THE REQUIREMENT FOR TWO COMPLEMENTING *Ir-GLΦ* IMMUNE RESPONSE GENES IN THE T-LYMPHOCYTE PROLIFERATIVE RESPONSE TO POLY-(Glu^{53}Lys^{36}Phe^{11})*

By RONALD H. SCHWARTZ, MARTIN E. DORF, BARUJ BENACERRAF, and WILLIAM E. PAUL

(From The Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014, and The Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115)

The ability of mice to form antibodies against the linear, random terpolymer, poly-(Glu^{53}Lys^{36}Phe^{11}) (GLϕ) has been shown to be under the control of two major histocompatibility complex (MHC)[1]-linked immune response *(Ir)* genes termed α and β (1, 2). The sites of expression of these *Ir* genes have not yet been elucidated. Recently, Munro and Taussig have studied another two-gene model, namely that for poly-(Tyr,Glu)-poly-D,L-Ala--poly Lys [(T,G)-A--L], and have postulated that in their system one gene is expressed in the thymus-derived (T) lymphocyte and the other in the bone marrow-derived (B) lymphocyte (3). To assess this possibility in the GLΦ system, we have examined the T-lymphocyte proliferative response to GLΦ in a variety of mouse strains using a newly developed assay system which employs nylon wool column-purified peritoneal exudate lymphocytes (4). Our results demonstrate that strains bearing responder alleles at only the α- or the β-locus are nonresponders as assessed by T-lymphocyte proliferation. Yet, similar to the requirement for antibody formation, strains possessing responder alleles at both loci are responders. The α- and β-genes can complement each other in both the *trans* position (F_1 hybrids) or the *cis* position (recombinant strains) to give responder phenotypes. The complementation in *cis* appears to be stronger than that in *trans*. These findings mitigate against the possibility that either of the *Ir* genes necessary for the GLΦ response is expressed solely in B lymphocytes because it would be anticipated that mice with a defect limited to the B lymphocyte would express a normal T-lymphocyte proliferative response.

Materials and Methods

Animals. Mice were purchased from The Jackson Laboratory, Bar Harbor, Maine or the Rodent and Rabbit Production Section of the Division of Research Services, National Institutes of Health. Several strains were raised either in the animal facilities of Harvard Medical School or those of the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases,

* This work was supported in part by grant AI-09920 from the National Institutes of Health, U. S. Public Health Service.

[1] *Abbreviations used in this paper:* MHC, major histocompatibility complexes; PETLES, peritoneal exudates, T-lymphocyte-enriched cells; PPD, purified protein derivitive.

National Institutes of Health. All mice were between 6 and 24 wk of age at the start of immunization.

Antigens. The random linear synthesis terpolymer poly-(Glu[53]Lys[36]Phe[11]), sample No. GF 6-23-8 (GLΦ), was synthesized from the *N*-carboxyanhydrides and donated by Dr. Elkan Blout, Department of Biological Chemistry, Harvard Medical School, Boston, Mass. The polymer was dissolved in 1 N NaOH, neutralized to pH 7.2 with 1 N HCl, and stored at −20°C. Purified protein derivative of tuberculin (PPD) was purchased from Connaught Medical Research Labs., Willowdale, Ontario, as a 2 mg/ml solution, and stored at −20°C. Before addition to the cultures both antigens were diluted to appropriate concentrations with culture medium.

Immunization. Mice were immunized with 20 μg of GLϕ in complete Fruend's adjuvant containing 1 mg/ml of *Mycobacterium tuberculosis,* strain H37 Ra (Difco Laboratories, Detroit, Mich.). The emulsion was distributed equally between the two hind footpads. The animals were sacrificed 3 to 6 wk later.

