RESTRICTION OF PRIMARY RESPONSES TO THE IgG CLASS AND DEPENDENCY OF IgM RESPONSES ON SECONDARY IMMUNIZATION FOR THE COPOLYMERS OF L-GLUTAMIC ACID, L-TYROSINE, AND L-ALANINE*

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The specific antibody responses of mice to thymus-dependent polypeptide antigens are controlled by autosomal dominant immune response (*Ir*) genes which map in the *I* region of the *H-2* complex (1). Primary responses in vivo or in vitro to the linear copolymers GAT[1] (L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}) and GA (L-glutamic acid^{50}-L-alanine^{50}) are restricted to the IgG class in responder mice (2–4). Similarly, in nonresponder mice primary responses to these antigens complexed to methylated bovine serum albumin (MBSA) and to GT (L-glutamic acid^{50}-L-tyrosine^{50}) as GT-MBSA are also restricted to the IgG class (2, 4, 5). These results are in sharp contrast with the early primary IgM responses to many conventional antigens such as sheep red blood cells (SRBC) bacterial antigens and to other synthetic polypeptide antigens (6, 7). Primary immunization with the linear terpolyper of L-glutamic acid, L-lysine, and L-phenylalanine (GLØ) stimulates production of antibodies of the IgM and IgG class in responder animals but no responses in mice lacking the appropriate α^+ and β^+ GLØ genes (8, 9), whereas the branched copolymers studied by McDevitt and Sela have been shown to stimulate IgM antibody responses in both responder and nonresponder animals and IgG antibodies, only in responder strains (10, 11). The prevalent immunological dogma that the synthesis of IgM antibodies always precedes the development of IgG-forming cells as the initial response to antigen should, therefore, be re-examined. In this study, we have investigated the conditions necessary for the synthesis of specific IgM antibodies against GAT in responder strains and against GAT-MBSA or GT-MBSA in nonresponder strains of mice. In contrast to many classical antigens referred to above, specific IgM antibody responses to these antigens require secondary immunization.

Previous studies from our laboratory demonstrated that immunization of nonresponder mice with GAT or GT stimulates the development of specific T cells capable of suppressing the primary IgG responses to GAT-MBSA (12) and GT-MBSA, respectively (5). The ability to elicit secondary IgM responses to these antigens permitted us to verify that such responses are also suppressed in nonresponder animals by preimmunization with the uncomplexed copolymers.

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; BGG, bovine gamma globulin; GA, copolymer of L-glutamic acid^{50}-L-alanine^{50}; GAT, terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GT, copolymer of L-glutamic acid^{50}-L-tyrosine^{50}; MBSA, methylated bovine serum albumin; PBS, phosphate-buffered saline; PFC, plaque-forming cells.

Materials and Methods

Mice. BALB/c mice were obtained from Health Research, Inc., West Seneca, New York. DBA/1 and C57BL/6 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Mice used in these experiments were 10–16 wk of age and were maintained on laboratory chow and acidified-chlorinated water ad lib.

Antigens. The random synthetic terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT), average mol wt 30,800 daltons and the copolymer of L-glutamic acid^{50}-L-tyrosine^{50} (GT), average mol wt 32,000, were purchased from Miles Laboratories, Inc., Miles Research Division, Elkhardt, Ind. Stock solutions (10 μg/ml) were prepared in normal saline containing 1% Na_2CO_3 at pH 9.0–9.5. MBSA was purchased from Worthington Biochemical Corp., Freehold, N.J.

Immunizations. Primary antibody responses were elicited by injecting mice intraperitoneally with 10, 50, or 100 μg GAT, 10 μg GAT as GAT-MBSA, or 10 μg GT as GT-MBSA in a mixture containing 2×10^9 killed *Bordetella pertussis* organisms and 10 μg of an aluminum-magnesium hydroxide gel (Maalox, W. H. Rorer, Inc., Fort Washington, Pa.). 7 days after injection, spleens were assayed for GAT- or GT-specific plaque-forming cells (PFC) (2). To investigate secondary antibody responses, mice were initially immunized intraperitoneally with 50 μg GAT, 50 μg GAT as GAT-MBSA or 50 μg GT as GT-MBSA in a mixture containing pertussis and/or Maalox or Maalox alone as indicated in the tables. A second injection of 10 μg of antigen followed 3 or 21 days later. Spleens from injected animals were assayed for GAT- or GT-specific PFC 4–11 days after second injection.

