GENETIC CONTROL OF SPECIFIC IMMUNE SUPPRESSION

II. *H-2*-Linked Dominant Genetic Control of Immune Suppression by the Random Copolymer L-Glutamic Acid^{50}-L-Tyrosine^{50} (GT)*

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In the companion paper, the random copolymer of L-glutamic acid^{50}-L-tyrosine^{50} (GT)[1] was shown to be unable to stimulate the formation of specific antibodies in 19 inbred and congenic resistant strains of mice (1). In several of these strains, however, GT complexed to methylated bovine serum albumin (GT-MBSA) was able to stimulate specific plaque-forming cell (PFC) responses. Furthermore, immunization with GT as early as 3 days and as late as 28 days before GT-MBSA was able to suppress a primary response to GT-MBSA in BALB/c mice. Unresponsiveness to GT-MBSA could be transferred to normal, syngeneic recipients with spleen cells or thymocytes from GT-primed BALB/c mice, demonstrating that GT is able to stimulate the development of suppressor cells in this strain of mouse.

In the present study, we have investigated whether GT preimmunization could inhibit the response to GT-MBSA in several inbred strains as well as congenic resistant strains of mice. Some strains of mice behave like BALB/c mice in this respect, whereas in other strains of mice, GT preimmunization does not have a tolerogenic effect on the response to GT-MBSA. The development of GT-specific unresponsiveness is inherited as a dominant trait. The development of specific immune suppression in response to GT immunization will be shown to be controlled by a gene or genes in the *H-2* major histocompatibility complex. In other experiments, we compared the specificity of the suppression induced by GT and by the copolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) on the response to these copolymers complexed with MBSA by mice bearing the nonresponder haplotypes *H-2*^{q} and *H-2*^{s}.

Materials and Methods

Mice. All mice were purchased from the Jackson Laboratories, Bar Harbor, Maine or the Health Research Laboratories, Buffalo, New York, or were bred in our animal facilities. Mice used

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[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; GAT, random terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GAT-MBSA, GAT complexed to methylated bovine serum albumin; GT, random copolymer of L-glutamic acid^{50}-L-tyrosine^{50}; GT-MBSA, GT complexed to methylated bovine serum albumin; MBSA, methylated bovine serum albumin; PFC, plaque-forming cells; SRBC, sheep red blood cells.

in these experiments were 2–8-mo old and were maintained on acidified chlorinated drinking water and laboratory chow ad libitum.

Antigens. Two preparations of GT with molar amino acid ratios of $G^{50}T^{50}$ and mol wt of 30,500 and 31,800 daltons and one preparation of $G^{60}A^{30}T^{10}$, mol wt 35,000 were purchased from Miles Laboratories Inc., Miles Research Div., Elkhart, Ind. Preparation of the solution of GT and GT complexed to MBSA was described in detail in the preceding paper. Solutions of GAT and suspensions of GAT complexed to MBSA were prepared as previously described (2).

Immunization. To investigate the suppressive properties of GT for different inbred strains, mice were injected intraperitoneally initially with 100 μg of GT in a mixture of magnesium and aluminum hydroxides (Maalox, William H. Rorer, Inc., Fort Washington, Pa.) or with Maalox alone and 3 days later with 10 μg of GT as GT-MBSA emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) according to the experimental protocol described in detail in the preceding paper (1). Selected nonresponder strains of mice, DBA/1 (*H-2*^{q}) and SJL (*H-2*^{s}), were injected intraperitoneally with 10 μg or 100 μg of GAT as GAT-MBSA in a mixture of Maalox and pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.) as previously described (3).

Hemolytic Plaque Assay. The antibody responses to GT and to GAT were measured by an indirect hemolytic plaque assay, which detects IgG specific PFC, using sheep red blood cells (SRBC) coated with GAT as indicator cells as described in detail in previous studies (1, 2).

