TsF

Group	Treatment of DBA/1 GAT-TsF	DBA/1	B10.S
		S_{50} U/ml*	
A	None	3,500	3,800
B	Absorbed by Anti-GAT-Sepharose	500	NT‡
C	Absorbed by GAT-Sepharose	<400	NT
D	Eluted from GAT-Sepharose (GAT-TsFP)	5,600	5,800
E	GAT-TsFP Absorbed by Anti-GAT-Sepharose	5,800	NT

* Suppressive activity was titrated on PFC responses to GAT-MBSA. Although not shown the immune responses to SRBC by DBA/1 or B10.S spleen cells were not suppressed by any of the GAT-TsF fractions tested at dilutions of ≥1/400.
‡ Not tested.

to GAT-MBSA by allogeneic nonresponder mice, GAT-TsF differs from the carrier specific TsF described by Tada et al. (7) in that GAT-TsF is associated with a fragment of immunologically recognizable antigen. An experiment was designed to determine whether association with a GAT fragment accounted for suppression of allogeneic recipient mice by GAT-TsF (Table V). GAT-TsF from DBA/1 mice was specifically suppressive for both DBA/1 and B10.S GAT-MBSA responses (group A). Association of immunoreactive GAT with GAT-TsF in crude extracts was verified by absorbing the crude extract with insolubilized

rabbit anti-GAT globulin (group B) which reduced the S_{50} titer by 85%. GAT-TsF was also bound by GAT-Sepharose (group C) and was eluted from GAT-Sepharose with 3.0 M KCl (group D). The apparent increase in activity of eluted GAT-TsF is frequently observed and is most likely due to removal of nonspecific enhancing material present in crude extracts (3). Purified GAT-TsF, eluted from GAT-Sepharose, is no longer absorbed by rabbit anti-GAT-Sepharose (group E). Nevertheless, the PFC response by B10.S spleen cells to GAT-MBSA was suppressed by crude as well as purified DBA/1 GAT-TsF (group D). This observation suggests that the fragment of GAT associated with GAT-TsF in crude extracts is not responsible for the ability of GAT-TsF to suppress anti-GAT-MBSA PFC responses across histocompatibility barriers.

Discussion

These experiments demonstrate that lymphoid cell extracts from GAT-primed H-2^p mice, like extracts from mice bearing the other nonresponder haplotypes, H-2^q and H-2^s (3), contain GAT-specific immunosuppressive factors (Fig. 1). In addition, GAT-specific suppression of responses by allogeneic (H-2^s) nonresponder spleen cells by DBA/1 (H-2^q) GAT-TsF has been confirmed (5) and extended to other allogeneic nonresponder recipient spleen cells (Table I). Suppression by allogeneic GAT-TsF is not restricted by the presence of responder non-H-2 genes in recipients, since DBA/1 GAT-TsF suppressed responses by spleen cells from SJL (H-2^s) and the congeneic strains of A.SW (H-2^s) and B10.S (H-2^s) equally (Table I). More importantly, all nonresponder strains of mice, regardless of the source of non-H-2 genes are suppressed by GAT-TsF from all other strains of mice bearing the nonresponder H-$2^{p,q,s}$ haplotypes (Tables I and II). Extracts prepared from mice bearing the H-2^s haplotypes on non-H-2 gene backgrounds of SJL, A, or C57BL/10 suppressed the GAT-MBSA response of DBA/1 spleen cells with no detectable differences in antigen specificity or level of activity (Table III) suggesting that non-H-2 genes do not modulate the production of GAT-TsF.

Previously published data, demonstrated that DBA/1 GAT-TsF bears determinants encoded by the I region of the H-2 gene complex (7). Absorption of A.SW GAT-TsF by insolubilized alloantisera specific for I subregion of the H-2^s, but not H-2^b, gene complex demonstrates that GAT-TsF from a second nonresponder strain of mice bears determinants that map between the H-$2K$ and H-$2D$ regions (Table IV). Furthermore, the substance in A.SW GAT-TsF that suppressed responses by allogeneic recipient spleen cells also contains determinants encoded by the H-2^s gene complex (Table V). This observation suggests, but does not prove, that suppression of responses by syngeneic and allogeneic spleen cells is mediated by the same molecules. The conclusion that suppression by GAT-TsF does not require that donors and recipients are identical at the I-J subregion is tenuous since the I-J subregions of H-2^p and H-2^q have not yet been identified by recombination. Thus, the argument could be made that I-J^p, I-J^q, and I-J^s genes encode molecules that contained shared specificities and that suppression by GAT-TsF across histocompatibility barriers actually represents cross-reactivity. Indirect evidence against this possibility has been obtained by Thèze et al. who have shown that suppressive factors obtained from BALB/c

and B10.BR mice primed with the copolymer L-glutamic-acid[50]-L-tyrosine[50] (GT) bear distinct alloantigens encoded by the *I-J* subregion and nevertheless reciprocally suppress GT-MBSA responses (9).