To investigate suppression of the secondary antibody response of nonresponder mice, the animals were first injected with 100 μg GT in Maalox (BALB/c) or 10 μg GAT in Maalox (DBA/1). 3 and 6 days later, mice were injected with GT-MBSA (BALB/c) and GAT-MBSA (DBA/1) according to the repeated immunization protocol described above.

Antigen-Binding Assay. The primary and secondary GAT-specific circulating antibody responses of BALB/c mice were measured. Serum samples were diluted 1/5 in phosphate-buffered saline (PBS) and then assayed by a modified Farr assay described previously (13).

Sephadex G-200 Gel Filtration. The separation of IgM and IgG anti-GAT antibodies was performed by gel filtration on a 1.5 × 90 cm Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, N. J.) column. The gel was equilibrated at 4°C in PBS, and the flow rate was maintained at 5 ml/h with a peristaltic pump. The column was calibrated using Blue dextran 2,000 (2×10^6 mol wt, Pharmacia Fine Chemicals), bovine gamma globulin (BGG, 150,000 mol wt), bovine serum albumin (BSA, 68,000 mol wt), and cytochrome *c* (12,500 mol wt) as markers.

The optical density of the eluate was recorded with an LKB UV recorder. BALB/c mice were immunized twice with GAT in Maalox pertussis as described above; 7 days after the second injection (day 10) the mice were bled. 0.5 ml of heat-inactivated (56°C 30 min) serum was loaded on the column. The optical density of the eluted material was recorded and the hemagglutinin titer of the various fractions measured using GAT-SRBC as indicator cells.

Hemolytic Plaque Assay. The antibody responses to GAT, GAT-MBSA, or GT-MBSA were assayed by a modification of the Jerne hemolytic plaque technique described previously (2). GAT was coupled to SRBC as described previously (2). As in previous studies, GAT-SRBC were used to detect GAT or GT-specific PFC because of the extensive cross-reactivity of the antibody produced. IgM PFC were enumerated after incubation of GAT-SRBC and spleen cells using guinea pig serum as a source of C^+. A goat anti-mouse μ-chain antiserum prepared against MOPC-104E and absorbed with λ-light chains (a gift from Dr. R. Asofsky) was used to inhibit IgM PFC (14). IgG PFC were developed with polyvalent rabbit anti-mouse IgG serum and C′ in the presence of goat anti-μ-chain antibody (2, 14). Both IgM and IgG antigen-specific plaques were determined by subtracting the number of PFC obtained in the presence of a suitable dilution of GAT (25 μg/ml) from the number of plaques detected on GAT-SRBC in the absence of the specific inhibitor (4).

Results

Effect of Repeated Immunization on the PFC Responses of BALB/c Mice to GAT. Previous studies have shown that genetic responder and nonresponder mice fail to develop a primary IgM GAT-specific PFC response in vivo or in vitro (2). Primary in vivo PFC responses to the copolymers GA and GT, likewise, have

TABLE I

Effect of Repeated Immunization on the IgM and IgG PFC Responses of BALB/c Mice to GAT

