GENETIC CONTROL OF SPECIFIC IMMUNE SUPPRESSION

I. Experimental Conditions for the Stimulation of Suppressor Cells by the Copolymer L-Glutamic Acid^{50}-L-Tyrosine^{50} (GT) in Nonresponder BALB/c Mice*

By PATRICE DEBRÉ,‡ JUDITH A. KAPP, AND BARUJ BENACERRAF

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

In earlier studies from our laboratory, mice that are nonresponders (*H-2^p*, *H-2^q*, *H-2^s*) to the terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT)[1] were shown to develop suppressor T cells capable of inhibiting GAT-specific responses to GAT complexed with methylated bovine serum albumin (GAT-MBSA) (1). This finding raised several important points: (*a*) Could suppressor T cells be demonstrated in nonresponder mice immunized with antigens other than GAT? (*b*) Do all nonresponder strains unable to form an antibody response to an antigen under *H-2*-linked *Ir* gene control develop suppressor cells? (*c*) If the ability to generate suppressor cells in response to an antigen is observed in some mouse strains but not in others, are these phenotypic differences under genetic control? (*d*) If such genetic control is observed, are the responsible genes linked to the major histocompatibility complex of the mouse in a manner similar to what has been demonstrated for the control of antibody responses by *H-2*-linked *Ir* genes (2, 3)?

We have selected the copolymer of L-glutamic acid^{50} and L-tyrosine^{50} (GT) to investigate these points for the following reasons. Firstly, it was reported that GT fails to induce antibody responses in any of the inbred strains of mice tested, although it is immunogenic in some individual random-bred Swiss mice (4), in strain 13 guinea pigs (5), and in some inbred rat strains (6). Secondly, the copolymer GT can be complexed readily with positively charged MBSA and, as will be shown, GT-MBSA is immunogenic for mice unable to form antibody responses to GT. Thirdly, we could use the same technique that was used

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

‡ Recipient of a fellowship from the French Ministry of Foreign Affairs.

[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; DNP-KLH, 2,4-dinitrophenyl conjugate of keyhole limpet hemocyanin; 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; GAT-SRBC, GAT coupled to sheep red blood cells; MBSA, methylated bovine serum albumin; PFC, plaque-forming cells; RAMG, polyvalent rabbit antimouse IgG; SRBC, sheep red blood cells; TNP-SRBC, 2,4,6-trinitriphenyl conjugate of sheep red blood cells.

successfully for the demonstration of GAT-specific T cells to investigate whether preimmunization with GT stimulated specific immune suppression (1, 7).

In this paper we verify that GT does not induce a detectable antibody response in a large number of inbred mouse strains and demonstrate that they can nevertheless respond to GT-MBSA. We then establish that preimmunization with GT inhibits primary and secondary responses to GT-MBSA in BALB/c mice, and that such unresponsiveness can be transferred to syngeneic recipients with spleen cells or thymocytes from GT-primed BALB/c mice.

In a companion paper, we will show that the development of specific immune suppression in mice injected with GT is under genetic control.

Materials and Methods

Mice. All mice were purchased from Jackson Laboratory, Bar Harbor, Maine or the Health Research Laboratories, Buffalo, New York, or were bred in our animal facilities. Mice used in these studies were from 2 to 8-mo old and were maintained on acidified chlorinated drinking water and laboratory chow ad libitum.

Antigens. Two preparations of GT with an average mol wt of 30,500 and 31,800 daltons, respectively, and one preparation of GAT, mol wt 35,000, were purchased from Miles Laboratories Inc., Miles Research Div., Elkhart, Ind. The two GT preparations were identical in their immunological properties. 10 mg per ml stock solutions were prepared in normal saline containing 1% Na_2CO_3 at pH 9.0 to 9.5. MBSA was prepared according to the method of Sueoka and Cheng (8).

The GT-MBSA was prepared using the techniques described for the preparation of GAT-MBSA complexes (9), i.e., MBSA (5 mg per ml) was added dropwise, while stirring, to a 1 mg per ml solution of GT. After a 20-min interval during which maximum floculation and aggregation occurred, the precipitate of GT-MBSA was washed three times with saline.

