CONTROL OF T-LYMPHOCYTE AND B-LYMPHOCYTE ACTIVATION BY TWO COMPLEMENTING *Ir-GLΦ* IMMUNE RESPONSE GENES*

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The capacity to develop antibody responses to most antigens requires the cooperative participation and interaction of thymus-derived (T) and bone marrow-derived (B) lymphocytes. These interactions constitute regulatory phenomena exerted by T cells on the B-cell precursors of antibody-producing cells (see references 1, 2 for reviews). Genetic analyses of both recognitive and regulatory functions in the immune system have revealed the control of these processes by genes in the major histocompatibility complex (MHC)[1] in several species. First, the capacity to form specific immune responses in individual animals and inbred strains of several species involves recognition events governed by dominant genes located in the MHC of these respective species (reviewed in references 3–5). Distinct histocompatibility or H-linked immune response *(Ir)* genes permit the development of immune responses to specific antigens and have been demonstrated in rodents (6–9), birds (10), and primates (11). In mice, *Ir* genes are located in the *I* region of the *H-2* complex (3–5). Second, the most effective physiologic regulatory interactions between T and B lymphocytes in the secondary response to hapten-carrier conjugates have also been shown to be controlled by genes in the MHC (reviewed in 5, 12, 13). These genes, termed cell interaction or *CI* genes (14), have been demonstrated most extensively in studies performed in inbred mice, and their intra-*H-2* localization has recently been mapped in the *I* region (15).

The fact that both *Ir* and *CI* genes are located in precisely the same region of the genome raises the interesting possibility that the the apparently distinct functions governed by these genes (i.e, antigen recognition and cell interactions) reflect the activities of product(s) of identical genes, or, alternatively, that multiple genes that have remained closely linked are responsible for these effects. For many years it was thought that individual *Ir* genes controlled the responses to specific antigens (3, 4). Recently, however, immune responses to certain antigens have been found to be controlled by two distinct H-linked *Ir* genes. The most extensively studied example is the *Ir* gene-controlled response of inbred mice to the linear synthetic terpolymer of L-glutamic acid, L-lysine, and L-phenylalanine (GLΦ). Thus, Dorf et al. previously demonstrated (16, 17) that F_1 hybrids derived from the matings of selected nonresponder parental strains (i.e., strains previously believed to be lacking the *Ir-GLΦ* gene) were phenotypic responders to GLΦ. Moreover, selected recombinant strains derived by crossover events between nonre-

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[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; *CI*, cell interaction genes or molecules; DNP, 2,4-dinitrophenyl; FγG, fowl gamma globulin; GLΦ, synthetic random linear terpolymer of L-glutamic acid^{53}-L-lysine^{36}-L-phenylalanine^{11}; KLH, keyhole limpet hemacyanin; MHC, major histocompatibility complex.

sponder parental strains were also found to be GLΦ responders (16). These observations demonstrated: *(a)* the existence of two genetically separable loci controlling responses to GLΦ; *(b)* that the nonresponder phenotype may reflect the absence of only one or both of the *Ir-GL*Φ genes; and *(c)* that complementation of two nonresponder alleles can occur to result in the responder phenotype when the genes are located in either the *cis* or *trans* position. The two *Ir-GL*Φ genes have been tentatively designated α and β with their respective alleles termed $\alpha(+)$, $\alpha(-)$ and $\beta(+)$, $\beta(-)$ (16). The β-genes have been mapped in the *I-A* subregion, and the α-genes have been tentatively mapped in a new subregion of *I* termed *I-F* located to the right of *I-C* (17).

Taking the aforementioned information collectively, one immediate question raised by the *(a)* close interrelationship between *Ir* and *CI* genes, and *(b)* the involvement of separable genes in the control of responses to a single defined antigen is the possibility that one gene is responsible for governing events in the T cell, and the other gene is predominantly concerned with B-cell function. This possibility has been recently speculated upon by others (18). If this were the case, then appropriately designed experiments should demonstrate selective functional defects in one or the other lymphocyte class depending on the presence or absence of the α- and β-*Ir-GL*Φ genes.

The experiments presented in this paper have been designed to test this possibility, and the results show that the functions of T lymphocytes and B lymphocytes and the cooperative interactions between T and B cells require the presence of both α- and β-genes in each respective cell type. Moreover, evidence has been obtained in these studies that suggests a preference for the α- and β-genes in the *cis* position to obtain the most effective T-B-cell interactions, a finding which corroborates observations in intact immunized animals reported in the two accompanying manuscripts (19, 20).

