Proc. Nat. Acad. Sci. USA
Vol. 72, No. 9, pp. 3671–3675, September 1975
Immunology

Complementation of *H-2*-linked *Ir* genes in the mouse

(immunogenetics/immune responses)

MARTIN E. DORF AND BARUJ BENACERRAF

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Contributed by Baruj Benacerraf, June 27, 1975

ABSTRACT **The immune response to the random linear terpolymer of L-glutamic acid, L-lysine, and L-phenylalanine (GLϕ) is under dominant *H-2*-linked *Ir* gene control in the mouse. Matings between two nonresponder strains produced responder F_1 hybrids, demonstrating complementation of the nonresponder alleles. This observation, coupled with the fact that several intra *H-2* recombinant strains derived by recombination between two nonresponder parental haplotypes are also GLϕ responders, indicated at least two dominant loci are concerned with responsiveness to this terpolymer. The complementary genes were termed α (tentatively localized in a new subregion of the *H-2* complex, *I-F*) and β, which maps in the *I-A* subregion. Generally, both the α(+) and β(+) alleles are required for responsiveness. However, in the (C57BL/6J × SJL)F_1 hybrid we noted complementation between two parental nonresponder strains, each of which carried β genes derived from different *H-2* haplotypes, yet lacked functional α genes. The possible cell levels at which these genes may function in the regulation of the immune response are discussed.**

The genetic study of the capacity to form specific immune responses has revealed that the recognition of thymus-dependent antigens by individual animals is governed by the product of dominant genes located in the genome in close relationship with the genes coding for the major histocompatibility specificities (1, 2). This has been verified in rodents (3–5), birds (6), and primates (7). These immune response genes have been termed histocompatibility or *H*-linked *Ir* genes, and map in the *I* region of the major histocompatibility complex (1), which also controls T (thymus-derived) and B (bone-marrow-derived) cell and macrophage and T cell interactions in immune responses (8–10). Single dominant *H*-linked *Ir* genes were thought to control the responses to specific antigen (1, 2). However, we recently demonstrated that at least two genetically separable loci, provisionally termed α and β, are required for immunological responsiveness to the linear random terpolymer of L-glutamic acid, L-lysine, and L-phenylalanine (GLϕ) (11).

Merryman *et al.* (12, 13) previously reported that the immune response to GLϕ was inherited as a Mendelian dominant character; the gene controlling GLϕ responsiveness was tentatively localized in the *I-C* region of the *H-2* complex. In a previous report (14), we tentatively mapped an *Ir-GL*ϕ gene in a different portion of the *I* region. The demonstration of the two-gene requirement for GLϕ responsiveness permitted a resolution of this apparent discrepancy. In this report, we localize in selected *H-2* haplotypes the α and β genes involved in GLϕ responsiveness and demonstrate that several nonresponder strains possess one of the two *Ir-GL*ϕ genes. The α genes were defined as those genes which can complement different β genes, but not complement each other. Correspondingly, β genes were defined as those genes capable of complementing a given α gene. Thus, by complementing the α gene derived from one nonresponder haplotype with a β gene derived from a different haplotype, F_1 hybrids derived from two appropriate nonresponder parental strains are shown to be GLϕ responders. In addition, certain recombinant strains in which the cross-over event occurred between two nonresponder haplotypes are also shown to be GLϕ responders. Surprisingly, complementation of α and β genes represents only part of the intricacy in the genetic control of the immune response to GLϕ. Complementation was also noted in (B6 × SJL)F_1 hybrids. The two parental strains carry only functional β genes in the absence of a functional α gene in either parent, yet the F_1 hybrids can make high levels of GLϕ antibody.

Abbreviations: In polypeptides, G is glutamate; L, lysine; ϕ, phenylalanine; T, tyrosine; Dnp, dinitrophenyl; PBS, phosphate-buffered saline; T cells, thymus-derived cells; B cells, bone-marrow-derived cells.

MATERIALS AND METHODS

Mice. Most inbred and congenic mice or F_1 hybrids were produced in our animal facilities. Those animals purchased from Jackson Laboratory, Bar Harbor, Me., are designated by the letter J in the tables. The abbreviation B10 is used for the C57BL/10J strain and B6 for the C57BL/6J strain. The QSR-1 mice were donated by Dr. D. C. Shreffler, the 8R and 21R recombinants were supplied by Dr. J. H. Stimpfling, and the (B6 × SJL)F_1 hybrids were provided by J. Kapp.

Animals were between 7 and 30 weeks of age at the beginning of immunization. Each experimental group consisted of at least three mice which were bled 7 days after secondary immunization.