Immunization. Mice were immunized intraperitoneally with 10 μg or 100 μg of GT. The copolymer was administered in a 10% solution of magnesium and aluminum hydroxides, Maalox (William H. Rorer, Inc., Fort Washington, Pa.), or in a mixture of Maalox and pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.), or emulsified with an equal volume of complete Freund's adjuvant containing 0.5 mg per ml of mycobacterium butyricum (CFA) (Difco Laboratories, Detroit, Mich.). Mice were immunized intraperitoneally with GT-MBSA containing 10 μg of GT in CFA.

Antigen-Binding Assay. A modification of the Farr technique, as previously described (10), was used to determine the antigen-binding capacity of the serum. Radioiodinated [^{125}I]GT was diluted with phosphate-buffered saline containing 1% normal mouse serum to a concentration of 2 $\times$ 10^{-8} M and a sp act between 0.2 and 4 Ci/g. Serum samples were diluted to 1:5 for assay.

Hemolytic Plaque Assay. The antibody response to GT and GT-MBSA was studied by enumerating the number of plaque-forming cells (PFC) per spleen at various times after immunization using a modification of the Jerne hemolytic plaque technique described by Pierce et al. (11). IgM PFC were enumerated after incubation of indicator red cells and spleen cells in agarose using guinea pig serum as a source of complement. IgG PFC were developed with polyvalent rabbit antimouse IgG (RAMG) and complement.

In preliminary experiments, it was observed that GT-coated sheep red blood cells (SRBC) could not be used as indicator cells to detect GT-specific PFC as they nonspecifically agglutinated. However, we observed that antibody responses to GT-MBSA could be assayed on SRBC coated with the cross-reacting terpolymer GAT (GAT-SRBC). The technique for the preparation of GAT-SRBC and the reliability in the hemolytic plaque assay for this antigen have been described elsewhere (12). In preliminary experiments, the number of GT-specific plaques was determined by subtracting the number of PFC inhibited by soluble GT from the number of plaques detected on GAT-SRBC in the absence of inhibition. It was verified, however, that one could use either GAT or GT as specific inhibitors of IgG PFC and obtain the same results. Therefore, for convenience and in accord with the previous procedure described for the GAT system, 50 μg of soluble GAT has been used to reproducibly inhibit all IgG-specific PFC induced by GT immunization. We have considered them operationally as GT-specific plaques.

Results

Antibody Response to GT or GT-MBSA In Vivo. Mice were immunized intraperitoneally with 100 μg GT in CFA, and 21 days later were challenged with the same dose of antigen. Individual sera obtained 7 days after secondary immunization were analyzed in duplicate. Of 11 inbred and 8 congenic resistant strains tested bearing 13 different *H-2* haplotypes, none showed a significant GT-specific antibody response, as detected by the binding of ^{125}I-labeled GT (Table I). In contrast, 3 out of 15 random-bred Swiss mice made detectable levels of anti-GT antibodies. We also studied the antibody response in selected mouse strains to GT, using the hemolytic plaque assay, 7 days after primary immunization with 100 μg of GT in Maalox-pertussis or CFA. The results of these experiments are presented in Table II. GT immunization with either Maalox-pertussis or CFA did not stimulate a significant PFC response detectable on GAT-coated SRBC in any of the four strains tested. Inasmuch as the plaques were developed with both RAMG and guinea pig complement, the assay should have detected both IgM and IgG plaques if they had been present in the spleens of GT-immunized mice. The same mouse strains responded to immunization with GT-MBSA in CFA with the production on day 7 of specific IgG plaques. The number of PFC per spleen detected was comparable in all four strains and was also of the same order of magnitude as the number of PFC per spleen that these mice usually produce after immunization with GAT-MBSA (12).

No specific IgM PFC were seen at either 4 or 7 days after immunization with GT-MBSA in CFA in A/J or BALB/c mice. Thus, the antibody response of mice to GT-MBSA is restricted for as yet undetermined reasons to the IgG classes, as was also observed in the response to GAT or GAT-MBSA.