Cell Cultures. The preparation of peritoneal exudate, T-lymphocyte-enriched cells (PETLES), and their in vitro culture with antigen has been described in detail elsewhere (4). Briefly, thioglycollate-induced peritoneal exudate cells were passed over nylon wool columns to obtain the PETLES. This population contained an average of only 2% B lymphocytes. The cells were cultured at 1×10^5 per well in microtiter plates containing 0.2 ml of EHAA medium supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). Control wells received no antigen, and antigen-stimulated wells received either 100 μg/ml of GLΦ or 20–40 μg/ml of PPD. The cells were cultured for 5 days, and proliferation was assessed by measuring the incorporation of a 1 μCi pulse of tritiated-methylthymidine (Amersham/Searle Corp., Arlington Heights, Ill.: Sp Act 5Ci/mmol). The data are expressed as counts per minute ± the standard error of the mean (SEM) for triplicate determinations. Strains were designated as responders to GLΦ if there was a statistically significant difference between antigen-stimulated and control cultures as measured by a Student's *t* test ($P \leq 0.05$).

Results

T-Lymphocyte Proliferative Response to GLΦ. GLΦ proved to be a potent stimulator of T-lymphocyte proliferation in the mouse. When PETLES were prepared from B10.D2 mice 3 wk after immunization with 20 μg of GLΦ emulsified in complete Freund's adjuvant, challenge with the antigen in vitro resulted in a substantial incorporation of tritiated thymidine by these cells. The mean incorporation, expressed as the difference between antigen-stimulated and control cultures, Δcpm, for the three such experiments shown in Table I was 110,000 cpm. The response to GLΦ in this strain was always equal to or greater than the response of the same B10.D2 cells to PPD. The GLΦ response was maximum at 3 wk after immunization and slowly declined thereafter (Table I, experiments 4 and 5).

The T-Lymphocyte Proliferative Response to GLΦ is under Genetic Control. Examination of the response to GLΦ by PETLES from a variety of inbred mouse strains revealed a division of the strains into responder and nonresponder categories. Strains were assigned a responder status if the incorporation of tritiated thymidine in the presence of GLΦ was significantly greater ($P < 0.05$) than the incorporation in the absence of GLΦ. Responder strains were further divided into marginal (±), weak (+), or strong responders (++) depending on the magnitude of the proliferative response.

Responsiveness appeared to be associated with *H-2* haplotype (Table II). Strains having the *d, j, q,* or *r* haplotype were responders to GLΦ,[2] whereas

[2] Schwartz, R. H., and W. E. Paul. 1976. T-lymphocyte-enriched murine peritoneal exudate cells. II. Genetic control of antigen-induced T-lymphocyte proliferation. *J. Exp. Med.* 143:529.

TABLE I

T-Lymphocyte Proliferative Response to Poly-(Glu^{53}Lys^{36}Phe^{11})

Exp. no.	Weeks after immunized	Proliferative response (cpm ± SEM) to:		
		Medium	GLΦ	PPD
1	3	1,900 ± 200	115,400 ± 17,800	108,900 ± 2,400
2	3	5,000 ± 3,400	92,400 ± 2,500	45,600 ± 3,900
3	3	1,700 ± 300	131,600 ± 3,100	67,500 ± 1,200
4	4	4,100 ± 500	44,700 ± 8,900	–
5	6	740 ± 320	29,600 ± 3,700	–

1×10^5 PETLES from B10.D2 mice, which had previously been immunized with GLΦ emulsified in CFA, were cultured with either 100 μg/ml of GLΦ, 20 μg/ml of PPD, or medium alone. Stimulation was assessed 5 days later by measuring the incorporation of a pulse of tritiated thymidine. The data are expressed as mean counts per minute ± SEM for triplicate determinations.

TABLE II

Strain Distribution of the T-Lymphocyte Proliferative Response to Poly-(Glu^{53}Lys^{36}Phe^{11})