Antigens. The random linear terpolymer (L-glutamic acid^{53},L-lysine^{36},L-phenylalanine^{11})$_n$ (sample no. GF6-23-8) was synthesized in Dr. Elkan Blout's laboratory, Department of Biological Chemistry, Harvard Medical School, Boston, Mass. Amino-acid analyses gave the values indicated. Dinitrophenyl hapten conjugates of GLϕ (Dnp-GLϕ) were prepared as described elsewhere (12). The terpolymer was emulsified in complete Freund's adjuvant containing 0.5 mg/ml of *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.). Primary and secondary immunizations with 0.2 ml of emulsion containing 100 μg of antigen were carried out intraperitoneally on days 0 and 21, respectively. Mice were bled on day 28. The sera were stored at −20° until tested.

Antigen-Binding Assay. The humoral response to GLϕ was measured by antigen-binding assay employing the cross-reactive linear terpolymer (L-glutamic acid^{57},L-lysine^{38},L-

Table 1. Humoral response to the terpolymer GLϕ

Strain	n*	Serum dilution binding 33% of ligand†		
		GLϕ	GL	GLT
A/J	4	0	0	0
B10.A	4	0	0	0
C57BL/10J	4	0	0	0
B10.D2	4	17 (2.7)	28 (1.7)	236 (1.4)
BALB/cJ	5	136 (1.9)	363 (2.6)	212 (2.5)
C3H.JK	4	265 (1.4)	189 (1.7)	249 (1.7)
C3H/HeJ	5	0	0	0
BDP/J	5	64 (3.1)	80 (4.0)	199 (4.3)
P/J	4	32 (2.4)	28 (1.2)	10 (1.2)
RIII/2J	4	1126 (2.0)	2323 (3.1)	1311 (4.2)
PL/J	4	144 (1.6)	159 (1.4)	143 (1.6)
B10.T (6R)	5	228 (2.1)	161 (4.2)	517 (2.4)

* Number of mice tested.
† Reciprocal of antiserum dilution binding 33% of radiolabeled ligand, standard deviation indicated in parentheses.

tyrosine$^5)_n$ (GLT), a gift from Dr. Paul H. Maurer, Jefferson Medical College, Philadelphia, Pa. GLT was iodinated by the chloramine-T method (11) with carrier-free ^{125}I (New England Nuclear Corp., Boston, Mass.) and separated from inorganic iodide by passage over 0.5 × 25 cm columns of Sephadex G-25F (Pharmacia Fine Chemicals, Piscataway, N.J.). Serum samples diluted 1:5 with phosphate-buffered saline (PBS) were assayed by a modified Farr assay which has been detailed previously (16). Serum anti-Dnp levels were measured using [^{3}H]Dnp-ϵ-amino-n-caproic acid as described elsewhere (8).

Antigen binding assays of anti-GLϕ antisera were carried out using our standard Farr procedure with [^{3}H]acetyl-GLϕ or with the [^{3}H]acetyl conjugate of the copolymer of L-glutamic acid and L-lysine (GL). These radiolabeled ligands were prepared by reacting 1 mCi of carrier-free [^{3}H]acetic anhydride (Schwarz/Mann, Van Nuys, Calif.) with 100 μg of antigen in 0.1 M $NaHCO_3$. The reaction was allowed to proceed for 30 min on ice. The material was dialyzed against 10 mM sodium acetate in PBS, then diluted with PBS containing 1% normal mouse serum to a concentration of about 2×10^{-8} M for use in the antigen-binding assay.

RESULTS

Initially a series of inbred mouse strains were immunized and boosted with 100 μg of GLϕ in complete Freund's adjuvant. The secondary sera were assayed for antigen binding with three different radiolabeled ligands; the homologous [^{3}H]acetylated terpolymer GLϕ, radioiodinated GLT, and the [^{3}H]acetylated copolymer GL. Sera from certain strains (A/J, B10.A, C57BL/10, and C3H) failed to bind detectable quantities of these ligands, while sera from other strains [B10.D2, C3H.JK, BDP, P/J, RIII, PL, B10.T(6R)] were capable of binding all ligands (Table 1). Some strains produced very high levels of antibody while others, such as P/J, responded with much lower levels. Generally, the antibody responses to GLϕ could be measured equally well with the GLT or GL polymers. There was no evidence of greater specificity for the homologous molecule than for GLT or GL using this assay. The predominant antibody specificity appears to be directed toward GL determinants. In experiments not shown, we determined that immunizations with 50–100 μg were optimal for humoral responses; doses of 1 and 10 μg resulted in lower antibody levels, while 0.1 μg was uniformly nonimmunogenic.