Effects of GT on the Immune Response to GT-MBSA. BALB/c mice were injected intraperitoneally with 10 μg or 100 μg of GT in Maalox or Maalox alone 3–7 days before immunization with GT-MBSA. All mice were sacrificed 7 days later and their spleens examined for GT-specific IgG PFC (Table III). Immunization with either 10 or 100 μg of GT 3, 5, or 7 days before GT-MBSA challenge caused a significant decrease in the antibody response of BALB/c mice to GT-MBSA. The tolerance induced by GT preimmunization was specific, since the administration of 100 μg of GT in Maalox failed to suppress the primary anti-DNP PFC response to an unrelated antigen DNP-KLH, as detected by an hemolytic plaque assay using TNP-conjugated SRBC as indicator cells (data not shown). To determine whether the inhibition of the primary response to GT-MBSA by GT preimmunization observed on day 7 was an absence of response or simply a delay in the antibody response, the PFC response to GT-MBSA of BALB/c mice pretreated with 100 μg GT in Maalox or with Maalox alone was assayed at various times up to 15 days after immunization. As shown in Fig. 1, the primary IgG PFC response to GT-MBSA can be detected on day 4, peaks on day 7, and decreases considerably by day 15. During all this time, the animals pretreated with GT failed to make a significant antibody response.

Transfer of GT-Specific Tolerance to Normal, Syngeneic Recipients. An experiment was performed to determine whether the inhibition of the antibody response to GT-MBSA in BALB/c mice caused by GT preimmunization was an active process resulting from the stimulation of specific suppressor cells. The

TABLE I
Immune Response to the GT Copolymer Measured by Antigen-Binding Assay

Strain	*H-2* haplotype	No. tested	Antigen binding (± SE)
B10.A	*a*	5	0.7 ± 1.4*
A/J	*a*	4	1.9 ± 3.9
C57BL/10J	*b*	4	−3.4 ± 2.4
B10.D2n	*d*	5	6.4 ± 4.0
BALB/c	*d*	3	−7.7 ± 3.7
B10.M	*f*	5	−6.3 ± 4.0
HTG	*g*	4	3.4 ± 5.0
C3H.JK	*j*	4	7.7 ± 4.3
C3H/HeJ	*k*	4	12.3 ± 3.1
B10.BR	*k*	5	−3.5 ± 3.2
BDP/J	*p*	6	−1.2 ± 0.8
P/J	*p*	3	2.1 ± 6.4
DBA/1J	*q*	8	8.6 ± 4.1
T138	*q*	3	−0.2 ± 2.5
SWR/J	*q*	2	−6.1 ± 5.7
B10.RIII	*r*	5	−9.7 ± 3.6
SJL/J	*s*	5	−3.3 ± 1.7
B10.PL	*u*	5	−12.0 ± 2.6
SM/J	*v*	5	9.5 ± 4.1
Swiss	random bred	3	44.8 ± 9.5
Swiss	random bred	12	6.8 ± 1.6

* Mean percentage of radiolabeled GT ligand-bound in Farr assay by a 1:5 dilution of serum ± SE, from M. Dorf and B. Benacerraf (unpublished observations).

ability of BALB/c mice rendered tolerant to GT to transfer specific unresponsiveness to GT-MBSA to syngeneic recipients was investigated. BALB/c mice were injected with either 100 μg of GT in Maalox or Maalox alone. 3 days later, 20×10^6 spleen cells or thymocytes obtained from these two groups of animals were transferred intravenously into normal syngeneic recipients, and all recipient mice were immunized with GT-MBSA in CFA. Their antibody response was assayed 7 days later. As can be seen in Table IV, the mice that received spleen cells or thymocytes from mice preimmunized with GT showed decreased PFC responses when compared to mice that received the same numbers of control spleen cells or thymocytes. The conclusion can, therefore, be made that GT induces suppressor cells in the spleen and thymus of BALB/c mice.

Kinetic Studies on the Stimulation and the Duration of Suppressor Effect. The data presented in Table III illustrate that specific suppression in BALB/c mice can be stimulated by GT immunization as late as 7 days and as early as 3 days before challenge with GT-MBSA. A more detailed study of the kinetics of this phenomenon was performed. 100 μg GT in Maalox was injected into six groups of BALB/c mice at times ranging from 28 days before to 1 day after GT-MBSA immunization. The 7-day PFC responses are shown in Fig. 2. The earliest that GT preimmunization causes a marked decrease in the response to GT-MBSA is 3 days before immunization with GT-MBSA. At that time, the suppressive effect is already maximal and is maintained unchanged, as deter-