Strain	H-2 haplotype	Proliferative response (cpm ± SEM) to:*			Responsiveness
		Medium	GLΦ	PPD	
A/J	*a*	1,500 ± 500	2,000 ± 1,000	38,000 ± 1,400	–
B10.A/SgSn	*a*	400 ± 100	450 ± 60	87,700 ± 6,500	–
C57BL/10Sn	*b*	1,000 ± 100	1,200 ± 200	51,500 ± 1,500	–
C57BL/6N	*b*	2,400 ± 800	4,900 ± 1,700	17,900 ± 2,800	–
BALB/cAnN	*d*	2,100 ± 300	28,700 ± 1,600	145,100 ± 3,100	++
B10.D2/nSn	*d*	1,900 ± 200	115,400 ± 17,800	108,900 ± 2,400	++
I/StN	*j*	37,600 ± 1,900	131,100 ± 2,600	87,400 ± 15,600	++
C3H/HeN	*k*	900 ± 250	300 ± 70	29,200 ± 1,900	–
B10.BR/SgSn	*k*	2,900 ± 600	5,500 ± 1,500	81,600 ± 2,500	–
DBA/1J	*q*	1,600 ± 50	64,600 ± 4,500	12,700 ± 500	++
SWR/J	*q*	2,500 ± 900	37,600 ± 5,200	17,400 ± 400	++
B10.RIII	*r*	3,100 ± 800	50,400 ± 4,000	5,100 ± 1,500	++
SJL/J	*s*	1,700 ± 700	800 ± 200	217,000 ± 20,700	–
B10.S	*s*	2,000 ± 500	4,200 ± 1,500	38,000 ± 2,900	–
B10.SM	*v*	1,800 ± 300	2,500 ± 500	11,300 ± 1,900	–

* Culture conditions were the same as described in Table I.

‡ Strains were assigned a nonresponder status (–) if the incorporation of tritiated thymidine in the presence of GLΦ was not significantly different (Student's *t* test) from the incorporation in the absence of GLΦ. Responder strains were divided into marginal (±), weak (+), or strong (++) responders depending on the magnitude of the proliferative response.

strains having the *a*, *b*, *k*, *s*, or *v* haplotype were nonresponders. Nonresponder strains had specific defects in responsiveness, since in every case the PETLES from such strains responded well to PPD. The proliferative response of congenic resistant strains located the genes controlling responsiveness to the MHC. For example, the B10.D2/nSn strain, which contains *H-2* genes of the same haplotype as the responder BALB/c strain associated with the non-*H-2* genes of the nonresponder C57BL/10 strain, was a responder to GLΦ. In all cases the designation of a strain as a responder or nonresponder for T-lymphocyte proliferation to GLΦ correlated exactly with the designation previously assigned those

TABLE III
Complementation of the H-2^a and H-2^b Haplotypes

Strain	MHC alleles *K,I-A,I-B,I-C,S,D*	Proliferative response (cpm ± SEM) to:*			Responsiveness‡
		Medium	GLΦ	PPD	
B10.D2/nSn	*d d d d d d*	1,900 ± 200	115,400 ± 17,800	108,900 ± 2,400	++
C57BL/10Sn	*b b b b b b*	1,000 ± 100	1,400 ± 200	109,900 ± 15,200	−
B10.A/SgSn	*k k k d d d*	2,300 ± 500	900 ± 200	123,200 ± 11,300	−
(B10 × B10.A)F$_1$	*b b b b b b* / *k k k d d d*	2,300 ± 200	130,000 ± 2,700	140,500 ± 6,600	++
B10.A(5R)/SgSn	*b b b d d d*	1,200 ± 300	79,900 ± 4,500	50,600 ± 4,200	++
C57BL/6N	*b b b b b b*	2,400 ± 800	4,900 ± 1,700	17,900 ± 2,800	−
A/J	*k k k d d d*	1,500 ± 500	2,000 ± 1,000	38,000 ± 1,400	−
(B6 × A)F$_1$	*b b b b b b* / *k k k d d d*	3,400 ± 1,200	49,300 ± 7,800	30,200 ± 7,800	++
(B10.A × A)F$_1$	*k k k d d d*	8,400 ± 3,100	5,500 ± 1,600	113,400 ± 11,700	−
B10.A(2R)/SgSn	*k k k d d b*	400 ± 120	550 ± 150	102,700 ± 11,600	−
B10.A(4R)/SgSn	*k k b b b b*	2,200 ± 500	2,500 ± 400	69,400 ± 2,400	−
B10.A(18R)	*b b b b b d*	6,200 ± 400	7,400 ± 1,300	121,200 ± 5,600	−

* Culture conditions were the same as described in Table I.
‡ See Table II.