Table 2. Strain distribution of GLϕ responsiveness

Strain	H-2 haplotype	n*	GLϕ response† (% binding ± SEM)
A/J	*a*	11	1.9 ± 1.6
A/St	*a*	4	−6.6 ± 1.0
B10.A	*a*	7	3.9 ± 2.1
C57BL/10J	*b*	13	0.5 ± 2.5
C3H.SW	*b*	3	−0.8 ± 5.1
A.By	*b*	4	−0.2 ± 0.5
D1.LP	*bc*	4	−2.4 ± 3.6
B10.D2	*d*	9	60.9 ± 4.7
DBA/2J	*d*	4	40.4 ± 7.9
BALB/cJ	*d*	4	59.2 ± 2.0
LG/J	*d*	3	87.2 ± 4.2
B10.M	*f*	5	1.0 ± 1.3
A.Ca	*f*	3	−0.6 ± 0.6
C3H.JK	*j*	4	97.0 ± 1.9
I/LnJ	*j*	6	80.3 ± 2.8
B10.WB	*ja*	3	79.3 ± 7.2
B10.BR	*k*	12	5.1 ± 3.0
C3H/HeJ	*k*	5	−6.8 ± 5.3
CBA/H	*k*	5	1.5 ± 3.2
AKR/J	*k*	4	1.7 ± 4.9
M523	*ka*	5	6.4 ± 3.4
B10.F	*n*	11	31.7 ± 7.8
C3H.NB	*p*	4	40.5 ± 8.0
P/J	*p*	5	45.4 ± 3.3
BDP/J	*p*	5	54.4 ± 9.9
B10.P	*p*	3	79.5 ± 1.9
B10.Y	*pa*	4	81.3 ± 0.6
C3H.Q	*q*	4	85.7 ± 8.1
DBA/1J	*q*	5	73.1 ± 3.3
T138	*q*	5	97.1 ± 1.2
SWR/J	*q*	5	94.7 ± 3.1
B10.RIII	*r*	5	68.6 ± 4.2
RIII/2J	*r*	4	100.0 ± 0.6
B10.S	*s*	7	−1.4 ± 1.3
SJL/J	*s*	4	−4.3 ± 0.9
A.Sw	*s*	5	1.6 ± 3.1
B10.PL	*u*	3	62.7 ± 6.1
PL/J	*u*	4	75.7 ± 1.0
B10.SM	*v*	3	8.9 ± 5.5
SM/J	*v*	7	5.1 ± 4.5

* Number of mice tested.
† Mean percentage of radiolabeled GLT bound in Farr assay by a 1:5 dilution of serum ± standard error of the mean.

The strain distribution of GLϕ responsiveness was determined among 40 inbred and congenic strains (Table 2). Following secondary immunization, mice carrying the *H-2^d*, *H-2^j*, *H-2^n*, *H-2^p*, *H-2^q*, *H-2^r*, and *H-2^u* haplotypes made high levels of anti-GLϕ antibody. In contrast, mice homozygous for *H-2^a*, *H-2^b*, *H-2^f*, *H-2^k*, *H-2^s*, and *H-2^v* made no detectable antibody responses after secondary challenge.

Thirty strains carrying recombinant H-2 haplotypes were screened for their capacity to make GLϕ responses (Table 3). The *Ir* genes appear to be localized in the *K*-end of *H-2*. The recombinant strains B10.HTG, R101, C3H.OL, and C3H.OH, in which the *K*-end was derived from the responder *H-2^d* haplotype or the DA, B10.DA, and 6R strains, in which the *K* and *I* regions are derived from the responder *H-2^q* haplotype, are all GLϕ responders. Likewise, those strains in which the entire *K*-end was derived from a nonre-