Results

Strain Differences in the Suppression by GT of the Primary Response to GT-MBSA. We compared the ability of preimmunization with GT to specifically inhibit the immune response to GT-MBSA in several inbred strains of mice. 100 μg GT in Maalox, or Maalox alone as control, were injected intraperitoneally 3 days before immunization with GT-MBSA. 7 days later the specific IgG PFC per spleen were enumerated (no IgM PFC were detected after immunization with GT or GT-MBSA, as was observed in the response to GAT or GAT-MBSA) (2). A 3-day interval was selected between the administration of GT and GT-MBSA in all experiments, as this was the earliest time when maximal suppression of the primary response was observed in the well-studied BALB/c mouse model.

The results listed in Table I illustrate five major points: (*a*) The 12 inbred and congenic resistant strains investigated were shown to produce a specific PFC primary response to GT-MBSA. The results confirm and extend to other nonresponder strains our earlier finding that inbred strains of mice can be stimulated to develop specific IgG PFC primary responses provided GT is administered as a complex with an immunogenic carrier (1).

(*b*) Preimmunization with GT of BALB/c (*H-2*^{d}), DBA/2 (*H-2*^{d}), D1.C (*H-2*^{d}), A.CA (*H-2*^{f}), SJL (*H-2*^{s}), and A.SW (*H-2*^{s}) mice causes a marked decrease in their PFC response to GT-MBSA.

(*c*) In contrast, GT immunization of A.By (*H-2*^{b}), 129/J (*H-2*^{b}), C57L (*H-2*^{b}), A/J (*H-2*^{a}), B10.A (*H-2*^{a}), and DBA/1 (*H-2*^{q}) mice fails to suppress the development of responses to GT-MBSA in these strains. To eliminate the possibility that the development of specific immune suppression might have been delayed in some of these strains, we investigated the effect of GT immunization 7 days before GT-MBSA in A/J mice. Whether administered 3 or 7 days before immunization with GT-MBSA, GT did not depress the specific PFC response of A/J mice. We conclude, therefore, that only some mouse strains manifest GT-specific suppression after immunization with this copolymer.

(*d*) The GT-MBSA responses of CAF_1 (*H-2*$^{a/d}$) and (DBA/1 × SJL)F_1 (*H-2*$^{q/s}$) mice were specifically suppressed after preimmunization with GT. These mice

TABLE I
Strain Differences in Suppression by GT of PFC Responses to GT-MBSA *

Strain	*H-2*	Number of mice per group	Maalox + GT-MBSA PFC/spleen	GT + GT-MBSA PFC/spleen	Suppression	*P* value
			Mean ± SE	*Mean ± SE*	%	
A/J	*a*	11	10,436 ± 1,952	12,593 ± 1,714	0	<0.4
B10.A‡	*a*	8	12,118 ± 1,746	15,631 ± 3,398	0	<0.3
A.By	*b*	8	11,075 ± 2,174	14,286 ± 2,179	0	<0.3
C57L/J	*b*	4	7,968 ± 986	10,275 ± 827	0	<0.1
129/J	*b*	4	5,225 ± 525	6,993 ± 2,070	0	<0.1
BALB/c	*d*	48	12,658 ± 750	2,566 ± 492	80	<0.000001
DBA/2	*d*	7	10,287 ± 1,766	2,007 ± 1,086	81	<0.001
D1.C	*d*	7	15,503 ± 3,001	3,753 ± 1,271	76	<0.002
A.CA	*f*	9	10,775 ± 2,049	<200	100	<0.00009
DBA/1	*q*	16	7,374 ± 1,028	9,562 ± 843	0	<0.1
SJL	*s*	16	10,401 ± 887	3,012 ± 652	72	<0.000001
A.SW	*s*	12	8,500 ± 1,300	2,800 ± 800	68	<0.001
CAF_1	*a/d*	16	10,437 ± 1,443	2,809 ± 1,075	74	<0.0001
$(DBA/1 \times SJL)F_1$	*q/s*	5	13,555 ± 2,019	1,360 ± 838	90	<0.0005

* 100 μg of GT or Maalox alone was administered intraperitoneally, followed 3 days later by 10 μg of GT complexed with MBSA. 7 days later the number of IgG-specific PFC per spleen were counted using SRBC coated with GAT.

‡ B10.A mice were immunized with GT-MBSA with Maalox and *B. Pertussis* as adjuvant.

are hybrids of A/J or DBA/1, which are not suppressed by GT, and BALB/c or SJL mice where GT has a suppressive effect. This demonstrates that the capacity to develop GT-specific suppression is inherited as a dominant trait.