Materials and Methods

Proteins, Polypeptides, and Hapten-Carrier Conjugates. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Bio-Marine Supply Co., Venice, Calif. Fowl gamma globulin (FγG) was precipitated from normal chicken serum with 33% saturated ammonium sulfate. The random linear terpolymer L-glutamic acid^{53}-L-lysine^{36}-L-phenylalanine^{11} (GLΦ) (No. GF6-23-8) was synthesized in the laboratory of Dr. Elkan Blout, Department of Biological Chemistry, Harvard Medical School, Boston, Mass. The composition was determined by amino acid analysis, and the polypeptide had a viscosity of 0.99 at a concentration of 0.5% in 0.2 M sodium chloride, pH 2.7.

The following 2,4-dinitrophenyl (DNP) conjugates were prepared as previously described (21, 22) and employed in these studies: DNP_{10}-KLH, DNP_{20}-FγG, and DNP_8-GLΦ. Subscripts refer to moles of DNP per 100,000 daltons of KLH and moles of DNP per mole of carrier for the remainder.

Mice and Immunizations. Inbred C57BL/10 (B10), congenic B10.A, and (C57BL/6 × A/J)F_1 hybrid mice (B6A)F_1 were purchased from The Jackson Laboratory, Bar Harbor, Maine. The 5R recombinant strain mice and (B10 × A)F_1 hybrids were bred and maintained in our own animal facilities. The *H-2* haplotypes and *Ir-GL*Φ genotypes and phenotypes of these strains are listed in Table I. Mice were immunized intraperitoneally (i.p.) at 8–14 wk of age with either 50 μg of DNP-FγG, 20 μg of KLH, or 100 μg of GLΦ, administered in complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich.). 3–4 wk after primary immunization, mice were boosted with the same antigen and dose in CFA, and their spleens were removed as a source of donor cells 2 wk thereafter.

Depletion of T Lymphocytes. The preparation of anti-θ serum, its characterization, and the method of anti-θ serum treatment of spleen cells are described elsewhere (23).

Adoptive Cell Transfers. The basic protocol followed in these experiments has been described in detail elsewhere (24, 25). Briefly, 50 × 10^6 spleen cells from either normal, KLH-primed, or GLΦ-primed donors are injected intravenously (i.v.) into nonirradiated, unprimed (B6A)F_1hybrid recipients. 24 h later, these mice are irradiated (500 R) and then injected i.v. with a second cell inoculum consisting of 20 × 10^6 DNP-FγG-primed, anti-θ serum plus complement-treated spleen

TABLE I

H-2 Haplotypes and Ir-GLΦ Genotypes and Phenotypes of the Strains Employed

Strain	*H-2* haplotype*	*H-2* regions‡							*Ir-GLΦ* genotype§		*Ir-GlΦ* phenotype§
		K	*I-A*	*I-B*	*I-C*	*S*	*G*	*D*	*α*	*β*	
B10.A	*k/d(a)*	*k*	*k*	*k*	*d*	*d*	*d*	*d*	+	−	Nonresponder
B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	−	+	Nonresponder
5R	*b/a(i5)*	*b*	*b*	*b*	*d*	*d*	*d*	*d*	+	+	Responder
(B6 × A)F_1	*b* × *a*	*b/k*	*b/k*	*b/k*	*b/d*	*b/d*	*b/d*	*b/d*	−/+	+/−	Responder
(B10 × A)F_1	*b* × *a*	*b/k*	*b/k*	*b/k*	*b/d*	*b/d*	*b/d*	*b/d*	−/+	+/−	Responder

* Parental *H-2* alleles of recombinant strains are separated by a slash. The designation of the recombinant haplotypes are indicated in parentheses.

‡ Modified from Shreffler and David (27). Letters indicate parental origin or the genes in each *H-2* region. Vertical bars indicate position of crossingover in recombinant strains.

§ From Dorf et al. (16).

cells (i.e, B lymphocytes) derived from one of various donor strains. Immediately thereafter, secondary challenge is performed i.p. with DNP-KLH (25 μg) or DNP-GLΦ (50 μg) precipitated with aluminum hydroxide gel (2 mg per animal), and the mice are bled 7 days later.