Table 3. Humoral responses to GLϕ by *H-2* recombinant strains*

Recombinant strains	*H-2* haplotype	*n*	*H-2* regions† *K*	*A*	*B*	*C*	*S*	*D*	GLϕ response (% binding ± SEM)
A.AL	*a1*	4	*k*	*k*	*k*	*k*	*k*	*d*	1.7 ± 0.6
HTG	*g*	5	*d*	*d*	*d*	*d*	*d*	*b*	88.5 ± 2.6
B10.HTG	*g*	14	*d*	*d*	*d*	*d*	*d*	*b*	59.7 ± 4.3
R101	*g101*	2	*d*	*d*	*d*	*d*		*b*	79.7 ± 8.6
D2.GD	*g4*	5	*d*	*d*	*b*	*b*	*b*	*b*	−3.6 ± 5.0
GD	*g4*	10	*d*	*d*	*b*	*b*	*b*	*b*	5.1 ± 3.6
2R	*h2*	4	*k*	*k*	*k*	*d*	*d*	*b*	−6.8 ± 3.7
4R	*h4*	4	*k*	*k*	*b*	*b*	*b*	*b*	1.0 ± 3.6
15R	*h15*	4	*k*	*k*	*k*	*d*	*d*	*b*	1.4 ± 3.5
3R	*i3*	5	*b*	*b*	*b*	*d*	*d*	*d*	58.5 ± 7.0
5R	*i5*	10	*b*	*b*	*b*	*d*	*d*	*d*	73.3 ± 5.0
18R	*i18*	5	*b*	*b*	*b*	*b*	*b*	*d*	4.7 ± 2.4
R106	*i106*	5	*b*	*b*	*b*	*b*	*b*	*da*	−6.0 ± 3.6
B10.AKM	*m*	4	*k*	*k*	*k*	*k*	*k*	*q*	4.9 ± 6.2
C3H.OL	*o1*	3	*d*	*d*	*d*	*d*	*k*	*k*	46.1 ± 12.4
C3H.OH	*o2*	3	*d*	*d*	*d*	*d*	*d*	*k*	77.1 ± 5.7
A.TL	*t1*	3	*s*	*k*	*k*	*k*	*k*	*d*	3.6 ± 2.9
A.TH	*t2*	3	*s*	*s*	*s*	*s*	*s*	*d*	8.7 ± 3.1
7R	*t2*	5	*s*	*s*	*s*	*s*	*s*	*d*	4.4 ± 1.8
B10.HTT	*t3*	5	*s*	*s*	*s*	*k*	*k*	*d*	76.5 ± 6.9
9R	*t4*	4	*s*	*s*	*s*	*d*	*d*	*d*	70.6 ± 9.5
BSVS	*t5*	8	*s*	*s*	*s*	*s*	*d*	*d*	43.8 ± 10.8
AQR	*y1*	4	*q*	*k*	*k*	*d*	*d*	*d*	−6.5 ± 2.0
6R	*y2*	7	*q*	*q*	*q*	*q*	*q*	*d*	69.1 ± 9.4
DA	*qp*	5	*q*	*q*	*q*	*q*	*q*	*s*	98.2 ± 0.5
B10.DA		3	*q*	*q*	*q*	*q*	*q*	*s*	68.0 ± 7.0
QSR-1		2	*s*	*s*	*s*	*s*	*q*	*q*	4.8 ± 3.2
8R		4	*k*	*k*	*k*	*s*	*s*	*s*	7.3 ± 2.1
21R		4	*b*	*b*				*s*	3.3 ± 4.2
LG/Ckc		5	*d*	*f*	*f*				−3.7 ± 2.5

* See legend, Table 2.

† Letters indicate parental origin of the genes in each *H-2* region. Vertical bars indicate position of crossing over.

sponder haplotype, e.g., 2R, 15R (*H-2*^{a}); 18R, R106 (*H-2*^{b}); A,AL, B10.AKM (*H-2*^{k}); and QSR-1, A.TH, and 7R (*H-2*^{s}), are all GLϕ nonresponders. The A.TL, AQR, and LG/Ckc strains are examples of crossing over within the *K*-end of the *H-2* complex between the *K* and *I* regions; as expected in each case, responsiveness segregated with the *I* region. The D2.GD and GD strains which carry the *H-2*^{g4} recombinant haplotype derived by recombination of the responder *H-2*^{d} and nonresponder *H-2*^{b} chromosomes failed to respond to GLϕ, suggesting that at least one *Ir-GL*ϕ gene in the *H-2*^{d} haplotype was localized to the right of the *I-A* subregion (Table 3). Surprisingly, several recombinant strains which were independently derived from crossovers between two nonresponder haplotypes were GLϕ responders. These include the 3R and 5R strains which were derived by recombination of the *H-2*^{a} and *H-2*^{b} haplotypes and the 9R, B10.HTT, and BSVS strains in which crossing over occurred between the *H-2*^{s} and *H-2*^{a} or *H-2*^{a1} haplotypes (17). It is important to note that the latter strains all represent examples of recombination within the *I* region, either between the *I-B* and *I-C* subregions or, in the case of the BSVS strain, between *I-C* and *S*.