Immunization*			No. of mice per group	GAT-specific PFC per spleen	
Day 0	Day 3	Day 21		IgM	IgG
				Arith. mean ± SE	
Exp. I					
–	–	–	5	600 ± 200	<400
–	GAT/MP‡	–	11	673 ± 220§	12,363 ± 4,590
–	GAT/MP‖	–	5	425 ± 175	14,700 ± 4,834
MP	GAT/MP	–	11	1,163 ± 484	5,218 ± 1,273
GAT/M	GAT/MP	–	11	9,473 ± 3,574	24,036 ± 5,968
GAT/MP	GAT/MP	–	11	21,163 ± 3,854	34,781 ± 4,908
Exp. II					
GAT/MP	GAT/MP	–	6	12,666 ± 3,324	22,766 ± 2,508
GAT/MP	–	GAT/MP	6	10,133 ± 985	19,766 ± 3,553

* Mice were immunized intraperitoneally, day 0, with 50 μg GAT in Maalox with or without 2×10^9 killed *Bordetella pertussis* organisms or with Maalox and pertussis alone. 3 or 21 days later the mice received 10 μg GAT in Maalox and pertussis. 7 days later, spleen cells were assayed for GAT-specific PFC.

‡ Mice received a single injection of 10 μg GAT in Maalox-pertussis.

§ IgM response not significantly different ($P = 0.461$) from no antigen control.

‖ Mice received a single injection of 100 μg GAT in Maalox-pertussis.

been found to be restricted to the IgG class (4). In addition, a primary IgM response to GT could not be detected in mice pretreated with cyclophosphamide in order to remove suppressor T cells (Waltenbaugh, unpublished observations). In experiments relating the structure of GAT with its antigenic properties, we have observed a marked enhancement in the number of GAT-specific PFC's in mice that had received two injections of GAT in Maalox pertussis (15). Mice receiving two injections of GAT, at a 3-day interval, display a two to threefold increase in the number of IgG PFC's (Table I, Experiment I). Moreover, after two injections of GAT, GAT-specific PFC's of the IgM class are elicited. The occurrence of IgM PFC's is due to repeated immunization with the antigen; Maalox pertussis followed by GAT in Maalox pertussis does not cause the formation of significant numbers of GAT-specific IgM plaques. The highest numbers of both IgM and IgG GAT-specific PFC's were seen after repeated injections of GAT in Maalox pertussis. The time period between the first and second injection of GAT in Maalox pertussis may be extended up to 3 wk (Table I, Experiment II), without any effect upon either the IgM or IgG PFC responses.

Effect of Repeated Immunization of Serum Antibody. We have analyzed the sera of animals receiving secondary immunization with GAT both by a modified Farr assay and passive hemagglutination. The data presented in Table II show that significant antigen binding, using the condition of our assay, is detected in the sera only after the secondary immunization with GAT. In contrast, the sera obtained after a single GAT injection do not show significant binding. In the hemagglutination assay, using GAT-SRBC as indicator cells, mice receiving two injections of GAT gave a titer of 1/1,024, whereas a titer of 1/32 was seen

TABLE II
Effect of Repeated Immunization with GAT on the Antibody Responses of BALB/c Mice

Immunization*		No. mice per group	Percent binding of GAT: Day 10	P value
Day 0	Day 3			
–	GAT/MP	7	4.8 ± 1.95	–
MP	GAT/MP	7	4.3 ± 0.68	0.80
GAT/MP	GAT/MP	7	33.9 ± 2.45	<0.001
GAT/M	GAT/MP	7	28.5 ± 5.25	0.001

* Mice were immunized intraperitoneally with 50 μg GAT in Maalox with or without pertussis or with Maalox and pertussis alone. 3 days later the mice received 10 μg GAT in Maalox and pertussis. 7 days later sera were collected and assayed at 1/5 dilution for antigen binding.

after single immunization. Pretreatment of these sera with 2-mercaptoethanol (16) completely abolishes the hemagglutination titer precluding the distinction between IgM and IgG antibodies by this method in early sera (17).