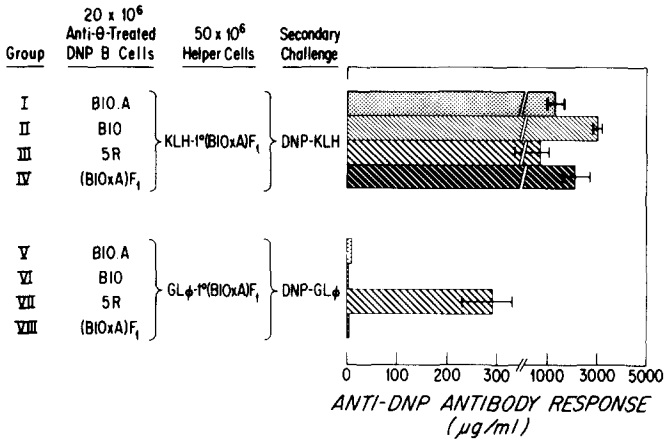
Antibody Determinations. Serum anti-DNP antibody levels were determined by a modified Farr technique (26) using [^{3}H]DNP-ε-amino-*N*-caproic acid (22). From standard curves constructed from calibrated anti-DNP antisera obtained from various strains of mice 4 wk after a standard immunization, percentage of binding was converted into amount of specific anti-DNP antibody in micrograms per milliliter of serum. It should be noted that since this binding assay reflects both affinity and quantity of antibody, the actual amounts of antibody may be either lower or higher than those recorded. However, since the sera from a given experiment are assayed on the same day and calculated on a comparable basis, the validity of the results is not affected by this method of depicting the data. Serum antibody levels were logarithmically transformed, and means and standard errors were calculated. In those mice in which no significant antibody could be detected in the serum, a value of 0.10 μg/ml was arbitrarily assigned to allow logarithmic transformation of the data. Group comparisons were made employing Student's *t* test.

Results

As reported previously and shown in Table I, B10.A *(H-2^a)* and B10 *(H-2^b)* mice are phenotypic nonresponders to GLΦ due to the fact that each strain lacks one of the *Ir-GL*Φ genes (16, 17). Thus, mice with the *H-2^a* haplotype possess the α(+) allele and lack the β(+) allele, whereas the reverse is true for *H-2^b* haplotype mice. Phenotypic responsiveness is obtained in the recombinant 5R strain as a result of the crossover event which occurred at some point between the *I-B* and *I-C* subregions, indicating that the two *Ir-GL*Φ loci are located to the right and left, respectively, of the crossover point. Moreover, complementation of the α- and β-genes can be demonstrated in *(H-2^a × H-2^b)*F_1 hybrids which are phenotypic responders to GLΦ.

To determine whether each gene is responsible for, or concerned with, events governing function in one or the other (T and B) lymphocyte class, we have taken advantage of certain parameters established in our earlier studies on genetic restrictions of physiologic T–B cell interactions. In brief, the two major points in this regard are as follows: *(a)* Reciprocal combinations of F_1 hybrid and parental T and B lymphocytes, primed, respectively, to carrier and haptenic determinants, can be shown to effectively interact in response to the appropriate hapten-carrier conjugate provided the carrier employed is one to which both parental strains are phenotypic responders; *(b)* Conversely, when the carrier antigen is one to which the response is governed by an H-linked *Ir* gene, then a (responder × nonresponder)F_1 hybrid will provide carrier-specific T-cell helper function for DNP-specific B cells of the responder, but not of the nonresponder, parent upon challenge with a DNP conjugate of the carrier antigen controlled by that *Ir* gene (28).

Upon this background, one can ask whether a (nonresponder × nonresponder)F_1 hybrid, itself a phenotypic GLΦ responder as a result of gene complementation, can provide GLΦ-specific helper T-cell function for DNP-specific B cells of one or the other, or neither, of the nonresponder parents in response to DNP-GLΦ. Likewise, in principal, the reciprocal question can be asked by determining whether either one of the nonresponder parents can provide GLΦ-specific T-cell helper function for the DNP-specific B cells of the F_1 hybrid. If each of the *Ir-GL*Φ genes were predominantly concerned with the

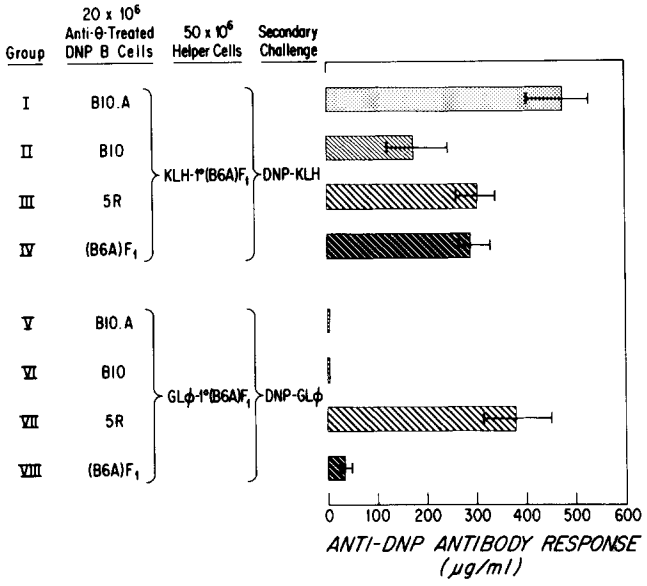


Strain	H-2 HAPLOTYPE *K*	*I-A*	*I-B*	*I-C*	*S*	*G*	*D*	*Ir*-GLΦ Genotype α	β	Phenotype
BIO.A	K	K	K	D	D	D	D	+	−	Non-Responder
BIO	B	B	B	B	B	B	B	−	+	Non-Responder
5R	B	B	B	D	D	D	D	+	+	Responder
(BIO x A) F_1	B/K	B/K	B/K	B/D	B/D	B/D	B/D	-/+	+/-	Responder