Table 4 illustrates the GLϕ responses of 21 F_1 hybrids following immunization with 100 μg of GLϕ. All F_1 hybrids between responder and nonresponder strains were responders. Two examples of the latter type of hybrid, the (DBA/1 × SJL)F_1 and (DBA/2 × 4R)F_1, are presented; additional examples have been published previously (11–13). Several hybrids which were the product of two nonresponder parental strains also responded to the GLϕ terpolymer. Thus, all F_1 hybrids which carried at least one β gene derived from the *I-A* subregion of the *H-2*^{b}, *H-2*^{d} haplotypes, or from *I-A* or *I-B* subregions of the *H-2*^{s} haplotype plus one α gene derived from the *I-C* or *S* chromosomal segments of the *H-2*^{a}, *H-2*^{d}, or *H-2*^{k} haplotype could respond to the GLϕ terpolymer. It is interesting to note that the F_1 hybrids involving β genes derived from the *H-2*^{s} haplotype showed weaker antibody responses than hybrids in which the β gene was derived from the *H-2*^{b} haplotype. The complementary genes responsible for GLϕ antibody responses were localized to the *H-2* complex, since congenic (B10 × 2R)F_1 and (B10.BR × B10.S)F_1 hybrids produced GLϕ antibody. A comparison of gene interaction in the *cis* and *trans* positions revealed that the β gene derived from the *H-2*^{s} haplotype complemented better with α genes in the *cis* (i.e., recombinant strains 9R, B10.HTT) than in the *trans* [i.e., (B10.BR × B10.S)F_1 hybrids]. This was not apparent in the case of the β genes from the *H-2*^{b} or *H-2*^{d} haplotype.

Table 4. Antibody responses of F_1 hybrids after immunization with GLϕ*

F_1 hybrid	*H-2*	*n*	GLϕ response (% binding ± SEM)
DBA/1 × SJL	*q* × *s*	8	96.2 ± 1.0
DBA/2 × 4R	*d* × *h4*	4	58.7 ± 5.2
A × B10.A	*a*	5	7.5 ± 2.3
A × B10.BR	*a* × *k*	5	0.3 ± 3.7
A.By × B10	*b*	7	7.8 ± 5.4
A × B10	*a* × *b*	5	62.8 ± 5.8
A × 18R	*a* × *i18*	10	64.1 ± 5.7
A × 4R	*a* × *h4*	5	1.4 ± 3.2
B10 × 2R	*b* × *h2*	3	68.7 ± 2.8
C3H × A	*k* × *a*	5	4.4 ± 2.5
C3H × B10	*k* × *b*	5	80.0 ± 1.7
SJL × C3H	*s* × *k*	6	48.8 ± 13.8
B10.BR × B10.S	*k* × *s*	5	28.2 ± 7.8
AKR × A.TH	*k* × *t2*	10	14.6 ± 5.5
B10.A × A.Sw	*a* × *s*	6	17.5 ± 7.8
B10.A × D2.GD	*a* × *g4*	5	46.4 ± 9.2
B10.M × D2.GD	*f* × *g4*	5	−4.0 ± 1.9
A × A.Ca	*a* × *f*	3	3.5 ± 2.6
A.Ca × B10	*f* × *b*	4	−0.5 ± 3.2
B10 × A.TL	*b* × *t1*	5	94.7 ± 1.3
B6 × SJL	*b* × *s*	9	71.1 ± 9.7

* Refer to legend, Table 2.

The *H-2*^{f} or *H-2*^{h4} haplotypes appear to lack both the α and β alleles. Attempts to complement the A.Ca or 4R strains by producing F_1 hybrids with either the A or B10 strains were uniformly unsuccessful (Table 4). Localization of the β gene in the *H-2*^{b} haplotype to the *K* or *I-A* subregions was shown by the inability of the (A × 4R)F_1 hybrid to complement, thus mapping the β gene of the *H-2*^{b} haplotype to the left of the *I-B* region. In addition, the ability of the (B10.A × D2.GD)F_1 hybrids to make anti-GLϕ antibody suggests the β gene derived from the *H-2*^{d} haplotype also lies in the *K* or *I-A* regions. Localization of the β gene of

Table 5. Immune responses to Dnp-GLϕ

Strain	*H-2*	*n*	Dnp-response* (% binding ± SEM)
A/J	*a*	4	−4.6 ± 1.6
B10.A	*a*	3	−7.3 ± 4.2
B10/J	*b*	5	−7.3 ± 3.6
B10.D2	*d*	5	38.4 ± 17.5
A.Ca	*f*	4	−3.1 ± 0.9
3R	*i3*	4	29.5 ± 4.2
18R	*i18*	3	5.1 ± 1.5
C3H.JK	*j*	5	38.1 ± 5.1
B10.BR	*k*	10	−1.8 ± 2.7
P/J	*p*	4	27.6 ± 2.8
DBA/1J	*q*	4	56.2 ± 9.3
B10.RIII	*r*	5	50.7 ± 3.9
A.Sw	*s*	5	−1.1 ± 2.6
B10.PL	*u*	4	34.2 ± 3.1
B10.SM	*v*	5	−8.7 ± 3.1
(B10 × A)F_1	*b* × *a*	5	31.1 ± 3.9

* Mean percentage of radiolabeled Dnp-ϵ-aminocaproic acid bound by a 1:5 dilution of serum ± standard error.