TABLE IV
Complementation of the H-2^k and H-2^s Haplotypes

Strain	MHC alleles *K,I-A,I-B,I-C,S,D*	Proliferative response (cpm ± SEM) to:*			Responsiveness‡
		Medium	GLΦ	PPD	
C57BL/10Sn	*b b b b b b*	1,000 ± 100	1,400 ± 200	109,900 ± 15,200	−
C3H/HeN	*k k k k k k*	900 ± 250	300 ± 70	29,200 ± 1,900	−
B10.BR/SgSn	*k k k k k k*	2,900 ± 600	5,500 ± 1,500	81,600 ± 2,500	−
SJL/J	*s s s s s s*	1,700 ± 700	800 ± 200	217,000 ± 20,700	−
B10.S	*s s s s s s*	5,400 ± 1,100	3,800 ± 1,100	29,500 ± 2,800	−
(B10.BR × B10.S)F$_1$	*k k k k k k* / *s s s s s s*	700 ± 150	4,400 ± 1,100	118,800 ± 12,100	+
(C3H × SJL)F$_1$	*k k k k k k* / *s s s s s s*	28,900 ± 4,300	52,700 ± 5,100	143,100 ± 5,200	+
B10.HTT	*s s s k k d*	860 ± 150	50,100 ± 6,500	33,900 ± 4,800	++
B10.S(9R)	*s s s d d d*	5,700 ± 1,100	66,600 ± 5,100	41,100 ± 2,100	++
B10.S(7R)	*s s s s s d*	8,200 ± 1,700	8,300 ± 2,100	77,800 ± 4,100	−

* Culture conditions were the same as described in Table I.
‡ See Table II.

strains on the basis of serum antigen-binding tests for antibody formation to GLΦ (1, 2).

Evidence for Two Ir Genes Controlling the T-Lymphocyte Proliferative Response to GLΦ. The antibody response to GLΦ has been shown to be under the control of two dominant MHC-linked *Ir* genes, α and β (1, 2). This was demonstrated by showing that different types of nonresponder strains could complement each other to give a responder phenotype if genetic material from each of them was brought together in either the *trans* position, by formation of F_1 hybrids, or the *cis* position, by recombination events. A similar approach was used for the study of the T-lymphocyte response to GLΦ.

The results in Table III illustrate again that strains of either the *H-2^a* or *H-2^b* haplotypes are nonresponders to GLΦ. However, a combination of these two

TABLE V
Complementation of the H-2^b and H-2^s Haplotypes

Strain	H-2 haplotype	Proliferative response (cpm ± SEM) to:*			Responsiveness‡
		Medium	GLΦ	PPD	
C57BL/6N	*b*	2,400 ± 800	4,900 ± 1,700	17,900 ± 2,800	−
C57BL/10Sn	*b*	1,000 ± 100	1,260 ± 200	51,500 ± 1,500	−
SJL/J	*s*	1,700 ± 700	800 ± 200	217,000 ± 20,700	−
B10.S	*s*	5,400 ± 1,100	3,800 ± 1,100	29,500 ± 2,800	−
(B6 × SJL)F_1	*b/s*	2,200 ± 300	7,200 ± 1,000	44,800 ± 18,300	+
(B10 × B10.S)F_1	*b/s*	5,300 ± 1,400	8,200 ± 100	41,600 ± 300	±

* Culture conditions were the same as described in Table I.
‡ See Table II.

MHC genomes in either the *trans* position, as seen with the (B10 × B10.A)F_1 hybrid, or the *cis* position, as seen with the B10.A(5R) recombinant strain, resulted in mice that responded to GLΦ. The magnitude of the response was similar to that of the B10.D2 responder strain. Other F_1 hybrids, such as the (B6 × A)F_1 were also responders to GLΦ, but the (B10.A × A)F_1 hybrid, in which complementation of only the non-*H-2* genes is achieved, was a nonresponder. Recombinants between the *H-2^a* and *H-2^b* haplotypes other than B10.A(5R), such as B10.A(2R), B10.A(4R), and B10.A(18R), were all nonresponders. These recombinants localize the *H-2^a* haplotype gene (α) to the region of the MHC between *I-B* and *D*, and the *H-2^b* haplotype gene (β) to the left of *I-C*.

