GENETIC CONTROL OF SPECIFIC IMMUNE SUPPRESSION

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

BY PATRICE DEBRÉ, JUDITH A. KAPP, MARTIN E. DORF, AND BARUJ BENACERRAF

(From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115)

In the companion paper, the random copolymer of L-glutamic acid\textsuperscript{50}-L-tyrosine\textsuperscript{50} (GT)\textsuperscript{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\textsuperscript{50}-L-alanine\textsuperscript{50}-L-tyrosine\textsuperscript{19} (GAT) on the response to these copolymers complexed with MBSA by mice bearing the nonresponder haplotypes H-2\textsuperscript{g} and H-2\textsuperscript{b}.

Materials and Methods

\textit{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

---

\* This investigation was supported by U.S. Public Health Service Grant AI-09920 from the National Institute of Allergy and Infectious Diseases.

\dagger Recipient of a fellowship from the French Ministry of Foreign Affairs.

\textit{Abbreviations used in this paper:} CFA, complete Freund’s adjuvant; GAT, random terpolymer of L-glutamic acid\textsuperscript{50}-L-alanine\textsuperscript{50}-L-tyrosine\textsuperscript{19}; GAT-MBSA, GAT complexed to methylated bovine serum albumin; GT, random copolymer of L-glutamic acid\textsuperscript{50}-L-tyrosine\textsuperscript{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 G50Tsu and mol wt of 30,500 and 31,800 daltons and one preparation of G50A300, mol wt 36,000 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^d), SJL (H-2^s), and A.SW (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 congeneric 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^b), D1.1C (H-2^b), A.CA (H-2^b), SJL (H-2^b), and A.SW (H-2^b) 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^b), B10.A (H-2^b), and DBA/1 (H-2^b) 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 CAF1 (H-2^{a/b}) and (DBA/1 × SJL)F1 (H-2^{a/b}) mice were specifically suppressed after preimmunization with GT. These mice
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^d), A.CA (H-2^a), 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^a) 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^a 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^d 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^d]). 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

### 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 Mean ± SE | GT + GT-MBSA PFC/spleen Mean ± SE | Suppression | P value |
|--------|-----|--------------------------|--------------------------------------|----------------------------------|-------------|--------|
| 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   | c   | 48                       | 17,503 ± 3,001                       | 3,753 ± 1,271                    | 76          | <0.002 |
| A.CA   | f   | 9                        | 19,775 ± 2,049                       | <200                             | 100         | <0.00009 |
| DBA/1  | q   | 16                       | 7,374 ± 1,026                        | 9,562 ± 843                      | 0           | <0.1   |
| SJL    | s   | 16                       | 10,401 ± 887                         | 3,012 ± 582                      | 72          | <0.000001 |
| A.SW   | s   | 12                       | 8,500 ± 1,300                        | 2,809 ± 800                      | 68          | <0.001 |
| CAF1   | a/d | 16                       | 10,437 ± 1,443                       | 2,809 ± 1,075                    | 74          | <0.0001 |
| (DBA/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.
induced by GAT, we compared the effect of immunization with GT and GAT in 
DBA/1 (H-2<sup>b</sup>) and SJL (H-2<sup>c</sup>) 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 suppres-
sion 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 re-
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<sup>b</sup>- 
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 investigated, but does 
stimulate specific antibody responses when administered complexed with an 
imunogenic 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" pheno-
types. 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<sub>1</sub> 
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
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 $I_s$ and $I_r$ genes.

Compared to the extensive information concerning mouse H-2-linked $I_r$ 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 |
|--------|-----|--------------------------|-------------------------------|---------------------------|---------------------------|
| 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 GTIs 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. 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\(^n\)-L-tyrosine\(^m\) (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) \(I_s\) genes. The strain distribution of GT induced suppression demonstrates that \(I_s\) 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.

We thank Mrs. Fern De La Croix for her excellent technical assistance, Dr. Zelig Eshhar for preparation of methylated bovine serum albumin, Dr. Carl W. Pierce for his generous gift of rabbit antimouse immunoglobulin sera, and Mrs. Charlene Small and Mrs. Barbara Teixeira for secretarial assistance in preparation of this manuscript.

Received for publication 11 August 1975.

References

1. Debré, P., J. A. Kapp, and B. Benacerraf. 1975. Genetic control of specific immune suppression. I. Experimental conditions for the stimulation of suppressor cells by the copolymer L-glutamic acid\(^n\)-L-tyrosine\(^m\) (GT) in nonresponder BALB/c mice. J. Exp. Med. 142:1436.
2. Kapp, J. A., C. W. Pierce, and B. Benacerraf. 1973. Genetic control of immune responses in vitro. I. Development of primary and secondary plaque-forming cell responses to the random terpolymer L-glutamic acid\(^n\)-L-alanine\(^m\)-L-tyrosine\(^o\) (GAT) by mouse spleen cells in vitro. J. Exp. Med. 138:1107.
3. Kapp, J. A., C. W. Pierce, and B. Benacerraf. 1974. Genetic control of immune responses in vitro. III. Tolerogenic properties of the terpolymer L-glutamic acid\(^n\)-L-alanine\(^m\)-L-tyrosine\(^o\) (GAT) for spleen cells from nonresponder (H-2\(^s\) and H-2\(^d\)) mice. J. Exp. Med. 140:172.
4. Pinchuck, P., and P. H. Maurer. 1965. Antigenicity of polypeptides (poly alpha amino acids). XV. Studies on the immunogenicity of synthetic polypeptides in mice. J. Exp. Med. 122:665.
5. Kapp, J. A., C. W. Pierce, S. Schlossman, and B. Benacerraf. 1974. Genetic control of immune responses in vitro. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid\(^n\)-L-alanine\(^m\)-L-tyrosine\(^o\) (GAT). J. Exp. Med. 140:648.
6. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility linked immune response genes. Science (Wash. D. C.). 175:273.
7. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* 11:31. 
8. Taussig, M. J. 1974. T cell factor which can replace T cells in vivo. *Nature (Lond.)* 248:234. 
9. Munro, A. J., and M. J. Taussig. 1975. Two genes in the major histocompatibility complex control the immune response. *Nature (Lond.)* 256:104. 
10. Tada, T., M. Taniguchi, and T. Takemori. 1975. Properties of primed suppressor T cells and their products. *Transplant. Rev.* In press. 
11. Zembala, M., G. L. Asherson, B. Mayhem, and J. Krijci. 1975. *In vitro* absorption and molecular weight of specific T cell suppressor factor. *Nature (Lond.)* 253:72. 
12. Kapp, J. A., C. W. Pierce, and B. Benacerraf. 1975. Role of suppressor T cells in an Ir-controlled immune response. In *Suppressor Cells in Immunity*. S. K. Singhal, editor. University of Western Ontario Press, Ontario. In press. 
13. Munro, A. J., M. J. Taussig, R. Campbell, H. Williams, and Y. Lawson. 1974. Antigen-specific T-cell factor in cell cooperation: physical properties and mapping in the left-hand (K) half of H-2. *J. Exp. Med.* 140:1579. 
14. Benacerraf, B., and D. H. Katz. 1975. The nature and function of histocompatibility-linked immune response genes. In *Immunogenetics and Immunodeficiencies*. B. Benacerraf, editor. Medical and Technical Publishing Co., Ltd., London. In press. 
15. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1975. Requirement for two H-2 complex Ir genes for the immune response to the L-Glu, L-Lys, L-Phe terpolymer. *J. Exp. Med.* 141:1459.