TABLE II
Immune Response to GT and GT-MBSA Measured by PFC Assay

Strain	*H-2*	Antigen	Adjuvant	No. tested	IgG-specific PFC/spleen
					Mean ± SE
A/J	*a*	GT	M/p	4	687 ± 520
		GT	CFA	8	653 ± 257
		GT-MBSA	CFA	19	8,955 ± 1,239
A.BY	*b*	GT	CFA	3	825 ± 825
		GT-MBSA	CFA	8	11,075 ± 2,124
BALB/c	*d*	GT	M/p	4	381 ± 264
		GT	CFA	4	400 ± 234
		GT-MBSA	CFA	57	12,252 ± 729
DBA/1	*q*	GT	M/p	4	950 ± 592
		GT	CFA	4	<200
		GT-MBSA	CFA	16	7,374 ± 1,208

100 μg of GT with Maalox and *B. pertussis* or CFA as adjuvant or 10 μg of GT complexed with MBSA with CFA was administered intraperitoneally. 7 days later specific IgG PFC were detected using GAT-SRBC. The number of IgG-specific PFC/spleen in A/J and DBA/1 mice immunized with GT do not differ statistically from the number in unimmunized animals (556 ± 258 and 312 ± 120, respectively).

TABLE III
Effect of GT Immunization on the Primary Response to GT-MBSA in BALB/c Mice in Vivo

Day of injection	Dose of GT in Maalox	IgG-specific PFC/spleen	Inhibition
	μg	*Mean ± SE*	%
	None	12,294 ± 2,129	
Day − 7	10	1,302 ± 576	90
Day − 5	100	1,000 ± 470	92
	10	2,737 ± 1,325	78
Day − 3	100	950 ± 660	93
	10	677 ± 341	95

10 μg or 100 μg of GT in alum was injected intraperitoneally 7, 5, or 3 days before immunization with 10 μg GT as GT-MBSA, using CFA as adjuvant. All mice were sacrificed 7 days later.

mined by in vivo experiments, up to 21 days after GT priming. The intensity of the suppression decreases by day 28.

Effect of GT Priming on the Development of Secondary Responses to GT-MBSA in BALB/c Mice. The effect of GT priming on the secondary response to GT-MBSA was investigated using three types of immunization protocols. All mice received two immunizations with GT-MBSA with an interval of 21 days.

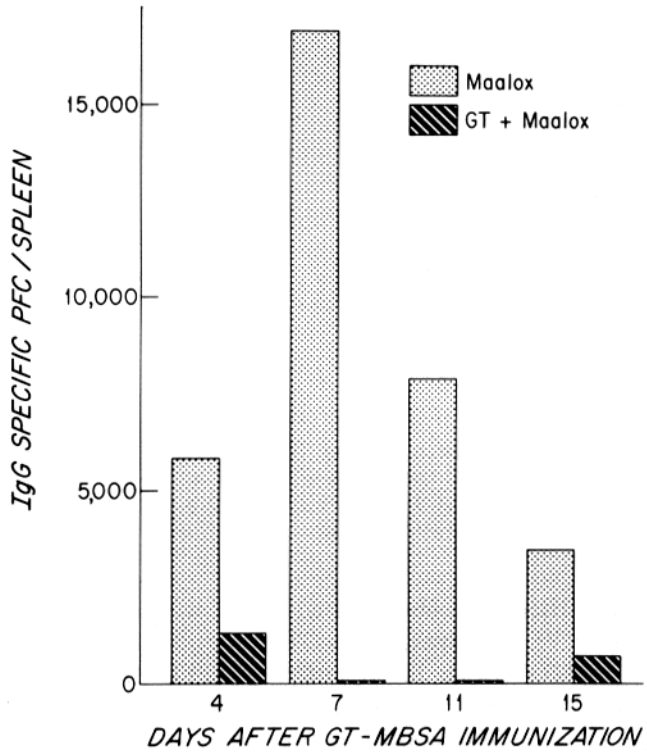

FIG. 1. Effect of GT preimmunization on the IgG PFC response of BALB/c mice to GT-MBSA assayed at various times after primary immunization. Mice were injected with 100 μg GT in Maalox or only Maalox 3 days before immunization with 10 μg GT as GT-MBSA in CFA. The graph shows the arithmetic mean of the PFC response of four mice per group.