Mice of the *H-2^k* and *H-2^s* haplotypes are both nonresponders to GLΦ. Table IV demonstrates that these haplotypes can also complement one another. In this case, however, the *cis* complementation is much more successful than the *trans* complementation. Thus, recombinants, such as B10.HTT, showed strong proliferative responses to GLΦ, whereas the F_1 hybrids, such as (B10.BR × B10.S)F_1 and (C3H × SJL)F_1, showed only weak, but significant, proliferative responses. The B10.S(9R) recombinant demonstrated that the *H-2^a* and *H-2^k* haplotypes share a similar *Ir* gene (α) capable of complementing a gene of the *H-2^s* haplotype. The lack of response by the B10.S(7R) recombinant located the α-gene of the *H-2^a* and *H-2^k* haplotypes to the region of the MHC between *I-B* and *D*.

Inasmuch as the *H-2^a* and *H-2^k* α-genes can be complemented by genes of the *H-2^b* and *H-2^s* haplotypes, respectively, the implication is that the *H-2^b* and *H-2^s* haplotypes possess responder alleles at the β-locus and nonresponder alleles at the α-locus. To further examine this point, we studied the response of *(H-2^b* × *H-2^s)*F_1 hybrids (Table V). In agreement with the serological data (2), PETLES from both the (B6 × SJL)F_1 and the (B10 × B10.S)F_1 strains gave weak, but statistically significant, responses to GLΦ, although the response of the former appeared to be somewhat greater than the response of the latter. Because the *H-2^k* and *H-2^s trans* complementations were also weak, it is possible that all *trans* complementations involving *H-2^s* genes are inefficient, and thus *(H-2^b* × *H-2^s)*F_1 strains fail to demonstrate the degree of complementation possible between genes of these haplotypes. Alternatively, it is possible that the meager

response of the (B10 × B10.S)F_1 strain, although statistically significant, is not biologically meaningful, and the partial complementation seen in the (B6 × SJL)F_1 strain represents some contribution of non-*H-2* genes of the SJL. The data, therefore, do not allow us to reach a definitive conclusion as to whether *H-2*^{b} and *H-2*^{s} haplotypes, both presumably of an α^-,β^+ phenotype, can complement each other. This point is, of course, important in the attempt to determine whether the β-alleles of *H-2*^{b} and *H-2*^{s} are the same or, indeed, whether a more complex system involving three polymorphic *H-2*-linked *Ir-GL*Φ may be operating.

Discussion

Our earlier studies of the genetic control of T-lymphocyte proliferation in the mouse using seven distinct antigens, responsiveness to which is controlled by MHC-linked *Ir* genes, indicated that the responder–nonresponder pattern was identical to that described for antibody-forming capacity[2]. Similarly, studies with guinea pigs have shown a concordance in responder status, as determined by both antibody responses and T-lymphocyte proliferation (5). Since all known antibody responses controlled by MHC-linked *Ir* genes are dependent on the participation of helper T lymphocytes, the simplest hypothesis to relate the two sets of observations would be to propose that *Ir* genes exert their effects in the process required for stimulation of the T lymphocyte. However, with the recent finding that the response to several antigens appears to be under the control of two independent, MHC-linked *Ir* genes (1–3), the situation becomes more complex. The goal of the present work was to examine the T-lymphocyte proliferative response in one of these systems (GLΦ) to determine whether only one or both of these *Ir* genes was required to obtain an immune response at the T-lymphocyte level.