Separation of IgM and IgG Antibodies in the Sera of BALB/c Mice Receiving Repeated GAT Immunization. We have used a Sephadex G-200 column to physically identify IgM anti-GAT antibodies in the sera of BALB/c mice that have received double GAT immunizations. Fig. 1 shows two peaks of hemagglutinating activity eluted from the column. The larger peak elutes in the void volume (marker, blue dextran) corresponding to the (19S) IgM class of antibodies. The smaller peak elutes at the same volume as BGG and thus corresponds to IgG anti-GAT antibodies. Since IgM antibodies are known to hemagglutinate more efficiently than IgG, the size of the hemagglutination peaks do not necessarily reflect the respective amount of antibodies.

Duration of the IgM PFC Responses of BALB/c Mice to GAT. Spleens of BALB/c mice immunized twice with GAT in Maalox pertussis show an IgM as well as an IgG PFC response 7 days after immunization. The data in Table III show that IgM and IgG GAT-specific PFC are seen at 4 and 11 days as well as 7 days after the second immunization. The appearance of GAT-specific IgM PFC is, therefore not a transient phenomenon.

Effect of Repeated Immunization in Responder and Nonresponder Mice. The data in Table IV show that another GAT-responder strain, C57BL/6, is capable of producing GAT-specific IgM PFC after immunization twice with GAT in Maalox-pertussis as described above. Although not shown, the primary response of a limited number of C57BL/6 mice was restricted to the IgG class, confirming previously reported results (2). Repeated immunization of a GAT nonresponder strain, DBA/1, with GAT in Maalox pertussis, however, fails to stimulate either IgM or IgG PFC responses.

A characteristic property of GT- or GAT-nonresponder animals is their ability to develop a GAT or GT-specific responses when injected with GAT or GT complexed to an immunogenic carrier such as MBSA. Primary responses to GT-MBSA and GAT-MBSA were found to be also restricted to IgG class of antibody (2, 5). However, nonresponder mice, BALB/c or DBA/1, injected twice with GT-MBSA or GAT-MBSA, respectively, can mount an IgM as well as an IgG antigen-specific PFC response (Table V).

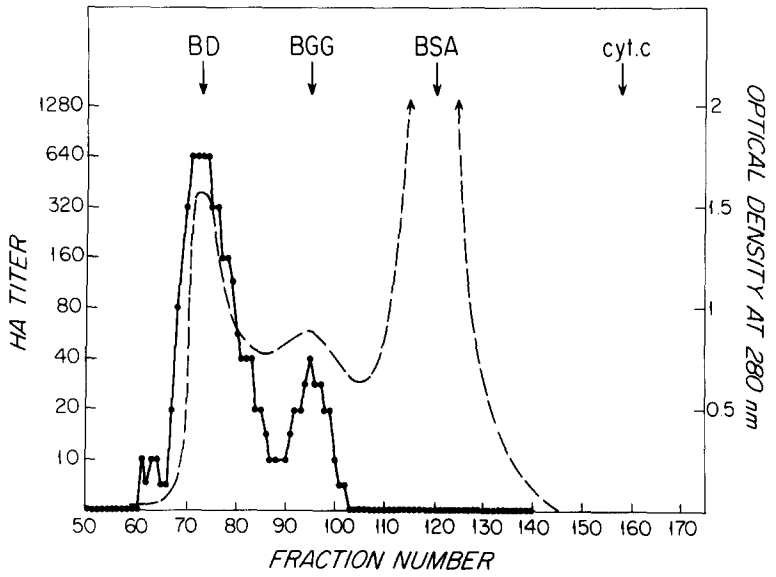

FIG. 1. Separation of IgM and IgG GAT-specific antibodies. A Sephadex G-200 column (1.5 × 20 cm) was equilibrated in PBS at 4°C. The column was calibrated using Blue dextran 2000 (BD, 2 × 10^6 mol wt), bovine gamma globulin (BGG, 150,000 mol wt), bovine serum albumin (BSA, 68,000 mol wt), and cytochrome *c* (cyt C, 12,500 mol wt) as markers. The serum from BALB/c mice immunized twice with GAT in Maalox pertussis was passed over the column. Optical density at 280 nm of the eluate was recorded and the hemagglutination titer (HA) of each fraction was determined using GAT-SRBC as indicator cells. (●–●–●) HA titer; (- - -) optical density.