TABLE IV

Transfer of GT-Specific Suppression to Normal Syngeneic Recipients (BALB/c Mice)

Cells transferred*	Specific IgG PFC/spleen	Inhibition‡
	Mean ± SE	%
Control spleen	19,468 ± 3,184	87
GT-primed spleen	2,687 ± 1,032	
Control thymus	22,781 ± 6,861	85
GT-primed thymus	3,587 ± 1,406	

* 20×10^6 cells from donors immunized 3 days before with Maalox or 100 μg of GT in Maalox were injected i.v. into normal syngeneic recipients. On the same day, these animals were intraperitoneally immunized with 10 μg GT complexed with MBSA in CFA. 7 days later, the number of Gt-induced IgG PFC per spleen was enumerated using GAT-SRBC.

‡ $P < 0.03$.

GT or Maalox was administered 3 days before GT-MBSA. In some animals GT was injected once before secondary immunization; in other animals once before primary immunization; and in a third group GT was administered before both primary and secondary immunization. No significant suppression of the second-

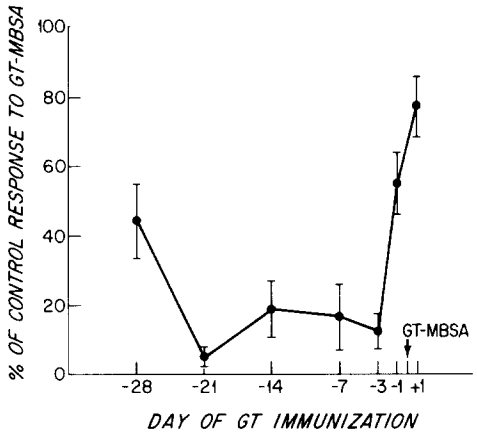

FIG. 2. Effect of the time interval between GT and GT-MBSA immunization on the suppression by GT of the IgG PFC response to GT-MBSA in BALB/c mice. Response was assayed 7 days after challenge with GT-MBSA in CFA. Data are presented as percentage of control response of animals preimmunized with Maalox alone, 7 days before GT-MBSA challenge.

ary response to GT-MBSA was observed when GT was injected before primary immunization (Table V). However, when GT was injected 3 days before secondary challenge with GT-MBSA, the specific IgG PFC response is clearly decreased on day 4 but not on day 7. If GT is administered twice, a first time 3 days before GT-MBSA priming and again 3 days before the secondary challenge, the secondary response to GT-MBSA is significantly decreased as compared to the control response both on days 4 and 7 after GT-MBSA challenge.

Discussion

The random copolymer of L-glutamic acid and L-tyrosine, which is immunogenic in strain 13 guinea pigs (5) and in rats bearing the *H-1*^{a} or *H-1*^{c} haplotype (6), stimulates antibody responses by some random-bred Swiss mice, but not by the 19 inbred strains investigated. Nor can GT elicit primary PFC responses in four selected inbred mouse strains bearing different *H-2* haplotypes. It would appear, therefore, that in mice *Ir* genes controlling antibody responses to GT are not widely represented. Nonresponder mice can, however, produce anti-GT antibodies after immunization with GT complexed with an immunogenic carrier, MBSA, as observed in other systems (9, 13). The restricted immunogenicity of GT for mice renders this antigen well suited for studies on the stimulation of specific immune suppression using the types of protocols which permitted the identification of GAT-specific suppressor T cells in mice bearing the *H-2*$^{p,\ q,\ s}$ haplotypes and unable to respond to GAT (1).

The experiments reported in this paper were designed to establish the conditions for demonstration of the suppressive properties of GT in a suitable mouse strain as a model, with the view to apply these techniques to the study of the genetic factors controlling the stimulation of specific immune suppression. The data document the tolerogenic properties of microgram amounts of GT on the specific response of BALB/c mice immunized with GT-MBSA in CFA. GT-specific tolerance could be transferred to normal, syngeneic recipients by spleen

TABLE V
Effect of GT Immunization on Secondary Response to GT-MBSA in BALB/c Mice in Vivo