The results were unequivocal. Mice of the *H-2*^{a} and *H-2*^{k} haplotypes, which possess a responder allele at the α, but not the β, *Ir-GL*ϕ locus, and mice of the *H-2*^{b} and *H-2*^{s} haplotypes, which possess a responder allele at the β, but not the α, *Ir-GL*Φ locus, were all nonresponders in the proliferation assay. That, in fact, there were two distinct gene defects accounting for nonresponsiveness in this system was shown by the analysis of F_1 hybrid and recombinant strains. PETLES from *(H-2*^{b} × *H-2*^{a}*)*F_1 hybrids and from B10.A (5R) recombinant mice were as responsive to GLΦ as were those from B10.D2 responders. Similarly, B10.HTT and B10.S(9R) recombinant mice, which were derived from the *H-2*^{s} and *H-2*^{a} or *H-2*^{k} nonresponder haplotypes, were excellent responders. PETLES from *(H-2*^{s} × *H-2*^{k}*)*F_1 hybrids also responded, although their response was much weaker than that of the other types of combinations. These complementation results thus demonstrate the operation of two *Ir* genes (α and β) in the T-lymphocyte response to GLϕ, and show that, in some cases, the *cis* complementation appears to be more successful than the *trans* complementation. These results are completely analogous to those observed when antibody responses are examined (1, 2, 6). In the present studies the possibility that the weaker complementation in the *trans* position might be explained by a gene dosage effect was not examined. However, we consider this possibility unlikely since serological analysis of the GLϕ response of the (B10.HTT × A.Ca)F_1 hybrid,

which is a cross between an $\alpha^+\beta^+$ responder and an $\alpha^-\beta^-$ nonresponder, demonstrated that both the α^+ and β^+ alleles are fully dominant (6).

The major conclusion from our experiments is that T lymphocytes from immunized mice possessing a responder allele at only one of the two *Ir*-GLϕ loci fail to proliferate in response to stimulation with GLΦ. In accord with this finding, Katz et al. (7), in a companion study, demonstrate that T-lymphocyte helper activity for DNP-FGG-primed B lymphocytes from (B6 × A)F_1 responders fails to develop in $\alpha^+\beta^-$ or $\alpha^-\beta^+$ parental donors primed with GLΦ. Thus, in two systems which B lymphocytes appear to play no role, the expression of both *Ir* genes is required for a T-cell response. These experiments indicate that neither the α-gene nor the β-gene can be expressed exclusively in B lymphocytes.

The proliferation data presented in this paper are compatible with the hypothesis that both *Ir* genes are expressed in T lymphocytes. An equally tenable hypothesis, however, is that one or both *Ir* genes are expressed in macrophages. Extensive studies in the guinea pig, using a similar T-lymphocyte proliferation assay, have indicated the requirement for antigen presentation by macrophages to obtain proliferation (8). In addition, it was shown for *Ir* gene-controlled systems, that T lymphocytes from (responder × nonresponder)F_1 animals could only be activated by antigen-pulsed macrophages from the responder parent (9). One way to interpret these results is to ascribe to the macrophage the ability to exert some control over the specificity of the immune response through the function of *Ir* genes.

The results in this paper appear to be at odds with the two-gene model proposed by Munro and Taussig (3) based on their interpretation of the data obtained with (T,G)-A--L. In their system one *Ir* gene is postulated to be expressed in T lymphocytes and the other in B lymphocytes. The evidence for this hypothesis is that some nonresponder strains are defective in the production of an antigen-specific, T-cell helper factor, while other nonresponder strains synthesize this factor but lack B lymphocytes capable of binding the factor (3). The apparent discrepancy is emphasized by the observation that the nonresponder strains which make the T-cell factor (e.g., *H-2*^{k}), nonetheless, fail to mount a measurable T-lymphocyte proliferative response to (T, G)-A--L. This was shown by us for the A/WySn, B10.A/SgSn, AKR/J, C3H/HeN, and B10.BR/SgSn strains[2] and by Lonai and McDevitt for the C3H/DiSn strain (10). The results with GLΦ potentially demonstrate the same point, although no analysis for production of T-cell helper factor in either type of GLΦ nonresponder strain has as yet been carried out.