FIG. 1. Failure of *(H-2^b × H-2^a)*F_1 T cells to provide GLΦ-specific helper function for B cells of either parental strain and demonstration that the most efficient T-B-cell interactions occur when the α- and β-*Ir-GL*Φ genes are located in the *cis* position. The protocol of the adoptive transfer system is described in Materials and Methods. The recipients for all cell combinations were (B6A)F_1 hybrids. Combinations and strain origins of T and B cells, the specificities of helper cells employed in various groups, and the antigen used for secondary challenge are indicated on the left. The genotypes and phenotypes of the strains employed are summarized beneath the protocol and data. Mean serum levels of anti-DNP antibody of groups of five mice on day 7 after secondary challenge with DNP-KLH or DNP-GLΦ are illustrated. Horizontal bars represent ranges of standard errors. Statistical comparison between the relevant groups yielded the following results: Groups V, VI, and VIII vs. Group VII had *P* values less than 0.001 in all cases.

function of one of the two lymphocyte classes, then one would predict that F_1 T cells would provide helper function for B cells of parent no. 1 but not of parent no. 2 and, reciprocally, T cells from parent no. 2 might provide GLΦ-specific helper function for F_1 B cells, whereas T cells from parent no. 1 would be unable to do so.

*Failure of (H-2^b × H-2^a)F_1 T Cells to Provide GLΦ-Specific Helper Function for B cells of either Parental Strain and Demonstration that the Most Efficient T-B-Cell Interactions Occur when the α- and β-Ir-GL*Φ *Genes are Located in the cis Position.* The experiments presented in Figs. 1 and 2 illustrate the basic design used to answer part of the question posed above. The left side of both figures depicts the protocols and various combinations of cell mixtures analyzed for cooperative responses to DNP-KLH and DNP-GLΦ. The *H-2* gene regions and *Ir-GL*Φ genotypes and phenotypes are summarized at the bottom of each



Strain	H-2 HAPLOTYPE K	I-A	I-B	I-C	S	G	D	Ir-GLϕ Genotype α	β	Phenotype
BIO.A	K	K	K	D	D	D	D	+	−	Non-Responder
BIO	B	B	B	B	B	B	B	−	+	Non-Responder
5R	B	B	B	D	D	D	D	+	+	Responder
(B6 x A) F_1	B/K	B/K	B/K	B/D	B/D	B/D	B/D	-/+	+/-	Responder

FIG. 2. Same as legend to Fig. 1. Statistical comparison yielded the following results: Groups V and VI vs. Group VII had P values of less than 0.001 in both cases; Group VIII vs. Group VII, $P = 0.0032$.

figure for convenience. Before discussing the data shown in Figs. 1 and 2, it is pertinent to cite the following data from control groups included in the experiments but not shown in the figures: *(a)* All of the DNP-primed spleen cell populations were capable of developing good secondary adoptive anti-DNP responses to the immunizing antigen, DNP-FγG, in parallel transfers utilizing spleen cells not treated with anti-θ serum. *(b)* Anti-θ serum treatment in the conditions employed effectively abrogated the capacity of such cells to mount an in vivo response in the absence of additional carrier-primed cells. *(c)* The substitution of normal cells for carrier-primed cells failed to permit development of responses to DNP-KLH or DNP-GLΦ.

The relevant results are presented on the right sides of Figs. 1 and 2. Groups I–IV demonstrate the capacity of semisyngeneic KLH-primed (B10 × A)F_1 or (B6A)F_1 T cells to provide excellent helper activity for the DNP-specific B cells of both parental strains, the 5R recombinant and the syngeneic F_1 hybrid donors in response to DNP-KLH within the environs of (B6A)F_1 irradiated recipients. In contrast, GLΦ-primed F_1 T cells fail to cooperatively interact with B cells from