(*e*) A.BY (*H-2*^{b}), A.CA (*H-2*^{f}), and A.SW (*H-2*^{s}) are three congenic resistant strains of mice on the A strain genetic background. As shown in Table I, these mouse strains are rendered specifically unresponsive or are not affected by GT immunization, depending upon their *H-2* haplotype, but not on their background genotype. The GT-MBSA responses of A.CA and A.SW, but not A/J and A.BY mice, are suppressed by GT preimmunization. Similarly the DBA/1 (*H-2*^{q}) and D1.C (*H-2*^{d}) congenic strain, which share the DBA/1 background but differ in their *H-2* haplotypes, also differ in their ability to be suppressed in this system. Furthermore, the strain distribution of mouse strains bearing the *H-2*^{d} or *H-2*^{s} haplotypes on different backgrounds are suppressed by GT (i.e., BALB/c, DBA/2, and D1.C [*H-2*^{d}] and SJL and A.SW [*H-2*^{s}]), whereas strains bearing *H-2*^{a} or *H-2*^{b} haplotypes are not suppressed by GT regardless of their background genotype (i.e., A/J and B10.A [*H-2*^{a}] and A.BY, C57L, and 129/J [*H-2*^{b}]). This demonstrates the critical role of a gene or genes in the *H-2* complex for specific immune suppression in response to GT immunization.

Differences in the Specificity of Immune Suppression Stimulated by GT or GAT on Specific PFC Responses to GAT-MBSA or GT-MBSA. GT and GAT are cross-reacting copolymers (4). Antibody responses to these two antigens can be detected by a hemolytic plaque assay using SRBC coated with GAT as indicator cells. To determine if suppression induced by GT is distinct from that

induced by GAT, we compared the effect of immunization with GT and GAT in DBA/1 (*H-2*^{q}) and SJL (*H-2*^{s}) mice on the PFC responses to these copolymers complexed with MBSA. These strains were selected for the experiments because GAT stimulates GAT-specific T cells capable of suppressing GAT-MBSA PFC responses in both strains (3, 5), whereas GT is able to suppress GT-MBSA responses in SJL, but not in DBA/1 mice.

100 μg GT in Maalox, 10 or 100 μg GAT in Maalox, or Maalox alone was injected intraperitoneally, followed 3 days later by 10 μg of GAT as GAT-MBSA in Maalox-pertussis or 10 μg of GT as GT-MBSA in CFA. The number of IgG specific PFC per spleen were enumerated 7 days later.

The results of preimmunization with GT or GAT on the GAT-specific PFC responses to GAT-MBSA are shown in Table II. Both GT and GAT suppress the GAT-MBSA response of SJL mice. In DBA/1 mice, however, GAT, but not GT preimmunization, is able to suppress the response to GAT-MBSA. The results of preimmunization with GT or GAT on the GT-MBSA PFC response are presented in Table III. GT, but not GAT, suppresses the GT-MBSA response of SJL mice. In DBA/1 mice neither GT nor GAT could effectively suppress the response to GT-MBSA. These results demonstrate: (*a*) that the pattern of immune suppression for the two related copolymers GT and GAT are distinct in different strains and (*b*) that the specificity of suppression induced by GT and GAT is distinct since in SJL mice GAT-specific suppressor cells inhibit only GAT-MBSA responses, whereas GT induced suppression inhibits both GT-MBSA and GAT-MBSA responses.

Discussion

The observation that immunization of nonresponder mice bearing the *H-2*^{p,q,s} haplotypes with GAT elicits GAT-specific suppressor T cells (5) raised the issue of whether nonresponder strains, in all systems under *H-2*-linked *Ir* gene control, could develop suppressor T cells, and whether the selective development of specific suppressor cells could indeed explain the unresponsiveness to these antigens. The copolymer GT was selected to investigate this critical point, since, as shown in the companion paper, GT does not stimulate detectable antibody responses in vivo in any of the 19 inbred strains of mice investigates, but does stimulate specific antibody responses when administered complexed with an immunogenic carrier, MBSA (1).