There are several possible ways of reconciling this apparent discrepancy. One is to postulate that the T-lymphocyte proliferation response requires the action of both *Ir* genes, whereas, the production of the T-cell factor requires the action of only one of the two genes. For example, one might propose that both *Ir* genes are expressed in a single cell and that one controls differentiation events while the other controls subsequent proliferative events. Thus, factor production might require only the onset of differentiation, whereas proliferation would require, in addition, the expression of the other *Ir* gene. Alternatively, it might be postulated that the T-lymphocyte proliferation assay requires the function of

two distinct cell types, and that only one of the *Ir* genes is expressed in each type of cell. For example, if the interaction of two subclasses of T lymphocytes was necessary for the proliferative response to antigens such as GLΦ and (T,G)-A--L, a phenotypic nonresponder could exist which possessed a T-cell subset capable of producing the helper factor. Another variation on this two-cell model would be to place the expression of one of the *Ir* genes in the macrophage. To make the data of Munro and Taussig consonant with thesis, it would be necessary to further postulate that the *Ir* gene expressed in macrophages was also expressed in B lymphocytes, so that animals lacking the B-cell *Ir* gene could still produce T-cell helper factor by utilizing the *Ir* gene expressed only in T lymphocytes. One would also have a postulate that "responder" macrophages were not required for the priming of factor-producing T lymphocytes nor for the production of factor on secondary antigenic stimulation.

Summary

The antibody response to poly-(Glu^{53}Lys^{36}Phe^{11}) (GLΦ) has been shown to be under the control of two independent, major histocompatibility-linked immune response genes, designated α and β. In the present work we demonstrate that the T-lymphocyte proliferative response is also under the control of these two immune response genes. Thus, mice of the *H-2^a*, *H-2^b*, *H-2^k*, and *H-2^s* haplotypes were all nonresponders to GLΦ. In contrast, F_1 hybrids between these strains, such as (B10 × B10.A)F_1 and (C3H × SJL)F_1, as well as several recombinant mice derived from the nonresponder haplotypes, such as B10.A(5R), B10.HTT, and B10.S(9R), were all responders to GLΦ. The complementation between nonresponder genomes appeared to be stronger in the *cis* position than in the *trans* position for some strain combinations. The failure of strains bearing only one of the two responder alleles to show a T-lymphocyte proliferative response to GLΦ, argues strongly that neither gene can be expressed exclusively in B lymphocytes. This conclusion is discussed in relation to another two gene model which has recently been proposed.

References

1. 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.
2. Dorf, M. E., and B. Benacerraf. 1975. Complementation of H-2-linked Ir genes in the mouse. *Proc. Natl. Acad. Sci. U. S. A.* 72:3671.
3. Munro, A. J., and M. J. Taussig. 1975. Two genes in the major histocompatibility complex control immune response. *Nature (Lond.).* 256:103.
4. Schwartz, R. H., L. Jackson, and W. E. Paul. 1975. T lymphocyte-enriched murine peritoneal exudate cells. I. Reliable assay for antigen-induced T lymphocyte proliferation. *J. Immunol.* 115:1330.
5. Shevach, E. M., S. Z. Ben-Sasson, F. Finkelman, W. E. Paul, and I. Green. 1975. Mechanisms of action of genes controlling the immune response. *Transplant. Proc.* 7:141.
6. Dorf, M. E., P. H. Maurer, C. F. Merryman, and B. Benacerraf. 1976. Inclusion group systems and *cis-trans* effects in responses controlled by the two complementing *Ir-GLΦ* genes. *J. Exp. Med.* 143:889.

7. Katz, D. H., M. E. Dorf, and B. Benacerraf. 1976. Control of T-lymphocyte and B-lymphocyte activation by two complementing *Ir-GL*Φ immune response genes. *J. Exp. Med.* 143:906.
8. Rosenthal, A. S., J. T. Blake, J. J. Ellner, D. K. Greineder, and P. E. Lipsky. 1975. The role of macrophages in T lymphocyte antigen recognition. *In* Immune Recognition. A. S. Rosenthal, editor. Academic Press, Inc., New York. 539.
9. Shevach, E. M., and A. S. Rosenthal. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. II. Role of the macrophage in the regulation of genetic control of the immune response. *J. Exp. Med.* 138:1213.
10. Lonai, P., and H. O. McDevitt. 1974. Genetic control of the immune response. In vitro stimulation of lymphocytes by (T,G)-A--L, (H,G)-A--L, and (Phe,G)-A--L. *J. Exp. Med.* 140:977.