either B10.A or B10 parental donors in response to DNP-GLΦ (Groups V and VI). This is not a refection of defective GLΦ-specific T-cell function on the part of the GLΦ-primed F_1 cells, as evidenced by the capacity of these cells to provide substantial helper activity for DNP-specific B cells from the 5R recombinant strain donors in both experiments (Group VII). An unexpected result in these experiments is illustrated by Group VIII in which GLΦ-primed F_1 T cells either failed to provide a detectable helper function (Fig. 1) or provided only low helper activity (Fig. 2) for DNP-specific B cells from syngeneic F_1 donors in response to DNP-GLΦ. These results cannot be explained on the basis of defective B-cell function on the part of the F_1, B10.A, or B10 donor B cells, since the same pools of cells were used for Groups I, II, and IV in which good antibody responses were elicited. In the experiment depicted in Fig. 2, the difference in magnitude of the F_1 B-cell response to DNP-GLΦ compared to that observed with 5R B cells was highly significant ($P = 0.0032$) and cannot be attributed to a lower general responsiveness on the part of F_1 B cells, since the magnitude of cooperative responses of 5R and (B6A)F_1 B cells with F_1 KLH-specific T cells to DNP-KLH were quite comparable (cf. Groups III and IV, Fig. 2). These data suggest a substantial difference in efficiency of T–B cell interactions when the *Ir* genes involved in the B cell are located in the *cis* versus the *trans* position.

Failure of Nonresponder Parental T Cells to Provide Helper Function for (Nonresponder × Nonresponder)F_1 B cells. In the preceding accompanying manuscript by Schwartz et al. (20), it was shown that neither B10.A nor B10 nonresponder parents, which had been previously primed with GLΦ were able to mount DNA synthetic responses upon exposure to GLΦ in vitro. Attempts to demonstrate GLΦ-specific helper T-cell function in either of the nonresponder parental strains in the present study were likewise unsuccessful, as shown by the experiment in Fig. 3. In this experiment, spleen cells from GLΦ-primed B10.A, B10, 5R, and (B6A)F_1 donors were tested for helper T-cell activity with DNP-primed B cells from (B6A)F_1 donor mice in response to DNP-GLΦ in the same type of adoptive transfer system utilized in the preceding experiments. In addition to the controls described in the preceding section, the population of DNP-primed (B6A)F_1 B cells employed in this experiment were capable of responding to DNP-KLH when admixed with KLH-primed helper T cells from B10.A, B10, 5R, and (B6A)F_1 donor mice (data not shown).

As shown in Fig. 3, neither B10.A nor B10 donors were capable of providing detectable GLΦ-specific helper activity for (B6A)F_1 B cells in response to DNP-GLΦ. In contrast, GLΦ-primed T cells from 5R donors provided helper function for DNP-specific F_1 B cells. A meager degree of helper activity was also obtained with (B6A)F_1 GLΦ-primed T cells, although this was substantially less than that observed with the 5R donor cells, a result which once again suggests the greater efficiency of the two *Ir-GL*Φ genes when located in the *cis* position in the responding T cells. It should be noted that the absolute levels of anti-DNP responses obtained, even with the 5R GLΦ-primed cells, are lower in this experiment than those observed in the preceding experiment and therefore are presented as mean percent binding at 1:10 dilutions of each serum. Nevertheless, the demonstration of GLΦ-specific helper activity in 5R and F_1 donors and

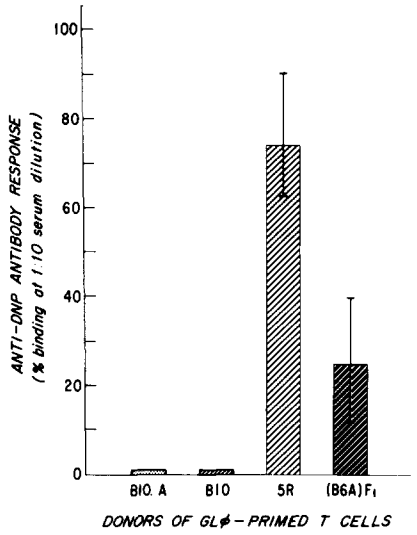


FIG. 3. Failure of nonresponder parental T cells to provide helper function for (nonresponder × nonresponder)F_1 B cells. The same type of adoptive transfer protocol used for the experiments in Figs. 1 and 2 was employed. The responses to DNP-GLΦ of mixtures of GLΦ-primed T cells from the various donor strains indicated with DNP-primed (B6A)F_1 B cells in (B6A)F_1 recipients are illustrated. The data are presented as mean serum levels of anti-DNP antibody of groups of five mice on day 11 after secondary challenge. The percent binding of 1×10^{-8} M [^{3}H]DNP-ε-amino-*N*-caproic acid by 1:10 dilutions of sera were recorded. Vertical bars represent ranges of standard errors. Statistical comparison of responses obtained with 5R helper cells with B10.A and B10 T cells yielded *P* values less than 0.001 in both cases; comparison of 5R and (B6A)F_1 GLΦ-specific helper activity yielded $P = 0.028$.

the absence of detectable activity in the case of either parental donor strongly indicate that both genes must be expressed for specific T-cell helper function to be successfully generated.