GT immunization*		IgG-specific PFC per spleen‡	
§Day − 24	Day −3	Day +4	Day +7
μg	μg		
None	None	21,095 ± 3,115	22,125 ± 2,594
None	100	2,185 ± 1,156 ($P < 0.0004$)	17,862 ± 3,182 (NS)
100	None	12,320 ± 4,048 (NS)	14,693 ± 4,455 (NS)
100	100	<200 ($P < 0.0001$)	5,625 ± 2,697 ($P < 0.004$)

* 100 μg of GT in Maalox was administered intraperitoneally 3 days before a primary immunization or a secondary immunization with 10 μg of GT as GT-MBSA in CFA. 4 days and 7 days after the secondary immunization with GT-MBSA, the number of IgG-specific PFC per spleen were enumerated using GAT-coated SRBC. *P* values between each group and the control group are shown in parentheses. BALB/c control animals, which received a primary injection of GT-MBSA on the same day as the above secondary immunization, develop 2,675 ± 1,760 IgG-specific PFC at day 4 and 6,881 ± 1,321 at day 7.

‡ Mean ± SE.

§ Day relative to second injection of GT-MBSA.

cells or thymocytes of GT-primed animals, demonstrating a role for suppressor cells in this phenomenon.

Before considering the properties of GT-specific immunosuppression in BALB/c mice, it is appropriate to discuss some of the experimental conditions which had to be used in this study and their implications. (*a*) Specific immune suppression is best shown on primary PFC responses when synthetic polypeptide antigens such as GAT or GT are used. (*b*) To assay GT-specific PFC responses, we used SRBC coated with GAT rather than with GT because GT-coated SRBC were not stable in the assay. The antibody responses to GT-MBSA detected were, therefore, those which are cross-reactive with GAT. This is no indication, however, that a large number of GT-specific plaques have been missed by the assay using GAT-SRBC. This statement is based on the large number of IgG PFC per spleen detected by this technique after immunization with GT-MBSA, a number which does not differ significantly from that observed using the same assay after immunization with GAT-MBSA (12). Furthermore, the conclusions concerning the stimulation of GT-induced specific suppression are not affected by the fact that antibody-forming cells elicited by GT-MBSA have been detected on GAT-SRBC. We have, therefore, considered the PFC detected with GAT-SRBC in mice immunized with GT-MBSA as GT-specific plaques operationally. (*c*) For reasons still undetermined, these linear copolymers and complexes of these copolymers with MBSA do not stimulate detectable IgM PFC in vivo or in vitro and elicit only IgG PFC responses (12).

The following conclusions were reached in our study of GT-specific suppres-

sion of the response to GT-MBSA of BALB/c mice. The stimulation of suppressor cells was observed with another synthetic polypeptide besides GAT in nonresponder mice. The suppression elicited by GT preimmunization of BALB/c mice resembles operationally the specific suppression stimulated by GAT in DBA/1 mice with respect to the degree of tolerance achieved, the dose range of antigen required, the time of appearance, and the length of time when suppression is effective in vivo after immunization (1, 7, 14).

The experiments on the effect of GT on the secondary response to GT-MBSA illustrate several interesting points. (*a*) Specific suppression of secondary responses is much more difficult to achieve than suppression of primary responses. 100 μg of GT administered 3 days before a primary challenge with GT-MBSA suppress effectively the primary antibody response, but was only able, when administered before a secondary challenge, to cause a short delay in the development of PFC response on day 4 and did not affect the level of the response observed on day 7. (*b*) If GT is administered 3 days before primary immunization with GT-MBSA, which effectively suppresses the primary antibody response, a characteristic secondary response is elicited by GT-MBSA challenge, indicating that GT immunization did not suppress priming for the secondary response. The activity of suppressor cells is, therefore, limited in time and can be overcome by repeated immunizations. (*c*) The most effective suppression of the secondary GT-MBSA response detectable on days 4 and 7 after secondary challenge required the administration of GT before both the primary and secondary injection with GT-MBSA. However, in these experiments with GT and in other experiments with GAT, no evidence was obtained that a high level of memory was achieved in the specific suppressor cell population by repeated injection (14).