Some, but not all, inbred nonresponder strains were found to develop GT-induced suppression of GT-MBSA responses. We may therefore conclude: (*a*) that immune suppression cannot account for nonresponder status in all cases and (*b*) that GT immunization permits us to identify two distinct phenotypes among inbred strains of mice that differ in their susceptibility to GT-induced suppression. We shall refer to these as "suppressor" and "nonsuppressor" phenotypes. The genetic analysis of the specific suppressor responses could only be carried out by using an antigen that does not stimulate antibody responses in a large number of mouse strains. The capacity to develop GT-induced suppression of GT-MBSA responses was shown to be inherited as a dominant trait in F_1 hybrids resulting from the mating of suppressors with nonsuppressor strains. This trait is, therefore, under the control of a gene or genes that we have

TABLE II

Strain Differences in the Specificity of Immune Suppression Stimulated by GT or GAT Active on GAT-Specific PFC Response to GAT-MBSA

Strain	*H-2*	Number of mice per group	Maalox and GAT-MBSA PFC/spleen	GAT and GAT-MBSA PFC/spleen	GT and GAT-MBSA PFC/spleen
			Mean ± SE	*Mean ± SE*	*Mean ± SE*
SJL	*s*	20	11,490 ± 1,512	3,135 ± 1,065	4,037 ± 846
DBA/1	*q*	24	13,408 ± 1,034	4,978 ± 928	13,189 ± 1,160

100 μg of GT or 10 μg or 100 μg GAT in Maalox was administered intraperitoneally, followed 3 days later by 10 μg GAT complexed with MBSA with Maalox and *B. pertussis* as adjuvant. 7 days later the number of GAT-specific IgG PFC per spleen were counted using GAT-coated SRBC.
In SJL mice, differences between groups immunized with GAT-MBSA and with GAT or GT following with GAT-MBSA were statistically significant. $P < 0.0001$. In DBA/1 mice, only the differences between groups immunized with GAT-MBSA and GAT followed with GAT-MBSA were statistically significant. $P < 0.000001$.

designated as specific immune suppression gene(s) (*Is* genes) to distinguish them from *Ir* genes. In contrast, the F_1 crosses between GAT responders and nonresponders behaved as responders to GAT, in keeping with the dominant character of *H-2*-linked *Ir* genes, indicating that responder phenotypes are dominant over suppressor phenotypes (2, 3).

The other major conclusion from the strain distribution of GT-induced suppression of GT-MBSA responses is that the *Is* gene(s) controlling these responses are coded for in the *H-2* complex. Mice bearing $H\text{-}2^d$, $H\text{-}2^f$, or $H\text{-}2^s$ haplotypes exhibit the GT specific "suppressor" phenotype, while mice possessing the $H\text{-}2^a$, $H\text{-}2^b$, or $H\text{-}2^q$ haplotypes are GT "nonsuppressors" irrespective of non-*H-2* background genotypes. The *H-2*-linked *Is* gene(s) controlling GT-induced immune suppression identified in this study are "specific" for this antigen. They are not concerned with the general capacity to develop suppression, since DBA/1 ($H\text{-}2^q$) mice which develop suppressor T cells after GAT immunization are not specifically suppressed by GT and, therefore, lack the GT *Is* gene. Genetic similarities between H-linked *Is* and *Ir* genes are illustrated by the following observations: (*a*) *Ir* genes have been shown to be concerned with the expression of helper function in T-cell-dependent responses (6, 7); (*b*) helper and suppressor activity of T cells appear to be two related aspects of the regulatory activity of these cells on specific immune responses; (*c*) the ability to mount antibody responses in systems under *Ir* gene control has been shown to be associated with the production of antigen-specific factors produced by responder T cells and endowed with helper activity for B cells (8, 9); (*d*) similar antigen-specific factors with specific suppressor activity have been obtained from suppressor T cells (10–12); and (*e*) both helper and suppressor factors have similar molecular size and possess antigenic determinants coded for in the *I* region of the *H-2* complex (10, 11, 13).