Discussion

During the past 2–3 yr in a series of experiments designed to investigate the genetic requirements for optimal T- and B-cell interactions in secondary immune responses to hapten-carrier conjugates, we have shown that identical genes in the MHC are required to obtain most effective T–B-cell cooperative interactions (24, 25). These *CI* genes have been mapped in the *I* region of *H-2* in the mouse (15). Based on these findings, we developed the hypothesis that there must exist on the surfaces of immunocompetent cells molecules, independent of specific receptors, that are primarily concerned with cell interactions (24, 25). In this scheme, we postulated that the T lymphocyte would interact via these molecules, either by membrane contact or release of the molecules, with comparable molecules on the B cell, which we originally termed "acceptor" sites, thereby providing a critical signal for induction of differentiation (24, 25).

Shortly thereafter it became apparent that a very close interrelationship existed between the *CI* genes controlling cell interactions and the genes control-

ling specific recognition by immunocompetent lymphocytes. This was first suggested by results of studies in our laboratory demonstrating that (responder × nonresponder)F_1 T cells primed to the synthetic terpolymer L-glutamic acid, L-lysine, and L-tyrosine (GLT), to which responses are controlled by two H-linked *Ir-GLT* genes, as indicated in the accompanying paper (19), were capable of providing helper function for DNP-specific B cells of responder, but not of nonresponder, parents in response to DNP-GLT (28). This observation was interpreted by us to indicate: (*a*) the involvement of *Ir*-gene function in controlling responses of B cells, in a nonclonal fashion; and/or (*b*) that activation of the *Ir* gene product(s) in the T cells determines the activation in turn of the CI molecules involved in T–B-cell interactions coded for by the same haplotype as that from which the *Ir* gene was derived (28). Since at that time there was no evidence for the existence of two allelic loci controlling the response to a single antigen, this possibility was not included among the alternatives.

More recently, studies performed by Mozes et al. (29) and Taussig and Munro (30) have provided evidence indicating that the genetic defect in nonresponder strains to the synthetic branched chain polymer (T,G)-A--L is in B cells in some strains and in both T and B cells in other strains. Moreover, the very recent studies performed by Dorf et al. (16, 17), which were described in the introduction and independently at the same time by Munro and Taussig (18, 31), have demonstrated that responses in mice controlled by the *Ir-GL*Φ and *Ir-(T,G)- A--L* genes, respectively, reflect control of such responses by two distinct genes and/or their products.

These observations opened the possibility that distinct *Ir* genes are responsible for controlling the functional responses of T and B lymphocytes, respectively (16–18). Indeed, an hypothesis has recently been developed to account for this by assuming that one *Ir* gene codes for the CI molecule and antigen receptor on the T cell, whereas the other *Ir* gene codes for the "acceptor" site on the B cell (18). A prediction of this model would be that reciprocal complementation of T- and B-cell interactions should be observed between appropriate F_1-parent mixtures concerned with the *Ir* genes under consideration.

The studies presented here were designed to determine the validity of the aforementioned prediction in the *Ir-GL*Φ system. Thus, F_1 hybrids derived from two GLΦ nonresponder strains, which themselves are GLΦ responders by virtue of inheriting the $\alpha(+)$ and $\beta(+)$ alleles from the B10.A and B10 parental strains, respectively, were tested for their capacity to (*a*) provide helper function and (*b*) "accept" helper function in cell mixtures with the two parental haplotypes. The results of such analyses appear to be conclusive in that GLΦ-primed F_1 T cells failed to provide helper function for DNP-specific B cells of either B10.A or B10 parental origin. This result is essentially analogous to the previous observation in the DNP-GLT system described above. The lack of cooperation between F_1 T cells with either of the parental B cells in response to DNP-GLΦ demonstrates, therefore, that the inheritance of only one of the two *Ir-GL*Φ alleles, irrespective of which it is, is not sufficient to permit effective interaction between such B cells and functional F_1 T cells. The validity of interpreting this observation so strongly is supported by the very effective helper function provided by GLΦ-

primed F_1 T cells to 5R recombinant B cells, thus demonstrating the functional capacity of such T cells.

Analysis of the reciprocal situation, i.e. the possibility of observing GLΦ-specific helper T-cell function from one or the other parent in responses of F_1 B cells to DNP-GLΦ, demonstrated the same phenomenon. Clearly, no helper function was observed in either parent-F_1 mixture, whereas, detectable helper function, albeit moderate, was exerted by GLΦ-primed 5R and (B6A)F_1 donors. The inability of either of the parental strains to manifest GLΦ-specific helper T-cell activity for F_1 B cells in this system is totally consistent with the results presented in the preceding accompanying manuscript by Schwartz et al. (20) which demonstrate an inability of either nonresponder parental strain to develop DNA synthetic responses to GLΦ in vitro. Indeed, the failure of either parental strain to manifest helper function, as shown herein, extend the aforementioned findings since it has been previously shown that helper function can be exhibited by primed T cells in the absence of DNA synthesis by such cells (32). Taken collectively, these observations demonstrate the requirement for inheritance of both *Ir-GL*Φ alleles to permit effective T-cell function in either antigen-induced proliferative responses or to perform in a helper capacity for B cells in the production of antibody.

