

COUPLED COMPLEMENTATION OF IMMUNE RESPONSE GENES CONTROLLING RESPONSIVENESS TO THE H-2.2 ALLOANTIGEN*

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Several histocompatibility-linked immune response genes (H-2-linked *Ir* genes) controlling responsiveness to synthetic polypeptides and to limiting doses of protein antigens have been identified in mice (1). In addition, in the mouse, many H-linked *Ir* genes have been discovered which control responsiveness to alloantigens (2-5), including the identification of *Ir* genes which influence the production of antibodies to selected H-2 alloantigens. Lilly and co-workers (6, 7) initially described the genetic control of the immune response to the H-2D^b alloantigen. Presumably a single H-linked *Ir* gene was involved, although the influences of additional genes not linked to the mouse histocompatibility complex were also established (8).

It has generally been assumed that a single histocompatibility linked *Ir* gene was required for responsiveness to a specific antigen. There were, however, indications that in some systems, two H-linked *Ir* genes were needed for responsiveness. The immune response of mice to the alloantigen, H-2.2, was one of the first systems postulated to be under the control of two interacting genes localized within the H-2 complex (9). We have reinvestigated this system and have confirmed these postulates. Furthermore, we have now established that the complementing *Ir* genes are highly polymorphic. Interactions between different alleles can result in asymmetric patterns of complementation, termed coupled complementation.

Materials and Methods

Mice. Animals used in these experiments were reared in the mouse colony of the McLaughlin Research Institute or Harvard Medical School. Mice used included the inbred strains C57BL/10Sg, B10.A/Sn, B10.A (2R), B10.A (3R), B10.A (5R), B10.A (18R), B10.D2n/Sn, B10.BR/Sn, B10.HTG, and HTI/Ao. These strains have been abbreviated to B10, B10.A, 2R, 3R, 5R, 18R, B10.D2, B10.BR, B10.HTG, and HTI, respectively. The H-2 region formulae for each of these strains is indicated in Table I.

Immunization. Groups of 8-15 mice were immunized with lymphoid cell preparations. Recipients were given biweekly injections of 2×10^7 spleen and lymph node cells. Sera were collected 1 wk after each injection, coded, and stored at -20°C until they were titered for hemagglutinating activity against suspensions of erythrocytes from B10, 2R, and B10.A mice. The method of testing for hemagglutinins was described earlier (10).

Mixed Lymphocyte Culture. Mixed lymphocyte reactions (MLR)¹ were performed as previously

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¹ Abbreviation used in this paper: MLR, mixed lymphocyte reaction.

TABLE I
H-2 Haplotypes of Relevant Strains

| Strain | <i>H-2</i> haplo- type | <i>H-2</i> region formulae | | | | | | | | |
|---------|---------------------------|----------------------------|------------|------------|------------|------------|------------|----------|----------|----------|
| | | <i>K</i> | <i>I-A</i> | <i>I-B</i> | <i>I-J</i> | <i>I-E</i> | <i>I-C</i> | <i>S</i> | <i>G</i> | <i>D</i> |
| B10 | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> |
| B10.A | <i>a</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> |
| 2R | <i>h2</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | ? | <i>b</i> |
| 3R | <i>i3</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> |
| 5R | <i>i5</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> |
| 18R | <i>i18</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | ? | <i>d</i> |
| B10.D2 | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> |
| B10.BR | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> |
| B10.HTG | <i>g</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | ? | <i>b</i> |
| HTI | <i>i</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | ? | <i>d</i> |

described (11, 12) with lymph node cell suspensions. Briefly, cell suspensions to be utilized as stimulators in the MLR assay were irradiated with 1,200 rad. The effectiveness of the irradiation in blocking DNA synthesis was routinely confirmed by the inability of irradiated cells to respond to phytohemagglutinin. All cell suspensions were cultured at a final concentration of 1.5×10^6 viable cells/ml/culture in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) containing 6% human serum (heated at 56°C for 30 min), 75 μ g L-arginine, 2 mM L-glutamine, 100 U penicillin G, and 35 μ g kanamycin sulfate/ml.

Results

We have tested seven strains for their ability to make hemagglutinating H-2.2 responses. As indicated in Table II, B10.A, HTI, 18R, and B10.D2 mice can be classified as non or low responders while 3R, 5R, and B10.BR mice can generally be distinguished after the third, fourth, or fifth immunization and are considered high responders. It should be noted, however, that the kinetics of the B10.BR response appear to be slightly delayed in comparison to the other responder strains (Table II).