Antigenic Suppression of the IgM and IgG PFC Responses of GAT and GT Nonresponder Mice. Earlier studies from our laboratory have shown that injection of GT or GAT into nonresponder mice bearing the *H-2*^{d} or *H-2*^{q} haplotype stimulate suppressor T cells capable of inhibiting antigen-specific IgG PFC responses to GT-MBSA or GAT-MBSA, respectively (5, 12). The effect of GT preimmunization on the IgM and IgG PFC responses of BALB/c mice is shown in Table VI. Both IgM and IgG responses are suppressed. Likewise, GAT preimmunization diminishes considerably the GAT-MBSA IgM and IgG PFC responses of DBA/1 mice. It is well documented that the inhibition of IgG PFC responses in the GAT and GT systems is due to active suppression mediated by T cells (18).

The data presented in Table VII illustrate that the transfer of 20 × 10^6 cells from GT-primed BALB/c mice results in the suppression of both the IgM and IgG PFC responses of BALB/c mice demonstrating, therefore, the presence of suppressor cells "active" on both IgM and IgG responses in GT-primed mice.

Discussion

Previous studies from our laboratory have shown that both responder and nonresponder mice develop only IgG primary responses to GAT, GT, and GA (2–5). In the present study, we report the appearance of IgM antibodies in secondary responses to GAT in responder and to GAT-MBSA or GT-MBSA in nonresponder mice. We have verified the presence of antigen-specific IgM antibodies

TABLE III
Secondary PFC Responses of BALB/c Mice to GAT at Various Times after the Second Immunization

Interval after second injection*	GAT-specific PFC per spleen‡	
	IgM	IgG
4 days	20,250 ± 4,360	75,825 ± 11,209
7 days	23,250 ± 5,603	67,640 ± 10,526
11 days	14,240 ± 5,124	22,240 ± 3,784

* Mice were immunized initially with 50 μg GAT in Maalox-pertussis i.p. followed 3 days later by 10 μg GAT in Maalox-pertussis. GAT-specific PFC were assayed either 4, 7, or 11 days after second injection of antigen.

‡ Numbers represent arithmetic mean ± SE for five mice per group.

TABLE IV
Effect of Repeated Immunization with GAT in Responder, C57BL/6, and Nonresponder, DBA/1, Mice

Strain	Immunization*		Number of mice/group	GAT-specific PFC per spleen‡	
	Day 0	Day 3		IgM	IgG
				Arith. mean ± SE	
C57BL/6	GAT/MP	GAT/MP	6	15,100 ± 2,822	145,225 ± 30,326
DBA/1	–	GAT/MP	4	<200	<200
DBA/1	GAT/MP	GAT/MP	4	<200	<200

* Mice received 50 μg GAT in Maalox pertussis intraperitoneally followed 3 days later by 10 μg GAT in Maalox pertussis, intraperitoneally. 7 days later, the number of GAT-specific PFC per spleen was determined.

‡ It was shown earlier that a primary immunization of C57BL/6 with GAT does not elicit an IgM response. Although not shown, these results were confirmed using a limited number of animals.

both functionally by the formation of direct PFC which are totally inhibitable by a well-characterized goat anti-mouse μ-chain antiserum (14) and physically by gel filtration on a Sephadex G-200 column. Simultaneous with the appearance of the IgM antibodies in the secondary response, we have observed a marked enhancement in the IgG antibody response, confirmed by both the plaque assay and by an antigen-binding assay.

The appearance of GAT- or GT-specific antibodies of the IgM class is dependent upon the repeated administration of antigen. The quantity of antigen does not appear to be the critical parameter in these experiments; BALB/c mice injected once with 10 μg GAT (Table I) develop only an IgG response, whereas a comparable amount of GAT in two injections stimulates an IgM as well as an enhanced IgG response. In other experiments (not shown) a single injection of BALB/c mice with 100 μg GAT in either Maalox pertussis or complete Freund's adjuvant did not induce IgM PFC while stimulating strong IgG PFC responses 4–7 days after primary immunization. IgM antibodies are induced even when the two injections of antigen are separated by a 21-day interval, characterizing this phenomenon as a secondary antibody response to these linear polymers. The appearance of IgM antibodies is not transient; IgM PFC responses can be detected in the plaque assay 4–11 days after the second injection of antigen.

