THE REQUIREMENT FOR TWO COMPLEMENTING Ir-GLΦ
IMMUNE RESPONSE GENES IN THE T-LYMPHOCYTE
PROLIFERATIVE RESPONSE TO POLY-(Glu53Lys36Phe11)*

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The ability of mice to form antibodies against the linear, random terpolymer, poly-(Glu53Lys36Phe11) (GLΦ) has been shown to be under the control of two major histocompatibility complex (MHC)-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 (F1 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,

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† Abbreviations used in this paper: MHC, major histocompatibility complexes; PETSLES, peritoneal exudates, T-lymphocyte-enriched cells; PPD, purified protein derivative.
T-LYMPHOCYTE RESPONSE TO POLY-(GLU\textsuperscript{53}LYS\textsuperscript{36}PHE\textsuperscript{11})

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\textsuperscript{53}Lys\textsuperscript{36}Phe\textsuperscript{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 Freund’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\textsuperscript{6} 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/mm). 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 ≤ 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\textsuperscript{2} whereas

\textsuperscript{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\textsuperscript{3}Lys\textsuperscript{3}Phe H)

| Exp. no. | Weeks | after immunized | Proliferative response (cpm ± SEM) to: |
|---------|-------|-----------------|---------------------------------------|
|         |       | Medium | GL\textsuperscript{\Phi} | 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\textsuperscript{6} PETLES from B10.D2 mice, which had previously been immunized with GL\textsuperscript{\Phi} emulsified in CFA, were cultured with either 100 \textmu g/ml of GL\textsuperscript{\Phi}, 20 \textmu g/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\textsuperscript{3}Lys\textsuperscript{3}Phe H)

| Strain       | H-2 haplotype | Medium Proliferative response (cpm ± SEM) to:* | GL\textsuperscript{\Phi} Proliferative response (cpm ± SEM) to:* | PPD Proliferative response (cpm ± SEM) to:* |
|--------------|---------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| A/J          | a             | 1,500 ± 500 | 2,000 ± 1,000 | 38,900 ± 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 GL\textsuperscript{\Phi} was not significantly different (Student's t test) from the incorporation in the absence of GL\textsuperscript{\Phi}. 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 GL\textsuperscript{\Phi}. In all cases the designation of a strain as a responder or nonresponder for T-lymphocyte proliferation to GL\textsuperscript{\Phi} correlated exactly with the designation previously assigned those
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 F₁ 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<sup>a</sup>* or *H-2<sup>b</sup>* haplotypes are nonresponders to GLΦ. However, a combination of these two strains on the basis of serum antigen-binding tests for antibody formation to GLΦ (1, 2).

### Table III

**Complementation of the *H-2<sup>a</sup>* and *H-2<sup>b</sup>* Haplotypes**

| Strain              | MHC alleles | Proliferative response (cpm ± SEM) to*: | Responsiveness† |
|---------------------|-------------|----------------------------------------|-----------------|
|                     | KJAJ-1J-1C-1S-D |                                        |                 |
|                     | Medium GLΦ PPD |                                      |                 |
| B10.D2/nSn          | d d d d d d    | 1,000 ± 200 115,400 ± 17,800          | ++              |
| C57BL/10Sn          | b b b b b b    | 1,000 ± 100 1,400 ± 200               | –               |
| B10.A/SgSn          | k k k k d d d  | 2,300 ± 500 900 ± 200                 | –               |
| (B10 × B10.A)F<sub>1</sub> | b b b b b b k k k k d d d | 2,300 ± 200 130,000 ± 2,700          | ++              |
| B10.A(5R)/SgSn      | b b b d d d    | 1,200 ± 300 79,900 ± 4,500            | +               |
| C57BL/6N            | b b b b b b    | 2,400 ± 800 4,900 ± 1,700             | –               |
| A/J                 | k k k d d d    | 1,500 ± 500 2,000 ± 1,000             | –               |
| (B6 × A)F<sub>1</sub> | b b b b b b k k k k d d d | 3,400 ± 1,200 49,300 ± 7,800        | ++              |
| (B10.A × A)F<sub>1</sub> | k k k d d d k k k k d d d | 8,400 ± 3,100 5,500 ± 1,600          | –               |
| B10.BR/SgSn         | k k k k k k k k | 400 ± 120 550 ± 150                   | –               |
| SJL/J               | s s s s s s s s | 1,700 ± 700 800 ± 200                 | –               |
| B10.HT              | s s s s k k d  | 5,400 ± 1,100 3,800 ± 1,100           | –               |
| (B10.BR × B10.S)F<sub>1</sub> | k k k k k k s s s s s s s s | 700 ± 150 4,400 ± 1,100             | +               |
| (C3H × SJL)F<sub>1</sub> | k k k k k k s s s s s s s s | 28,900 ± 4,300 52,700 ± 5,100       | +               |
| B10.S(9R)           | s s s s k k k k | 860 ± 150 50,100 ± 6,500              | ++              |
| B10.S(7R)           | s s s s s s d | 5,700 ± 1,100 66,600 ± 5,100         | ++              |
| B10.S(18R)          | b b b b b b b b s s s s s s s s | 8,200 ± 1,700 8,300 ± 2,100       | –               |

