Brief Definitive Report

GENETIC CONTROL OF SPECIFIC IMMUNE SUPPRESSION
III. Mapping of *H-2* Complex Complementing Genes Controlling Immune Suppression by the Random Copolymer L-Glutamic Acid^{50}-L-Tyrosine^{50} (GT)*

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Earlier studies from our laboratory demonstrated that the terpolymer of L-glutamic acid, L-alanine, and L-tyrosine (GAT) stimulated the development of T cells capable of specifically suppressing the antibody responses in vivo and in vitro of nonresponder strains (bearing the *H-2^s*, *H-2^q*, and *H-2^p* haplotypes) to GAT complexed with an immunogenic carrier, methylated bovine serum albumin, MBSA (1, 2). We then extended these findings to another antigen, the copolymer of L-glutamic acid and L-tyrosine (GT). None of 19 inbred or congenic resistant mouse strains developed antibody responses to GT after immunization with this synthetic polypeptide in adjuvants. All the strains investigated, however, developed IgG plaque-forming cells (PFC) primary responses to GT complexed with MBSA (3). This permitted us to determine that: (*a*) preimmunization with GT suppressed the response to GT-MBSA in certain but not in all strains; (*b*) the suppression could be transferred by thymocytes and spleen cells from GT-primed animals; (*c*) the development of GT-specific suppressor cells is under dominant control of *H-2*-linked gene(s) which have been designated specific immune suppressor genes (Is genes); (*d*) the Is genes are antigen specific since GAT-MBSA responses are suppressed by GAT in strains carrying the *H-2^q* haplotype, while GT-MBSA responses are not suppressed by the related polymer GT in these same strains (3, 4).

The experiments reported in this study map the Is genes responsible for GT-specific suppression within the *H-2* complex. The data indicate that the *K* and *D* loci are not concerned with GT-specific suppression, and that this phenomenon is controlled by complementing or interacting genes which map on either side of cross-over events between the *IB* and *IC* subregions.

Materials and Methods

Mice. Mice were purchased from The Jackson Laboratory, Bar Harbor, Maine or the Health Research Laboratories, Buffalo, N. Y., or were bred in our animal facilities. Mice used in these experiments were 2–8 mo old and were maintained on acidified chlorinated drinking water and laboratory chow ad libitum.

Antigens. A preparation of GT with molar amino acid ratios Glu^{50}Tyr^{50} and average mol wt of 30,800 daltons was used for immunization. Several preparations of GAT with amino acid ratios Glu^{60}Ala^{30}Tyr^{10} and average mol wt ranging from 35,000 to 47,000 were used for sensitizing sheep red cells. All polymers were purchased from Miles Laboratories, Inc., Miles Research Division, Elkhart, Ind.

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MBSA was prepared according to the method of Sueoka and Cheng (5). The GT and GAT solutions and the complexes of these antigens with MBSA were prepared as described previously (1, 4).

Immunization. To investigate the suppressive properties of GT for different mouse strains, mice were injected intraperitoneally initially with 100 μg GT in a mixture of 1 mg magnesium and aluminum hydroxides (Maalox, William H. Rorer, Inc., Fort Washington, Pa.) or with Maalox alone. 3 days later the mice were immunized intraperitoneally with 10 μg of GT as GT-MBSA. Since our experience demonstrated that different strains of mice required different adjuvants (complete Freund's or pertussis vaccine) for optimal PFC responses, the appropriate adjuvant was selected for each strain as indicated in the Tables. When complete Freund's adjuvant was used, the antigen was emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). When pertussis vaccine was used, mice were injected with antigen in 1 mg Maalox with 2×10^9 killed *Bordetella pertussis* organisms (Eli Lilly and Co., Indianapolis, Ind.).

Hemolytic Plaque Assay. The antibody response to GT-MBSA was assayed 7 days after immunization using a modification of the Jerne hemolytic plaque technique described by Pierce et al. (6). As in previous studies the GT-MBSA response was assayed on sheep red blood cells coupled with the cross-reacting polymer GAT, GAT-SRBC (3).

Since it was noted in our previous studies that the PFC responses in the spleens to GT-MBSA are restricted to the IgG class, the plaques were developed using suitable rabbit antimouse Ig antisera at the appropriate dilution and guinea pig complement as described (3). GT-specific plaques were determined by subtracting the number of PFC remaining after inhibition by a suitable dilution of GAT from the number of plaques detected on GAT-SRBC in the absence of the specific inhibitor (1, 3). All assays were done in duplicate and the number of PFC per spleen recorded.