$H\text{-}2^d$ in the *I-A* or *I-B* subregions resulted from comparisons of the anti-GLϕ responses of the A.TL and B10.HTT recombinant strains (Table 3).

Surprisingly, matings between strains carrying only the β gene (lacking functional α genes), i.e., C57BL/6 mice carrying the $H\text{-}2^b$ haplotype with the SJL strain bearing the $H\text{-}2^s$ haplotype, produced GLϕ responder hybrids. Thus, (B6 × SJL)F_1 hybrids, which possess two different β genes, yet lack functional α genes, can produce high levels of anti-GLϕ antibody. In contrast, matings between two strains which carry different α genes, yet lack the β gene, fail to make GLϕ humoral responses, e.g., (A × B10.BR)F_1 and (C3H × A)F_1 hybrids.

The immune responses to the dinitrophenyl (Dnp) hapten conjugate of GLϕ were studied in 15 different strains, including many congenic lines representing a variety of different *H-2* haplotypes. Anti-Dnp antibodies were present in mice bearing the $H\text{-}2^d$, $H\text{-}2^{i3}$, $H\text{-}2^j$, $H\text{-}2^p$, $H\text{-}2^q$, $H\text{-}2^r$, and $H\text{-}2^u$ haplotypes, while inbred strains carrying the $H\text{-}2^a$, $H\text{-}2^b$, $H\text{-}2^f$, $H\text{-}2^{i18}$, $H\text{-}2^k$, $H\text{-}2^s$, and $H\text{-}2^v$ haplotypes were nonresponders to Dnp-GLϕ (Table 5). Thus, identical *H-2* distributions were noted for responses to GLϕ as for its Dnp-conjugate. Again there was evidence for the two-gene control of responsiveness, since the (B10 × A)F_1 hybrid between two parental nonresponder strains produced anti-Dnp antibody. In addition, the results with the 3R and 18R recombinant strains mirror those obtained with the GLϕ terpolymer and localize the α and β genes in the $H\text{-}2^a$ and $H\text{-}2^b$ haplotypes, respectively, in the same fashion as described above.

DISCUSSION

It has generally been assumed that a single histocompatibility-linked *Ir* gene was required for responsiveness to an antigen. There were, however, indications that in some systems two *H*-linked *Ir* genes were needed for responsiveness. In 1972, Stimpfling and Durham (18) first postulated that the immune response of mice to the alloantigen H-2.2 might be controlled by two interacting genes localized within the *H-2* complex. Rare instances had been reported where F_1 hybrids between two low responder strains gave responses higher than either parental strain (5, 6, 19).

Table 6. Mapping of the α gene to the I-F subregion*

Strain	GLϕ response	*H-2* regions: *K*	*I-A*	*I-B*	*I-C*	*I-F*	*S*	*D*
9R	70.6 ± 9.5	*s*	*s*	*s*	↳ *d*	*d*	*d*	*d*
B10.HTT	76.5 ± 6.9	*s*	*s*	*s*	↳ *k*	*k*	*k*	*d*
7R	4.4 ± 1.8	*s*	*s*	*s*	*s*	*s*	*s* ↲	*d*
A.TH	8.7 ± 3.1	*s*	*s*	*s*	*s*	*s*	*s* ↲	*d*
QSR-1	4.8 ± 3.2	*s*	*s*	*s*	*s*	*s* ↲	*q*	*q*
BSVS	43.8 ± 10.8	*s*	*s*	*s*	*s*	↳ *d*	*d*	*d*

* Refer to legend, Table 3.

A documented example of dual *H*-linked *Ir* gene control of the response to a single antigen was demonstrated in our laboratory using the GLϕ system (11). Our preliminary observations have been confirmed and extended by the data presented in this report. Localization of the genes controlling GLϕ responsiveness to the *H-2* complex has been confirmed by studies of strain distribution of responsiveness and of congenic strains. Although additional quantitative differences in the magnitude of the immune response occur among responder strains (Table 1), these appear unrelated to the *H-2* complex, as has been noted in other *Ir* systems (20).