TABLE V

Effect of Repeated Administration of GT-MBSA or GAT-MBSA in Genetic Nonresponder BALB/c and DBA/1 Mice

Strain	Immunization*		Antigen-specific PFC per spleen‡			
	Day 0	Day 3	IgM	*P* value	IgG	*P* value
			Arith. mean ± SE		*Arith. mean ± SE*	
BALB/c		GT-MBSA	205 ± 5.5		4,708 ± 923	
BALB/c	GT-MBSA	GT-MBSA	12,261 ± 1,709	<0.001	11,283 ± 2,029	0.009
DBA/1		GAT-MBSA	178 ± 8.8		4,305 ± 573	
DBA/1	GAT-MBSA	GAT-MBSA	16,477 ± 3,635	<0.001	10,569 ± 1,544	0.001

* Mice were immunized intraperitoneally with either 50 μg of GT as GT-MBSA or 50 μg GAT as GAT-MBSA in Maalox pertussis initially followed 3 days later by an i.p. injection of 10 μg GT as GT-MBSA or 10 μg GAT as GAT-MBSA in Maalox pertussis. On day 10, spleens from these mice were assayed for antigen-specific PFC.

‡ Nine mice were used per data group.

Furthermore, the induction of IgM antibodies is not limited to responder animals; nonresponder animals produce antigen-specific IgM when either GT or GAT complexed to MBSA are administered twice.

The restriction of primary responses to GAT, GA, GAT-MBSA, and GT-MBSA to the IgG class together with the requirement of repeated immunization to stimulate IgM antibody responses to these antigens raise important issues concerning the regulation of class-specific antibody responses and challenge the generally accepted belief that IgM antibody responses (when they occur) are the earliest antibody responses and always precede IgG antibody responses, particularly after primary immunization. These conclusions were based upon the responses to particulate antigens such as SRBC and bacteria and also upon the result of immunization with antigens that have a thymus-independent as well as a thymus-dependent component, such as (T,G)-A--L or polymerized flagellin. The strong possibility must be entertained that the commitment to early IgM responses is a property of thymus-independent antigens, whereas thymus-dependent antigens which require the activity of helper T cells and often elicit the regulatory activity of specific suppressor T cells may sometimes stimulate primary IgG and secondary IgM responses as seen in the current experiments. The appearance of IgM in the secondary antibody response in our experiments may be explained by the existence of two helper T-cell populations, one specific for IgM responses and the other for IgG. Differential activation of these two populations could explain why IgG helper cells might be triggered after only a single injection of antigen, while IgM helpers would require a second injection of antigen for stimulation. An alternative hypothesis would be that injection of antigen into responder animals leads to the stimulation of both antigen-specific helper and suppressor T cells. The suppressor cells stimulated after a single injection of antigen may preferentially affect the IgM response, therefore, the effect of the helpers would lead to the production of only IgG antibodies. According to this hypothesis, a second injection of antigen would shift the helper-suppressor balance in favor of the helper cells explaining the appearance of IgM and the enhancement of IgG antibody levels. Both hypotheses also apply to genetic nonresponder animals when the relevant linear polymer is coupled to an immunogenic carrier-protein such as MBSA.