The detailed analysis of the structural and biological relationships of antigen-specific helper and suppressor factors from T cells should permit a better understanding of the function of *H-2*-linked *Is* and *Ir* genes.

Compared to the extensive information concerning mouse *H-2*-linked *Ir* genes

TABLE III

Strain Differences in the Specificity of Suppression on GT-Specific PFC Response to GT-MBSA

Strain	*H-2*	Number of mice per group	Maalox and GT-MBSA PFC/spleen	GT and GT-MBSA PFC/spleen	GAT and GT-MBSA PFC/spleen
			Mean ± SE	*Mean ± SE*	*Mean ± SE*
SJL	*s*	16	10,401 ± 887	3,012 ± 652	11,425 ± 1,249
DBA/1	*q*	12	7,374 ± 1,208	9,562 ± 843	8,510 ± 1,063

The same experimental protocol as described in Table II was used. However, GT-MBSA was administered in CFA instead of GAT-MBSA.

Only in SJL mice the differences between groups immunized with GT-MBSA and GT followed with GT-MBSA were statistically significant. $P < 0.000001$.

In DBA/1 mice no statistical differences between the groups were found.

and their precise mapping in subregions of *I* (14), our understanding of *Is* genes is still very limited. It is important to determine whether *Is* genes can be identified with control responses to other antigens besides GT. The GT *Is* gene or genes should be precisely mapped within the *H-2* complex of the mouse. Furthermore, we must determine whether two cooperative *Is* genes are needed to develop specific suppression as was shown to be the case in two systems for antibody responses under *H-2*-linked *Ir* gene control (9, 15). We must also determine at which cell levels the *Is* genes operate. Finally, conclusive data is needed to clarify the relationship between *H-2*-linked *Ir* genes and *Is* genes and their products. The following important questions should be resolved: (*a*) Are specific helper T cells and suppressor T cells two different cell populations or two different stages of differentiation of the same regulatory cells? (*b*) The helper and suppressor products are probably, as stated earlier, very similar, but what are the crucial differences responsible for their distinct biological properties and the genetic distinction between *Ir* and *Is* genes? (*c*) How is the antigen specificity of the helper and suppressor factor determined, and how are the antigen-related genetic restrictions explained? (*d*) Are the helper and suppressor factors stimulated by the same antigenic determinants, and do both factors possess identical combining sites? (*e*) What is the relationship between the specificity and combining site of helper and suppressor factors and that of immunoglobulin antibodies? (*f*) What is the nature of the acceptor molecule for both the helper and suppressor T-cell factors? (*g*) What is the nature of the *Is* "nonsuppressor" defect? Do lymphocytes from nonsuppressor animals fail to recognize antigen, fail to make suppressor factor, or lack a receptor for suppressor factor?

Irrespective of the answers to these critical questions, we may consider the stimulation of helper and suppressor cells, as well as the production by these cells of antigen-specific factors capable of mediating helper or suppressor activity on lymphocytes, as the phenotypic expression of the regulating activity of specific "*Is* genes," "*Ir* genes," and of related "cell interactions genes," all of which are coded for by the major histocompatibility complex.

Summary

Several inbred as well as congenic resistant strains of mice, which fail to respond to the random copolymer of L-glutamic acid^{50}-L-tyrosine^{50} (GT), were shown to develop specific PFC responses when stimulated by GT complexed to an immunogenic carrier such as methylated bovine serum albumin (MBSA). In these studies we have found that GT preimmunization has a tolerogenic effect on the response to GT-MBSA in some mouse strains; whereas in other strains of mice, GT fails to inhibit the GT-MBSA response. We may, therefore, conclude that immune suppression cannot account for nonresponsiveness in all cases. The development of specific immune suppression in response to GT was shown to be inherited as a dominant trait in F_1 hybrids resulting from the mating of suppressor with nonsuppressor strains. This trait is, therefore, under the control of a gene or genes that we have designated as specific immune suppression gene(s) *Is* genes. The strain distribution of GT induced suppression demonstrates that *Is* genes are coded for in the *H-2* complex. Furthermore, immune suppression by the two related copolymers, GT and GAT, are distinct in different strains of mice. The significance of these data for our understanding of the regulation of the immune response is discussed.

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