The first criteria in the identification of complementing H-linked *I_r* genes is the demonstration that *F*₁ hybrids prepared between parental low responder strains yield high level immune responses. Thus, the congenic resistant 18R and B10.A mice which both produce low level anti-H-2.2 hemagglutinating responses can be complemented as evidenced by the high response of (18R \times B10.A)*F*₁ hybrid mice (Table II). In these experiments, the control of immune responsiveness to the alloantigen H-2.2 is limited to genes in the *H-2* complex since these experiments have generally been performed by using mice carrying the same genetic background.

The second point to be determined is whether complementation is the result of interactions between two genetically distinct loci. That two distinct loci are involved has been proven by the identification of the 3R and 5R high responder strains which were derived by recombination between parental strains having low responder haplotypes. These *H-2* recombinant strains also permit the mapping of the *I_r-H-2.2* genes within the *H-2* complex (Table III). As in other systems (13-15) we have termed the locus residing in the I-C, S, or G segment of the chromosome, α and the locus in or adjacent to the I-A subregion, β . The *I_r-H-2.2- α* locus is bordered on the left side by the crossover position in the B10.A

TABLE II
Strain Distribution of *H-2.2* Responsiveness

| Recipient | | Cell donor | Hemagglutinin titer \pm SD (\log_2) | | |
|-------------------------------|------------|------------|---|-------------------|-------------------|
| Strain | H-2 | | 3rd bleed- ing | 4th bleed- ing | 5th bleed- ing |
| B10.A | <i>a</i> | 2R | <2.0 \pm 0.0 | <2.0 \pm 0.0 | <2.3 \pm 0.6 |
| 18R | <i>i18</i> | B10 | <2.6 \pm 1.0 | <2.0 \pm 0.0 | <2.0 \pm 0.0 |
| HTI | <i>i</i> | B10 | <2.0 \pm 0.0 | <2.1 \pm 0.3 | <2.2 \pm 0.4 |
| B10.D2 | <i>d</i> | B10.HTG | <2.0 \pm 0.0 | <2.1 \pm 0.3 | <2.2 \pm 0.3 |
| (B10.D2 \times B10.A) F_1 | <i>d/a</i> | B10.HTG | <2.0 \pm 0.0 | <2.0 \pm 0.0 | <2.1 \pm 0.4 |
| 3R | <i>i3</i> | B10 | 3.6 \pm 1.4 | 5.0 \pm 2.3 | 7.1 \pm 0.9 |
| 5R | <i>i5</i> | B10 | 5.8 \pm 2.8 | 4.2 \pm 3.0 | 6.5 \pm 2.6 |
| B10.BR | <i>k</i> | 2R | 2.1 \pm 0.3 | 4.9 \pm 2.0 | 6.7 \pm 1.5 |
| (18R \times B10.A) F_1 | <i>i18</i> | B10 | 4.6 \pm 1.9 | 5.8 \pm 2.1 | 6.4 \pm 0.6 |

TABLE III
Localization of the *Ir-H-2.2* Genes

| Strain | <i>H-2</i> haplo- type | <i>H-2</i> region formulae | | | | | | | | | <i>H-2.2</i> response ($\log_2 \pm$ SD) |
|--------|---------------------------|----------------------------|------------|------------|------------|------------|------------|----------|----------|----------|---|
| | | <i>K</i> | <i>I-A</i> | <i>I-B</i> | <i>I-J</i> | <i>I-E</i> | <i>I-C</i> | <i>S</i> | <i>G</i> | <i>D</i> | |
| 18R | <i>i18</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>d</i> | <2.0 \pm 0.0 |
| HTI | <i>i</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>d</i> | <i>d</i> | <2.2 \pm 0.4 |
| 3R | <i>i3</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | 7.1 \pm 0.9 |
| 5R | <i>i5</i> | <i>b</i> | <i>b</i> | <i>b</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | 6.5 \pm 2.6 |
| B10.A | <i>a</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <2.3 \pm 0.6 |
| B10.BR | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | 6.7 \pm 1.5 |

strain (between the I-E and I-C subregions and on the right margin by the recombination position of the HTI and 18R strains, i.e., between S and D). The high response of the 5R strain indicates that the *Ir-H-2.2-beta* locus maps to the left of the I-J subregion.