One mechanism by which GAT-TsF suppresses GAT-MBSA PFC responses is by stimulation of GAT-Ts cells in the recipient (10). In addition, crude extracts containing GAT complexed to GAT-TsF stimulates GAT-Ts cells in recipient mice whereas purified GAT-TsF containing no detectable GAT does not (11). Furthermore, the addition of small amounts of GAT, in concentrations too low to stimulate Ts cells independently, to purified GAT-TsF restores the capacity to stimulate GAT-Ts cells (11). This observation raised the possibility that GAT-TsF complexed to a fragment of GAT (as it is found in crude extracts) might suppress across histocompatibility barriers by virtue of stimulating autologous GAT-Ts cells in the recipient and thereby masking potential restrictions between donors and recipients. To test this possibility, crude DBA/1 GAT-TsF was analyzed for the presence of immunoreactive GAT, and another portion of this extract was absorbed to GAT-Sepharose and purified GAT-TsF that was subsequently eluted from GAT-Sepharose was re-examined for the presence of a fragment of GAT. Each of these fractions were assayed for functional activity (Table V). Crude GAT-TsF containing GAT and purified GAT-TsF containing no detectable GAT determinants suppressed allogeneic as well as syngeneic responses (Table V). Thus, the ability of GAT-TsF to suppress across histocompatibility barriers cannot be attributed to the residual GAT complexed to GAT-TsF.

To date all antigen-specific suppressor T-cell factors bear determinants encoded by the *H-2* gene complex (reviewed in 12). Comparison of those TsF that bear alloantigens encoded by the *I-J* region indicates variability in strain restrictions between donors and recipients. The discrepancy between requirements for syngenicity at I-J between GAT-TsF and carrier-specific TsF remains unresolved. Data presented here demonstrate that suppression of syngeneic and allogeneic PFC responses are mediated by the same molecules and that the association of GAT-TsF with a fragment of GAT does not mask inherent strain restrictions between donors and recipients of GAT-TsF. At least two significant differences remain between the GAT-TsF and carrier-specific TsF systems. First, carrier-specific TsF is analyzed by measuring suppression of hapten-specific responses stimulated by complexes of hapten and homologous carriers, whereas suppression by GAT-TsF is measured on PFC response to GAT itself. Second, the activity of GAT-TsF is analyzed on primary-immune responses whereas the activity of carrier-specific TsF is routinely analyzed on secondary PFC responses. Data from several other systems demonstrate that efficient collaboration between antigen-primed T cells and macrophages (Mϕ) or B cells requires that these cells are syngeneic at the *I* region of the major histocompatibility complex; whereas these restrictions do not appear to govern interactions between Mϕ and virgin T cells or B cells, (Reviewed in 13–16). By analogy, the discrepancy between requirements for syngenicity between donors and recipients of GAT-TsF and carrier-specific TsF may be a function of the immunological status of the recipient rather than a fundamental difference between the mediators. Experiments to resolve this issue are currently in progress.

Summary

The synthetic terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) fails to stimulate development of GAT-specific antibody responses in nonresponder mice but stimulates development of GAT-specific suppressor T cells that inhibit the development of normal anti-GAT plaque-forming cell responses to GAT complexed to methylated bovine serum albumin (MBSA). Extracts from lymphoid cells of GAT-primed but not control, nonresponder (DBA/1) mice contain a T-cell factor (GAT-TsF) that also specifically suppresses responses to GAT-MBSA by normal syngeneic spleen cells. The experiments reported in this communication demonstrate that: (a) extracts from all GAT-primed nonresponder mice tested contain GAT-TsF; (b) non-*H-2* genes do not restrict the production of GAT-TsF; (c) all nonresponder strains of mice regardless of their non-*H-2* genes are suppressed by GAT-TsF from all other strains bearing the nonresponder *H-2*^{p,q,s} haplotypes; (d) suppression of GAT-MBSA responses by both syngeneic and allogeneic nonresponder spleen cells is mediated by a molecule encoded by the *H-2* gene complex; and (e) both syngeneic and allogeneic nonresponder mice are suppressed by purified GAT-TsF that lacks immunoreactive GAT.

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