The unexpected observations in these experiments was the relative inefficiency of F_1 GLΦ-primed T cells in providing helper function for F_1 B cells in response to DNP-GLΦ. This result cannot be explained on the basis of a relatively weak GLΦ-specific F_1 helper cell population since, in the case of the experiments presented in Figs. 1 and 2, these cells provided quite adequate helper activity for B cells from 5R recombinant donors. Indeed, the substantial difference observed between $F_1 \leftrightarrow$ 5R and $F_1 \leftrightarrow F_1$ T- and B-cell interactions suggests the preferential efficiency of a *cis* chromosomal relationship of the $\alpha(+)$ and $\beta(+)$ alleles in the respective interacting lymphocyte classes. It should be pointed out, however, that we cannot rule out the possibility that these effects may be the result of gene dosage as discussed in the previous paper (19).

Several examples of more effective *Ir* gene complementation in antibody responses of intact animals to GLΦ in the *cis* rather than *trans* position have been presented and discussed in the accompanying paper for haplotypes other than $H\text{-}2^a$ and $H\text{-}2^b$ (19). It is of interest that the exception to the *cis–trans* phenomenon were the comparable anti-GLΦ responses observed in B10.A(5R) and in (C57BL/6 × A/J)F_1 mice. However, as shown in this study, when passive transfer experiments involving a limited number of cells are carried out, the presumed *cis* or F_1 effect can also be demonstrated with the α- and β-genes of the $H\text{-}2^a$ and $H\text{-}2^b$ haplotypes.

In addition to the *cis-trans* effect, several important issues raised by these and previous experiments with systems under *Ir* gene control need to be clarified to permit a definitive understanding of the genetic control of T- and B-cell interactions:

(*a*) Are the primed F_1 B cells resulting from the interactions with parental T cells restricted or preferentially conditioned to interactions with helper T cells from the same parental origin as compared with helper T cells bearing the other

parental haplotype, a phenomenon which we term "haplotype preference"?

(*b*) Are hapten-specific memory B cells selected to interact best in the secondary response with helper T cells specific for the carrier used in the primary response?

These questions are presently being investigated in our laboratory.

Summary

The possibility that the two complementing α- and β-*Ir-GL*Φ genes are independently responsible for controlling events in T lymphocytes and B lymphocytes, respectively, has been tested in double adoptive transfer experiments utilizing cells from appropriate inbred strains of mice. The results of these studies show that the functions of T lymphocytes and B lymphocytes and the cooperative interactions between T and B cells require the presence of both α- and β-genes in each respective cell type. Moreover, evidence has been obtained in these studies that indicates a preference for the α- and β-*Ir-GL*Φ genes in the *cis* position to obtain the most effective T-B-cell interactions. The possible implications of these findings are discussed.

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References

1. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* 15:1.
2. Gershon, R. K. 1973. T cell control of antibody production. *Contemp. Top. Immunobiol.* 3:1.
3. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* 175:273.
4. Benacerraf, B., and D. H. Katz. 1975. The histocompatibility-linked immune response genes. *Adv. Cancer Res.* 21:121.
5. Benacerraf, B., and D. H. Katz. 1975. The nature and function of histocompatibility-linked immune response genes. *In* Immunogenetics and Immunodeficiency. B. Benacerraf, editor. Medical and Technical Publishing Co., Ltd., London. 117.
6. McDevitt, H. O., and A. Chinitz. 1969. Genetic control of the antibody response: relationship between immune response and histocompatibility (H-2) type. *Science (Wash. D. C.)* 163:1207.
7. Ellman, L., I. Green, W. J. Martin, and B. Benacerraf. 1970. Linkage between the poly-L-lysine gene and the locus controlling the major histocompatibility antigens in strain 2 guinea pigs. *Proc. Natl. Acad. Sci. U. S. A.* 66:322.
8. Günther, E., E. Rüde, and O. Stark. 1972. Genetic control of the immune response in rats. *Eur. J. Immunol.* 2:151.
9. Armerding, D., D. H. Katz, and B. Benacerraf. 1974. Immune response genes in inbred rats. II. Segregation studies of the GT and GA genes and their linkage to the major histocompatibility locus. *Immunogenetics.* 1:340.
10. Günther, E., J. Balcarova, K. Hala, E. Rüde, and T. Hraba. 1974. Evidence for an association between immune responsiveness of chicken to (T,G)-A--L and the major