Further analysis of the complementing patterns of the *Ir-H-2.2-alpha* and β -alleles derived from the *H-2^b*, *H-2^d*, and *H-2^k* haplotypes are illustrated in Fig. 1. The data demonstrate that the β -alleles obtained from different haplotypes are not equivalent in their ability to complement, and the same is true for α -alleles derived from different haplotypes. Thus, the *Ir-H-2.2-beta^k* allele complements with the *Ir-H-2.2-alpha^k* allele (e.g., the high responder B10.A mice), but does not complement the *Ir-H-2.2-alpha^d* allele, i.e., B10.BR mice. However, the *Ir-H-2.2-alpha^d* allele is perfectly functional since it can complement with the *Ir-H-2.2-beta^b* allele as evidenced by the high level response noted in the 3R and 5R strains. We refer to this phenomenon as coupled complementation.

Finally, we compared the ability of T cells from high (5R) and low (18R) responder strains to respond to B10 lymphoid cells by the MLR. As indicated in Table IV, cells from 18R and 5R mice responded equally well to B10 stimulator cells.

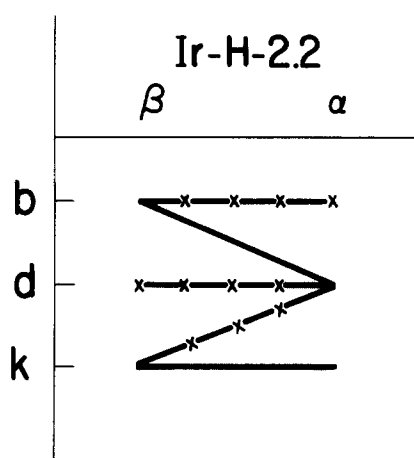


FIG. 1. Gene complementation of *Ir-H-2.2* α - and β -alleles derived from *H-2^b*, *H-2^d*, and *H-2^k* haplotypes (indicated in margin) are illustrated. Effective complementation for the development of anti-*H-2.2* hemagglutinating responses are indicated by solid lines. Broken lines with x's indicate the absence of gene complementation. The restricted interactions of specific α - and β -alleles for effective gene complementation is referred to as coupled complementation.

TABLE IV
Comparison of Mixed Lymphocyte Response by Using High and Low Responder Strains to H-2.2

| Responder | Stimulator | Proliferative response | | |
|-----------|------------|------------------------|--------------------|--------------------------------|
| | | Control | Experimental | Stimulation ratio [†] |
| 18R | B10 | 2,168 \pm 111* | 13,444 \pm 848 | 6.2 |
| 5R | B10 | 2,180 \pm 934 | 12,403 \pm 2,152 | 5.7 |

* The data represent the mean cpm \pm SE of two independent experiments, each performed in triplicate.

[†] Stimulation ratios expressed as the ratio of cpm in the allogeneic mixture divided by the cpm in the syngeneic control.

Discussion

Recently, there have been several documented examples of dual H-linked *Ir* gene control of the response to a single antigen. The usual criteria for the demonstration of complementing *Ir* genes is the ability of F_1 hybrids produced between parental low responder strains to give immune responses higher than either parental strain. This has been demonstrated for the immune response genes controlling humoral responses to the linear synthetic polypeptides of GL Φ (13), GLT (14), GLIeu (15), the branched polypeptide (T,G)-A--L (16), and the protein antigens lactic dehydrogenase B (17) and ovalbumin (18), as well as the alloantigen Thy-1 (3). In this report, we present one additional example of dual H-linked gene control of the response to a single antigen. Thus, the congenic resistant 18R and B10.A mice which are both classified as low responders to H-

2.2 can be complemented as evidenced by the high response of the (18R × B10.A)_F₁ hybrid mice (Table II). In addition, we have demonstrated that the complementing *Ir* loci are genetically distinct as evidenced by the high response of the 3R and 5R recombinant strains, in which crossing over occurred between the α - and β -loci.