Our recent studies have demonstrated the stimulation of specific suppressor T

TABLE VI

Effect of GT or GAT Preimmunization on the IgM or IgG PFC Responses of BALB/c or DBA/1 Mice to GT-MBSA or GAT-MBSA, Respectively

Strain	Immunization		Number of mice/group	Antigen-specific PFC per spleen*			
				IgM	*P* value	IgG	*P* value
				Arith. mean ± SE		*Arith. mean ± SE*	
BALB/c	–	GT-MBSA*	10	23,140 ± 4,209		12,680 ± 1,331	
BALB/c	GT‡	GT-MBSA	10	620 ± 265	0.002	360 ± 22	<0.001
DBA/1	–	GAT-MBSA*	9	17,677 ± 3,577		9,822 ± 1,371	
DBA/1	GAT‡	GAT-MBSA	10	3,710 ± 1,710	0.002	475 ± 34	<0.001

* Mice were immunized intraperitoneally with 50 μg GT as GT-MBSA or 50 μg GAT as GAT-MBSA in Maalox pertussis followed 3 days later by 10 μg GT as GT-MBSA or 10 μg GAT as GAT-MBSA in Maalox pertussis. 7 days after second antigen-MBSA injection the number of PFC per spleen was determined.

‡ Mice were preimmunized with 100 μg GT or 10 μg GAT in Maalox.

TABLE VII

Effect of Transfer of Normal or GT-Primed Spleen Cells on the Primary and Secondary PFC Responses of BALB/c Mice to GT-MBSA

Cells transferred:*	Immunization‡		Number of mice/group	GT-specific PFC per spleen			
Day 0	Day 3	Day 6		IgM	*P* value	IgG	*P* value
				Arith. mean ± SE		*Arith. mean ± SE*	
–	–	GT-MBSA	4	ND§		10,750 ± 900	
GT primed	–	GT-MBSA	4	ND		450 ± 250	<0.001
–	GT-MBSA	GT-MBSA	6	13,700 ± 2,328		13,433 ± 2,577	
Normal	GT-MBSA	GT-MBSA	6	10,841 ± 821	0.27	11,875 ± 1,669	0.62
GT primed	GT-MBSA	GT-MBSA	6	275 ± 25	0.001	270 ± 50	0.001

* BALB/c spleen cells (20×10^6) from normal mice or mice that had received 100 μg GT in Maalox 3 days earlier were injected intravenously into normal BALB/c mice.

‡ Mice were immunized with 1 or 2 injections of GT-MBSA. Primary responses were elicited by injecting 10 μg of GT as GT-MBSA, i.p. Mice were initially injected intraperitoneally with 50 μg of GT as GT-MBSA followed 3 days later by an i.p. injection of 10 μg GT as GT-MBSA for secondary responses. 7 days after the 10 μg injection spleens were assayed for antigen-specific PFC.

§ ND, not determined.

cells in nonresponder mice by GAT and in selected nonresponder strains by GT. These suppressor T cells were found to suppress the primary IgG responses to GAT-MBSA and GT-MBSA (5, 12, 18). In this report, we have shown that preimmunization with the appropriate copolymer inhibits the secondary IgM and IgG responses in nonresponder animals to GAT-MBSA or GT-MBSA. The tolerance observed is due to the induction of suppressor cells as demonstrated by cell transfer experiments (Table VII). We can extend the above two hypotheses to explain suppression in nonresponder animals. Suppression can be explained either by two populations of suppressor cells each acting separately on the IgM and IgG responses or by one population of suppressor cells working on both responses. Experiments are currently in progress to resolve these two models.

Summary

Primary responses to the linear polymers of L-glutamic acid, L-tyrosine, and L-alanine are restricted to the IgG class of antibodies. The appearance of specific IgM antibodies against these antigens is dependent upon secondary immunization, in contrast to many classical antigenic systems. The presence of an IgM response was verified by a direct plaque-forming cell assay, the inhibition of direct plaques by an antiserum specific for mouse μ-chain, and the physical

separation of IgM and IgG GAT-specific antibodies by gel filtration. Preimmunization of the appropriate nonresponder strain with GAT or GT inhibits both the secondary IgM and IgG responses to GAT-MBSA and GT-MBSA, respectively. The tolerance observed is due to the induction of suppressor cells as demonstrated by cell transfer experiments.

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