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

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### Table IV

**Complementation of the *H-2<sup>a</sup>* and *H-2<sup>b</sup>* Haplotypes**

| Strain              | MHC alleles | Proliferative response (cpm ± SEM) to*: | Responsiveness† |
|---------------------|-------------|----------------------------------------|-----------------|
|                     | KJAJ-1J-1C-1S-D |                                        |                 |
|                     | Medium GLΦ PPD |                                      |                 |
| C57BL/10Sn          | b b b b b b    | 1,000 ± 100 1,400 ± 200               | –               |
| C3H/HeN             | k k k k k k k k | 900 ± 250 300 ± 70                    | –               |
| B10.BR/SgSn         | k k k k k k k k | 2,800 ± 600 5,500 ± 1,500             | –               |
| SJL/J               | s s s s s s s s | 1,700 ± 700 800 ± 200                 | –               |
| B10.S               | s s s s s s s s | 5,400 ± 1,100 3,800 ± 1,100           | –               |
| (B10.BR × B10.S)F<sub>1</sub> | k k k k k k s s s s s s s s | 700 ± 150 4,400 ± 1,100             | +               |
| (C3H × SJL)F<sub>1</sub> | k k k k k k s s s s s s s s | 28,900 ± 4,300 52,700 ± 5,100       | +               |
| B10.HT              | s s s s k k d  | 860 ± 150 50,100 ± 6,500              | ++              |
| B10.S(9R)           | s s s s d d d | 5,700 ± 1,100 66,600 ± 5,100         | ++              |
| B10.S(7R)           | s s s s s s d | 8,200 ± 1,700 8,300 ± 2,100         | –               |

* 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)F1 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 F1 hybrids, such as the (B6 × A)F1, were also responders to GLΦ, but the (B10.A × A)F1 hybrid, in which complementation of only the non-H-2 genes is achieved, was a nonresponder. Recombinants between the H-2α and H-2β 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-2α haplotype gene (α) to the region of the MHC between I-B and D, and the H-2β haplotype gene (β) to the left of I-C.

Mice of the H-2k and H-2" 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 F1 hybrids, such as (B10.BR × B10.S)F1 and (C3H × SJL)F1, showed only weak, but significant, proliferative responses. The B10.S(9R) recombinant demonstrated that the H-2α and H-2" haplotypes share a similar Ir gene (α) capable of complementing a gene of the H-2" haplotype. The lack of response by the B10.S(7R) recombinant located the α-gene of the H-2α and H-2" haplotypes to the region of the MHC between I-B and D.

Inasmuch as the H-2α and H-2" α-genes can be complemented by genes of the H-2β and H-2" haplotypes, respectively, the implication is that the H-2β and H-2" 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-2β × H-2")F1 hybrids (Table V). In agreement with the serological data (2), PETLES from both the (B6 × SJL)F1 and the (B10 × B10.S)F1 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-2α and H-2" trans complementations were also weak, it is possible that all trans complementations involving H-2α genes are inefficient, and thus (H-2β × H-2")F1 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)F₁ strain, although statistically significant, is not biologically meaningful, and the partial complementation seen in the (B6 × SJL)F₁ 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-2b and H-2s 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-2b and H-2s are the same or, indeed, whether a more complex system involving three polymorphic H-2-linked Ir-GLΦ 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 capacity². 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-2a and H-2k haplotypes, which possess a responder allele at the α, but not the β, Ir-GLΦ locus, and mice of the H-2b and H-2s haplotypes, which possess a responder allele at the β, but not the α, Ir-GLΦ 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 F₁ hybrid and recombinant strains. PETLES from (H-2b × H-2s)F₁ 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-2s or H-2k nonresponder haplotypes, were excellent responders. PETLES from (H-2s × H-2k)F₁ 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)F₁ hybrid,
which is a cross between an $\alpha^+\beta^+$ responder and an $\alpha^-\beta^-$ nonresponder, demonstrated that both the $\alpha^+$ and $\beta^+$ 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-GLcP loci fail to proliferate in response to stimulation with GLcP. In accord with this finding, Katz et al. (7), in a companion study, demonstrate that T-lymphocyte helper activity for DNP-FOG-primed B lymphocytes from (B6 × A)F1 responders fails to develop in $\alpha^+\beta^-$ or $\alpha^-\beta^+$ parental donors primed with GLcP. 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 $\alpha$-gene nor the $\beta$-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)F1 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 and by Lonai and McDevitt for the C3H/DiSn strain (10). The results with GLcP potentially demonstrate the same point, although no analysis for production of T-cell helper factor in either type of GLcP 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-(Glu3Lys3Phe11) (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-2a, H-2b, H-2d, and H-2k haplotypes were all nonresponders to GLΦ. In contrast, F1 hybrids between these strains, such as (B10 × B10.A)F1 and (C3H × SJL)F1, 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.

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