Results and Discussion

The ability of a variety of inbred, congenic resistant, and *H-2*-recombinant strains to show suppressed GT-MBSA primary PFC responses as a result of preimmunization with GT is presented in Table I. The development of GT-specific suppression is controlled by genes in the *H-2* complex, as reported earlier (4). Mouse strains bearing the $H\text{-}2^k$, $H\text{-}2^d$, or $H\text{-}2^s$ haplotypes but not $H\text{-}2^b$ or $H\text{-}2^q$ haplotypes are specifically suppressed by GT preimmunization regardless of the genes contributed by the B10 or the A backgrounds in the congenic resistant strains.

A most intriguing finding presented in Table I is the failure of GT to suppress GT-MBSA response in the recombinant strains B10.A and A/J and 9R and B10.HTT. The B10.A and A/J strains bear natural *H-2* recombinants between the two "GT suppressor" haplotypes $H\text{-}2^d$ and $H\text{-}2^k$, whereas B10.HTT is a recombinant between the "GT suppressor" haplotypes $H\text{-}2^s$ and $H\text{-}2^u$. In addition, the 9R strain is a recombinant comprising the left side of $H\text{-}2^s$ and the right side of $H\text{-}2^q$ (which is thought to be identical to the right side of the "GT suppressor" $H\text{-}2^d$ haplotype). The recombinant events in all these strains occurred between the *IB* and *IC* subregions. These observations indicate that: (*a*) GT-specific suppression is controlled by at least two complementing or interacting loci; (*b*) that there are restrictions in the ability of GT suppressor genes to complement when they come from different haplotypes (we shall refer to this phenomenon as the requirement for coupled complementation); and (*c*) that the genes responsible for the control of GT-specific suppression are different in $H\text{-}2^d$, $H\text{-}2^k$, and $H\text{-}2^s$ suppressor haplotypes. The patterns of responsiveness of the HTG (Table II) and 7R (Table I) recombinant strains establish that the *D* locus is not concerned with GT-specific suppression. Conversely the finding that the

TABLE I

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

Strain	Mice/ group	*H-2* type	*H-2* region formulae						Maalox + GT-MBSA (mean ± SE)	GT + GT-MBSA (mean ± SE)	Suppression of the GT-MBSA response	*P* value
			K	*IA*	*IB*	*IC*	*S*	*D*				
									PFC/spleen	*PFC/spleen*	%	
BALB/c	48	*d*	*d*	*d*	*d*	*d*	*d*	*d*	12,658 ± 750	2,566 ± 492	80	<0.000001
B10.D2	4	*d*	*d*	*d*	*d*	*d*	*d*	*d*	9,187 ± 977	3,325 ± 1,920	64	<0.03
B10.BR	16	*k*	*k*	*k*	*k*	*k*	*k*	*k*	7,765 ± 1,133	1,371 ± 496	82	<0.00001
C57BR	8	*k*	*k*	*k*	*k*	*k*	*k*	*k*	10,334 ± 1,819	3,346 ± 1,018	68	<0.006
SJL	16	*s*	*s*	*s*	*s*	*s*	*s*	*s*	10,401 ± 887	3,012 ± 652	71	<0.000001
B10.S	9	*s*	*s*	*s*	*s*	*s*	*s*	*s*	6,358 ± 2,178	847 ± 355	87	<0.01
A.SW	12	*s*	*s*	*s*	*s*	*s*	*s*	*s*	8,500 ± 1,300	2,800 ± 800	67	<0.001
A.BY	8	*b*	*b*	*b*	*b*	*b*	*b*	*b*	11,079 ± 2,174	14,286 ± 2,179	0	NS
B10	7	*b*	*b*	*b*	*b*	*b*	*b*	*b*	14,489 ± 3,147	13,096 ± 2,351	10	NS
A/J	27	*a*	*k*	*k*	*k* |	*d*	*d*	*d*	9,021 ± 894	10,315 ± 982	0	NS
B10.A	8	*a*	*k*	*k*	*k* |	*d*	*d*	*d*	12,118 ± 1,746	15,631 ± 3,398	0	NS
9R	11	*t4*	*s*	*s*	*s* |	*d*	*d*	*d*	8,677 ± 2,441	6,406 ± 1,644	26	NS
B10.HTT	14	*t3*	*s*	*s*	*s* |	*k*	*k*	*d*	8,119 ± 1,197	8,446 ± 1,012	0	NS
HTG	10	*g*	*d*	*d*	*d*	*d*	*d* |	*b*	15,807 ± 1,922	3,117 ± 1,258	80	<0.00003
7R	10	*t2*	*s*	*s*	*s*	*s*	*s* |	*d*	5,817 ± 1,205	725 ± 310	88	<0.0006
A.TL	8	*t1*	*s* |	*k*	*k*	*k*	*k*	*d*	10,693 ± 2,322	3,196 ± 1,326	71	<0.01

NS, not significant. Vertical bar indicates location of crossing over event.