This report presents the first documented example of the restricted interactions of specific α - and β -alleles for effective gene complementation. This specific form of complementation has been termed coupled complementation. It is characterized by the asymmetry of the complementation patterns between specific α - and β -alleles. This is most probably a consequence of the extensive polymorphism which exists at the *Ir* α - and β -loci. Such polymorphism is not surprising since most other loci in the major histocompatibility complex can also be characterized as highly polymorphic. We have previously termed the α - and β -alleles as either (+) or (-); this nomenclature system must be modified to account for the polymorphism of these loci. Therefore, we now refer to the α - and β -alleles by indicating the haplotype from which the allele was derived. Alternatively, we cannot exclude the possibility that only one *Ir* gene (located in the I-C region) is required for responsiveness in mice carrying the *H-2^k* haplotype, while two genes are required for responsiveness in the other combinations tested.

What is the nature of the *Ir* gene defect in the H-2.2 low responder mice? An interesting feature of the Ir-H-2.2 system is the ability of low antibody producing strains to respond to the same sensitizing lymphoid cells in other assay systems, i.e., the mixed lymphocyte reaction. This measure of T-cell reactivity has shown no apparent difference between the high and low responder strains (Table IV). Generally, T-cell defects, especially in the inability to provide helper function are observed in *Ir* gene systems (1). In the Ir-H-2.2 system, the inability of low responders to make humoral responses to the H-2.2 determinants may also be interpreted as a lack of helper function although other interpretations (such as B-cell defects) are equally possible. Alternatively, one may question whether the determinants recognized by the MLR and by the serological methods are coded for by the same locus or are very closely linked but inseparable by using the presently available recombinant strains.

In our previous studies of the *H*-linked genes controlling immune suppression to the copolymer of L-glutamic acid, L-tyrosine (Is-GT genes) we also described the phenomenon of coupled complementation (19). In this report, we have extended this concept to include H-linked *Ir* gene systems. However, it is not clear whether the asymmetric complementation patterns noted in the Ir-H-2.2 system represent recognition of different determinants on the H-2.2 molecule. Coupled complementation has been postulated for the Ir-GL Φ (20) and Ir-GLleu (21) systems suggesting this may be a generalized genetic mechanism. Additional *Ir* gene systems may demonstrate this mode of genetic control particularly those which have been tentatively classified as being controlled by a single gene mapping in the I-B subregion, most notably, the *Ir-IgG* gene (2). One can offer an alternative interpretation for the Ir-IgG data by proposing that this *Ir* gene system is controlled by two genes which also demonstrate coupled complementation. Since the I-B subregion is presently defined only by *Ir* gene markers,

the latter interpretation would no longer necessitate the presence of the I-B subregion.

Recently, Taniguchi et al. (22) and Waltenbaugh et al. (23), studying the genetics of immune suppression and suppressor factors, have observed complementation between one gene residing within the *H-2* complex and another gene or genes lying outside the *H-2* complex, presumably on a different chromosome. The concept of coupled complementation may also account for the asymmetric restrictions noted in the above systems (23). Thus, coupled complementation may be a generalized genetic phenomenon. One evolutionary advantage of maintaining such a mechanism of interacting polymorphic gene systems would be to increase the potential for specificity in the regulation of immune responsiveness.

The interactions of the complementing *Ir* genes could be manifested at either the gene level or at the level of the gene products, particularly if the *Ir* gene products were Ia antigens which are expressed on the cell surface. That *I* region genes code for products (presumably Ia antigens) which interact on the cell surface to create new determinants is supported by the observations of Fathman and Nabholz (24) who demonstrated unique stimulating determinants on F₁ cells by using secondary mixed lymphocyte cultures. Genetic analyses indicated that hybrid determinants were coded for by genes which mapped in the same region as the α - and β -loci. Such hybrid molecular complexes may be critically involved in the presentation of antigen to T cells. Complementation may, therefore, serve as a mechanism for increasing the specificity of such Ia-antigen interactions.

Summary

The ability of various B10 congenic resistant strains to respond to the alloantigen H-2.2 was tested. High and low antibody-producing strains were distinguished by their anti-H-2.2 hemagglutinating responses. However, these strains do not differ in their ability to respond to these antigenic differences in the mixed lymphocyte culture. The humoral response to the H-2.2 alloantigen was shown to be controlled by two interacting genes localized within the *H-2* complex. Thus, F₁ hybrids prepared between parental low responder strains could yield high level immune responses. In addition, strains bearing recombinant *H-2* haplotypes were used to map the two distinct genes controlling the immune response. The alleles at each locus were shown to be highly polymorphic as evidenced by the asymmetric complementation patterns observed. The restricted interactions of specific alleles was termed coupled complementation. The significance of the results in the terms of mechanisms of *Ir* gene control are discussed.

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