The finding that the mating of two nonresponder strains produces responder F_1 hybrids is evidence of complementation of the nonresponder alleles (Table 4). This observation, coupled with the fact that the 3R, 5R, 9R, and B10.HTT recombinant strains (which are all derived by crossing over between two nonresponder parental haplotypes) are GLϕ responders, indicates that at least two dominant *Ir* loci which can function in either the *cis* or *trans* position are concerned with responsiveness to GLϕ. However, responses to GLϕ resulting from gene complementation in the *trans* position are in some instances ($H\text{-}2^s$ with $H\text{-}2^a$ or $H\text{-}2^k$) clearly lower than those resulting from interaction of the same genes in the *cis* position (i.e., the 9R and B10.HTT recombinant strains).

Tentative mapping of the *Ir-GLϕ* genes is based on the responses of intra *H-2* recombinant strains. The β gene of the $H\text{-}2^s$ has been localized in the *I-A* or *I-B* subregions (see *Results* section). In the $H\text{-}2^b$ haplotype, the β gene can be mapped more precisely to the *I-A* subregion by comparing the antibody responses of the (A × B10)F_1 and (A × 4R)F_1 hybrids (Table 4). The complementation observed with the (B10.A × D2.GD)F_1 mice is also consistent with the localization of the β gene to the *I-A* subregion of $H\text{-}2^d$.

Comparisons of the immune responses of the 3R and 5R recombinants with the 18R and R106 strains provided evidence that *Ir* genes are present in the *I-C* or *S* regions of the *H-2* complex (8). This second *Ir-GLϕ* gene, termed α, can also be mapped to the *I-C* or *S* regions using the 9R or B10.HTT and A.TH or 7R recombinant strains (Table 3). Analysis of the GLϕ responses of the QSR-1 and BSVS strains provides additional data localizing the α gene. The data suggest that a previously undefined region codes for the α gene, which, according to the suggestions of Dr. D. C. Shreffler, shall tentatively be termed *I-F* (Table 6). The BSVS strain which is critical for the localization of the α gene is considered a "natural recombinant strain" presumably involving crossing over between the $H\text{-}2^s$ and $H\text{-}2^a$ ha-

plotypes. The major histocompatibility complex of the BSVS strain has been previously characterized using mixed lymphocyte culture and tissue typing techniques (21). Subdivision of the *I-C* region into 2 segments termed *I-C* [with marker genes coding for the Ia.7 (17) and Lad_2 (21) determinants] and *I-F* coding for the α gene provides the simplest explanation of the mapping data.

One of the most surprising findings concerning the two-gene concept of *Ir* gene control was the ability of two strains, each of which lack a functional α gene, to complement each other. Thus, (C57BL/6 × SJL)F_1 hybrids produce high levels of anti-GLϕ antibody. Thus, either complementary non-*H-2* genes in the C57BL/6 and SJL strains can substitute for the α gene, or the *Ir-GL*ϕ genes coded for by the *H-2*^{b} and *H-2*^{s} haplotypes are different from each other. The high levels of antibody noted in F_1 hybrids between *H-2*^{b} mice with any $\alpha(+)$ nonresponder strain compared with F_1 hybrids involving *H-2*^{s} mice suggest differences in the GLϕ genes of the *H-2*^{b} and *H-2*^{s} haplotypes (Table 4). Preliminary data using congenic mice suggest that the *H-2*^{b} and *H-2*^{s} haplotypes can complement to permit low level GLϕ responses. In contrast, the *H-2*^{a} and *H-2*^{k} haplotypes, which represent examples of $\alpha(+)$ and $\beta(-)$ genotypes, do not complement each other, yet they complement all $\alpha(-)$ and $\beta(+)$ strains tested. The *H-2*^{f} haplotype is an example of the $\alpha(-)$ and $\beta(-)$ genotype and fails to complement with all nonresponder alleles tested.

There are several possible explanations for the finding that (B6 × SJL)F_1 hybrids carrying the *H-2*^{b} and *H-2*^{s} haplotypes can respond to GLϕ. One can postulate, although we do not favor this hypothesis, that three interacting *Ir-GL*ϕ genes are required for immune responsiveness. Thus, the α gene may consist of two genes (referred to as α_1 and α_2 for convenience). The C57BL/6 mice would then be represented as $\beta(+)\alpha_1(-)\alpha_2(+)$, while the SJL strain would carry the $\beta(+)\alpha_1(+)\alpha_2(-)$ alleles. If the *Ir-GL*ϕ genes code for units of the variable region of a postulated T cell receptor, at least two different variable region units may be required for the recognition of antigen in responder animals. This postulates that Ir gene products may interact in a random fashion accounting for the specificity of responsiveness with a limited number of genes, and permitting two different β genes to complement for a GLϕ response.