*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 specific IgG PFC per spleen were counted using GAT-coated SRBC. All strains except B10, B10.D2, B10.S, B10.A, and 7R were immunized with complete Freund's adjuvant. B10, B10.D2, B10.S, B10.A, and 7R mice were immunized using Maalox and *B. pertussis* as adjuvant.

TABLE II

*Gene Complementation in the Trans Position Controlling GT-Specific Suppression**

Strain	Mice/ group	*H-2* type	*H-2* region formulae						Maalox + GT-MBSA (Mean ± SE)	GT + GT-MBSA (Mean ± SE)	Suppression GT-MBSA response	*P* value
			K	*IA*	*IB*	*IC*	*S*	*D*				
									PFC/spleen	*PFC/spleen*	%	
HTG	10	*g*	*d*	*d*	*d*	*d*	*d* |	*b*	15,807 ± 1,922	3,117 ± 1,258	80	<0.00003
D2.GD	10	*g4*	*d*	*d* |	*b*	*b*	*b*	*b*	13,140 ± 1,312	11,387 ± 2,563	14	NS
B10.A	8	*a*	*k*	*k*	*k* |	*d*	*d*	*d*	12,118 ± 1,746	15,631 ± 3,398	0	NS
(D2.GD × B10.A)F_1	11	*g4/a*	*d/k*	*d/k*	*b/k*	*b/d*	*b/d*	*b/d*	7,243 ± 1,087	3,544 ± 1,312	51	<0.04

NS, not significant. Vertical bar indicates location of crossing over event.

* Immunization doses and procedures were the same as described in Table I. HTG and D2.GD strains were immunized with complete Freund's adjuvant. B10.A and (D2.GD × B10.A)F_1 were immunized with Maalox and *B. pertussis* as adjuvant.

A.TL strain can be suppressed in its GT-MBSA response by GT preimmunization rules out a contribution by the *K* locus to this phenomenon and establishes that the complementing suppressor genes map in the *I* and/or *S* regions of the *H-2* complex and more probably in the *I* region, known to be concerned with the regulation of immune responses to thymus-dependent antigens (7). The data

presented in Table II suggest that, when genes from the same "suppressor" *H-2*^{d} haplotype capable of complementation for GT-specific suppression are found in the *trans* configuration as in the (D2.GD × B10.A)F_1, GT-specific suppression of GT-MBSA primary PFC responses can be observed. Collectively, these various experiments may be interpreted to indicate that complementing genes in *I-C* and genes in *I-A* and/or *I-B* are responsible for the development of GT-specific suppression. This situation is, in several respects, analogous with the genetic control of antibody responses (8) and of in vitro stimulation of DNA synthesis (9) in animals immunized with the terpolymer of L-glutamic acid, L-lysine, and L-phenylalanine (GLϕ) except that in the case of the GLϕ Ir genes, complementation was demonstrable by the responsiveness of recombinant strains derived from strains bearing nonresponder haplotypes (8); in the case of GT Is genes, we are faced with the reverse situation, i.e., recombinants between strains bearing suppressor haplotypes fail to show suppression and yet cannot be demonstrated to behave as unequivocal GT responder strains (3). The data imply, therefore, a very close if not, as yet, an absolute reverse relationship between the control of specific responsiveness and specific suppression by complementing *I*-region genes. The close relationship between Ir and Is genes is also supported by the observations that (*a*) weak but definite T-cell helper activity has been reported in GAT nonresponder strains under appropriate experimental conditions (10); and (*b*) the development of transient PFC responses to GT in nonresponder *H-2*^{d} BALB/c mice treated with an appropriate dose of cyclophosphamide known to destroy suppressor T-cell activity (11).

The ability of preimmunization with GAT or GT to suppress the response of genetic nonresponder strains to GAT-MBSA or GT-MBSA, respectively, can be transferred by antigen-specific factors obtained from thymocytes or spleen cells from animals primed with the copolymers (12, and footnote 1). The complementary genes concerned with the control of GT-specific suppression could control the synthesis and/or release of the suppressor factor or its ability to exert its regulatory activity on cells of the immune system. Studies in progress to explore these possibilities suggest strain restrictions in the ability of GAT-specific and GT-specific suppressor factors to exert their effects in recipient animals (12, and footnote 1).

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