The study of specific suppressor cells and of their specific active products is one of the most fascinating and potentially useful areas of cellular immunology. Much remains to be learned concerning these phenomena. The use of the copolymers of L-amino acids such as GT and GAT to stimulate the formation of specific suppressor cells in nonresponder strains may prove to be the most effective and reliable technique to investigate specific immune suppression. These products, in contrast to conventional antigens, stimulate suppressor cells when administered in microgram amounts, even in the presence of adjuvants, without the development of specific antibody which may complicate the interpretation of the results.

Summary

In the present studies we have confirmed that the random copolymer of L-glutamic acid^{50}-L-tyrosine^{50} (GT) fails to induce an antibody response in a large number of inbred strains of mice. Nevertheless, GT complexed to methylated bovine serum albumin (MBSA) elicits a GT-specific IgG PFC response in vivo. Furthermore, injection of BALB/c mice with 10 to 100 μg of GT specifically decreases their ability to develop anti-GT PFC responses to a subsequent challenge with GT-MBSA. GT-specific tolerance can be transferred to normal, syngeneic recipients by spleen cells or thymocytes of GT-primed animals. These results indicate that the stimulation of suppressor cells can be observed in

nonresponder mice with another synthetic polypeptide besides GAT. Various parameters of GT-specific immunosuppression in BALB/c mice are described. The application of these techniques to the study of the genetic factors controlling the stimulation of specific immune suppression is discussed.

We thank Mrs. Fern De La Croix for her excellent technical assistance, Dr. Zelig Eshlar for preparation of methylated bovine serum albumin, Dr. Carl W. Pierce for his generous gift of rabbit antimouse immunoglobulin sera, Mrs. Annette Benacerraf for performing the Farr assays, and Mrs. Barbara Teixeira for her secretarial assistance for the preparation of this manuscript.

Received for publication 11 August 1975.

References

1. 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^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). *J. Exp. Med.* **140**:648.
2. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* **175**:273.
3. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* **11**:31.
4. Merryman, C. F., and P. H. Maurer. 1973. Genetic control of immune response of mice to GA, GT, GL, GAT, and GLT. *Fed. Proc.* **32**:4372 (Abstr.).
5. Bluestein, H. G., I. Green, and B. Benacerraf. 1971. Specific immune response genes of the guinea pig. I. Dominant genetic control of immune responsiveness to copolymers of L-glutamic acid and L-alanine and L-glutamic acid and L-tyrosine. *J. Exp. Med.* **134**:458.
6. Armerding, D., D. H. Katz, and B. Benacerraf. 19743. Immune response genes in inbred rats. I. Analysis of responder status to synthetic polypeptides and low doses of bovine serum albumin. *Immunogenetics.* **1**:329.
7. 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^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) for spleen cells from nonresponder (*H-2^s* and *H-2^q*) mice. *J. Exp. Med.* **140**:172.
8. Sueoka, N., and T-Y. Cheng. 1962. Fractionation of nucleic acid with the methylated albumin column. *J. Mol. Biol.* **4**:161.
9. Gershon, R. K., P. H. Maurer, and C. F. Merryman. 1973. A cellular basis for genetically controlled immunologic unresponsiveness in mice: tolerance induction in T cells. *Proc. Natl. Acad. Sci. U. S. A.* **70**:250.
10. Dunham, E. K., M. E. Dorf, D. C. Shreffler, and B. Benacerraf. 1973. Mapping the H-2 linked genes governing respectively the immune responses to a glutamic acid-alanine-tyrosine copolymer and to limiting doses of ovalbumin. *J. Immunol.* **111**:1621.
11. Pierce, C. W., B. M. Johnson, H. E. Gershon, and R. Asofsky. 1971. Immune responses in vitro. III. Development of primary γM, γG, and γA plaque-forming cell responses in mouse spleen cell cultures stimulated with heterologous erythrocytes. *J. Exp. Med.* **134**:395.
12. 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^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) by mouse spleen cells in vitro. *J. Exp. Med.* **138**:1107.

13. Green, I., W. E. Paul, and B. Benacerraf. 1966. The behavior of hapten-poly L-lysine conjugates as complete antigens in genetic responder and as haptens in nonresponder guinea pigs. *J. Exp. Med.* 123:859.
14. Benacerraf, B., J. A. Kapp, P. Debré, C. W. Pierce, and F. De La Croix. 1975. The stimulation of specific suppressor T cells in genetic nonresponder mice by linear random copolymers of L-amino acids. *Transplant. Rev.* In press.