All GLϕ responder strains made anti-Dnp antibody following secondary immunization with the hapten conjugate of GLϕ (Table 5). Nonresponsiveness to the GLϕ carrier determines that to the Dnp hapten as well in nonresponder strains (Table 5). Since carrier recognition is a function of thymus-derived lymphocytes, one or both of the *Ir-GL*ϕ genes probably operate on some level in the T-cell regulation of immunity.

Using the branched synthetic polypeptide poly(Tyr,Glu)-poly(Ala)- -poly(Lys) [(T,G)-A- -L], Munro and Taussig (22) have also obtained evidence for the dual gene control of immune responsiveness. These authors have suggested that in this system one gene codes for the ability to produce an antigen-specific T cell factor, while the second gene acts at the level of the B cell, presumably by coding for a B cell acceptor molecule. All the gene complementations observed in the GLϕ responses, including the complementation of (B6 × SJL)F_1 hybrids, cannot be explained by the expression of one of the genes in the T cell and the other in the B cell as proposed by Munro and Taussig for their system. If the (C57BL/6 × SJL)F_1 mice represent complementation between two β genes in the absence of an α gene, it is difficult to envision how these genes can function exclusively in only one lymphocyte subpopulation. The expression of α and/or β complementing genes in the T cell and B cell populations remains to be investigated.

We appreciate the skilled technical assistance of Mrs. M. Twigg, Mrs. A. Benacerraf, and Mr. D. Walsh, and the secretarial assistance of Ms. D. Siner. This study was supported by National Institutes of Health Grant AI-09920.

1. Benacerraf, B. & Katz, D. H. (1975) *Adv. Cancer Res.*, in press.
2. Benacerraf, B. & McDevitt, H. O. (1972) *Science* **175**, 273–279.
3. McDevitt, H. O. & Chinitz, A. (1969) *Science* **163**, 1207–1208.
4. Ellman, L., Green, I., Martin, W. J. & Benacerraf, B. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 322–328.
5. Rüde, E. & Günther, E. (1974) *Prog. Immunol. II* **2**, 223–233.
6. Günther, E., Balcarova, J., Hala, K., Rüde, E. & Hraba, T. (1974) *Eur. J. Immunol.* **4**, 548–553.
7. Dorf, M. E., Balner, H. & Benacerraf, B. (1975) *Transplant. Proc.* **7**, 21–24.
8. Katz, D. H., Graves, M., Dorf, M. E., DiMuzio, H. & Benacerraf, B. (1975) *J. Exp. Med.* **141**, 263–268.
9. Shevach, E. M. & Rosenthal, A. S. (1973) *J. Exp. Med.* **138**, 1213–1229.
10. Erb, P. & Feldman, M. (1975) *J. Exp. Med.* **142**, 460–472.
11. Dorf, M. E., Stimpfling, J. H. & Benacerraf, B. (1975) *J. Exp. Med.* **141**, 1459–1463.
12. Merryman, C., Maurer, P. H. & Bailey, D. W. (1972) *J. Immunol.* **108**, 937–940.
13. Merryman, C. F. & Maurer, P. H. (1975) *Immunogenetics* **1**, 549–559.
14. Dorf, M. E., Lilly, F. & Benacerraf, B. (1974) *J. Exp. Med.* **140**, 859–864.
15. Benacerraf, B. & Levine, B. (1962) *J. Exp. Med.* **115**, 1023–1035.
16. Dorf, M. E., Balner, H. & Benacerraf, B. (1975) *J. Exp. Med.*, in press.
17. Shreffler, D. C. & David, C. S. (1975) *Adv. Immunol.* **20**, 125–195.
18. Stimpfling, J. H. & Durham, T. (1972) *J. Immunol.* **108**, 947–951.
19. Zaleski, M., Fuji, H. & Milgrom, F. (1973) *Transplant. Proc.* **5**, 201–204.
20. Dorf, M. E., Dunham, E. K., Johnson, J. P. & Benacerraf, B. (1974) *J. Immunol.* **112**, 1329–1336.
21. Dorf, M. E., Plate, J. M. D., Stimpfling, J. H. & Benacerraf, B. (1975) *J. Immunol.* **114**, 602–605.
22. Munro, A. J. & Taussig, M. J. (1975) *Nature* **256**, 104–112.