histocompatibility system. *Eur. J. Immunol.* 4:548.
11. Dorf, M. E., H. Balner, M. L. deGroot, and B. Benacerraf. 1974. Histocompatibility-linked immune response genes in the rhesus monkey. *Transplant. Proc.* 6:119.
12. Katz, D. H., and D. Armerding. 1975. Evidence for the control of lymphocyte interactions by gene products of the *I* region of the *H-2* complex. *In* Immune Recognition. Proceedings of the Ninth Leukocyte Culture Conference. A. S. Rosenthal, editor. Academic Press, Inc., New York. 727.
13. Katz, D. H., M. E. Dorf, D. Armerding, and B. Benacerraf. 1975. The role of products of the histocompatibility gene complex in immune responses. *In* Molecular Approaches to Immunology, Miami Winter Symposia. E. E. Smith and D. W. Ribbons, editors. Academic Press, Inc., New York. 9:211.
14. Katz, D. H., and B. Benacerraf. 1975. Hypothesis. The function and interrelationships of T cell receptors, Ir genes, and other histocompatibility gene products. *Transplant. Rev.* 22:175.
15. Katz, D. H., M. Graves, M. E. Dorf, H. DiMuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VII. Cooperative responses between lymphocytes are controlled by genes in the *I* region of the *H-2* complex. *J. Exp. Med.* 141:263.
16. 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.
17. 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.
18. Munro, A. J., and M. J. Taussig. 1975. Two genes in the major histocompatibility complex control immune response. *Nature (Lond.).* 256:103.
19. Dorf, M. E., P. H. Maurer, C. F. Merryman, and B. Benacerraf. 1975. Inclusion group systems and *cis-trans* effects in responses controlled by the two complementing *Ir-GL*Φ genes. *J. Exp. Med.* 143:889.
20. Schwartz, R. H., M. E. Dorf, B. Benacerraf, and W. E. Paul. 1975. The requirement for two complementing *Ir-GL*Φ genes in the T-lymphocyte proliferative response to poly-($Glu^{53}Lys^{36}Phe^{11}$). *J. Exp. Med.* 143:897.
21. Benacerraf, B., and B. B. Levine. 1961. Immunological specificity of the delayed and immediate hypersensitivity reactions. *J. Exp. Med.* 113:571.
22. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in antihapten immune responses. I. Enhancement of primary and secondary antihapten antibody responses by carrier preimmunization. *J. Exp. Med.* 132:261.
23. Katz, D. H., and D. P. Osborne, Jr. 1972. The allogeneic effect in inbred mice. II. Establishment of the cellular interactions required for enhancement of antibody production by the graft-versus-host reaction. *J. Exp. Med.* 136:455.
24. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. *J. Exp. Med.* 137:1405.
25. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. III. Demonstration that the H-2 gene complex determines successful physiologic lymphocyte interactions. *Proc. Natl. Acad. Sci. U. S. A.* 70:2624.
26. Green, I., B. Benacerraf, and S. H. Stone. 1969. The effect of the amount of mycobacterial adjuvants on the immune response of strain 2, strain 13 and Hartley strain guinea pigs to DNP-PLL and DNP-GL. *J. Immunol.* 103:403.
27. Shreffler, D. C., and C. S. David. The *H-2* major histocompatibility complex and the *I*

immune response region: genetic variation, function, and organization. *Adv. Immunol.* 20:125.

28. Katz, D. H., T. Hamaoka, M. E. Dorf, P. H. Maurer, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (*Ir*) gene in the control of lymphocyte interactions in responses controlled by the gene. *J. Exp. Med.* 138:734.
29. Mozes, E., R. Isac, and M. J. Taussig. 1975. Antigen-specific T-cell factors in the genetic control of the immune response to poly(Tyr, Glu)-polyDLala--polylys. Evidence for T- and B-cell defects in SJL mice. *J. Exp. Med.* 141:703.
30. Taussig, M. J., and A. J. Munro. 1974. Antigen-specific T-cell factor in cell cooperation and genetic control of the immune response. In Immune Recognition. A. S. Rosenthal, editor. Academic Press, Inc., New York. 791.
31. Taussig, M. J., and A. J. Munro. 1975. Antigen-specific T cell factor in cell cooperation and genetic control of the immune response. Cellular and Soluble Factors in the Regulation of Lymphocyte Activation. FASEB Symposium, April 1975. *Fed. Proc.*
32. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1970. Radioresistance of the cooperative function of carrier-specific lymphocytes in anti-hapten responses. *Science (Wash. D. C.)* 170:462.