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 recognition 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 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 F1 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; FvG, fowl gamma globulin; GLΦ, synthetic random linear terpolymer of L-glutamic acid43-L-lysine45-L-phenylalanine11; 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-GLΦ 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-GLΦ genes have been tentatively designated α and β with their
respective alleles termed α(+), α(−) and β(+), β(−) (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 Cl 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 specu-
lated 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-GLΦ 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 (FyG)
was precipitated from normal chicken serum with 33% saturated ammonium sulfate. The random
linear terpolymer L-glutamic acid-L-lysine-L-phenylalanine (GLΦ) (No. GP6-23-8) was synthe-
sized 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 polypep-
tide 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: DNP50-KLH, DNP20-FyG, and DNP8-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 x A/J)F1
hybrid mice (B6A), were purchased from The Jackson Laboratory, Bar Harbor, Maine. The 5R
recombinant strain mice and (B10 x A)F1 hybrids were bred and maintained in our own animal
facilities. The H-2 haplotypes and Ir-GLΦ 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-
FyG, 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 x 10⁶ spleen cells from either normal, KLH-primed, or
GLΦ-primed donors are injected intravenously (i.v.) into nonirradiated, unprimed (B6A)F1 hybrid
recipients. 24 h later, these mice are irradiated (500 R) and then injected i.v. with a second cell
inoculum consisting of 20 x 10⁶ DNP-FyG-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§ |
|------------|--------------------------------|------------------|------------------|-------------------|
| B10.A      | k/d(a)                         | K k I-A I-B I-C  | +                | Nonresponder     |
| B10        | k/d(a)                         | b b b b b       | -                | Nonresponder     |
| 5R         | b/a(i5)                        | b b b k d d     | +                | Responder        |
| (B6 × A)F₁ | b × a                          | b/k b/k b/k     | -/+              | Responder        |
| (B10 × A)F₁| b × a                          | b/k b/k b/k     | -/+              | 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-GLcP (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 [3H]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-2a) and B10 (H-2b) mice are phenotypic nonresponders to GLcP due to the fact that each strain lacks one of the Ir-GLcP genes (16, 17). Thus, mice with the H-2a haplotype possess the α(+)-allele and lack the β(+) allele, whereas the reverse is true for H-2b 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-GLcP 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-2a x H-2b)F1 hybrids which are phenotypic responders to GLcP.

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 F1 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 x nonresponder)F1 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 x nonresponder)F1 hybrid, itself a phenotypic GLcP responder as a result of gene complementation, can provide GLcP-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-GLcP. Likewise, in principal, the reciprocal question can be asked by determining whether either one of the nonresponder parents can provide GLcP-specific T-cell helper function for the DNP-specific B cells of the F1 hybrid. If each of the Ir-GLcP genes were predominantly concerned with the
function of one of the two lymphocyte classes, then one would predict that 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, B cells, whereas T cells from parent no. 1 would be unable to do so.

**Failure of (H-2b × H-2a)F1, 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-GLΦ 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-GLΦ genotypes and phenotypes are summarized at the bottom of each
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-F\(_\gamma\)G, in parallel transfers utilizing spleen cells not treated with anti-\(\theta\) serum. (b) Anti-\(\theta\) 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-GL\(\Phi\).

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 \(\times\) 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, GL\(\Phi\)-primed F\(_1\) T cells fail to cooperatively interact with B cells from
either B10.A or B10 parental donors in response to DNP-GLΔ (Groups V and VI). This is not a reflection of defective GLΔ-specific T-cell function on the part of the GLΔ-primed F1 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 GLΔ-primed F1 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 F1 donors in response to DNP-GLΔ. These results cannot be explained on the basis of defective B-cell function on the part of the GLΔ-primered F1 cells, as evidenced by the capacity of these cells to provide substantial helper activity for DNP-specific B cells from syngeneic F1 donors in both experiments (Group VH). An unexpected result in these experiments is illustrated by Group VIII in which GLΔ-primed F1 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 F1 donors in response to DNP-GLΔ. These results cannot be explained on the basis of defective B-cell function on the part of the F1, 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 F1 B-cell response to DNP-GLΔ 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 F1 B cells, since the magnitude of cooperative responses of 5R and (B6A)F1 B cells with F1 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)F1 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 GLΔ were able to mount DNA synthetic responses upon exposure to GLΔ in vitro. Attempts to demonstrate GLΔ-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 GLΔ-primed B10.A, B10, 5R, and (B6A)F1 donors were tested for helper T-cell activity with DNP-primed B cells from (B6A)F1 donor mice in response to DNP-GLΔ 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)F1 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)F1 donor mice (data not shown).

As shown in Fig. 3, neither B10.A nor B10 donors were capable of providing detectable GLΔ-specific helper activity for (B6A)F1 B cells in response to DNP-GLΔ. In contrast, GLΔ-primed T cells from 5R donors provided helper function for DNP-specific F, B cells. A meager degree of helper activity was also obtained with (B6A)F1 GLΔ-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-GLΔ 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 GLΔ-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 GLΔ-specific helper activity in 5R and F1 donors and
FIG. 3. Failure of nonresponder parental T cells to provide helper function for (nonresponder × nonresponder)F₁, 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)F₁, B cells in (B6A)F₁ 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 x 10⁻⁸ M [3H]DNP-e-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)F₁, GLφ-specific helper activity yielded P = 0.028.

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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-
Two-Gene Control of T- and B-Cell Functions

Ling specific recognition by immunocompetent lymphocytes. This was first suggested by results of studies in our laboratory demonstrating that (responder × nonresponder)F₁ 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-GLP 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 F₁-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-GLP system. Thus, F₁ hybrids derived from two GLP nonresponder strains, which themselves are GLP responders by virtue of inheriting the α(+) and β(+) 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 GLP-primed F₁ 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 F₁ T cells with either of the parental B cells in response to DNP-GLP demonstrates, therefore, that the inheritance of only one of the two Ir-GLP alleles, irrespective of which it is, is not sufficient to permit effective interaction between such B cells and functional F₁ T cells. The validity of interpreting this observation so strongly is supported by the very effective helper function provided by GLP-
primed F₁ 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 F₁ B cells to DNP-GLΦ, demonstrated the same phenomenon. Clearly, no helper function was observed in either parent-F₁ mixture, whereas, detectable helper function, albeit moderate, was exerted by GLΦ-primed 5R and (B6A)F₁ donors. The inability of either of the parental strains to manifest GLΦ-specific helper T-cell activity for F₁ 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-GLΦ 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 F₁ GLΦ-primed T cells in providing helper function for F₁ B cells in response to DNP-GLΦ. This result cannot be explained on the basis of a relatively weak GLΦ-specific F₁ 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 F₁ ↔ 5R and F₁ ↔ F₁ T- and B-cell interactions suggests the preferential efficiency of a cis chromosomal relationship of the α(+) and β(+) 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-2a and H-2b (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)F₁ mice. However, as shown in this study, when passive transfer experiments involving a limited number of cells are carried out, the presumed cis or F₁ effect can also be demonstrated with the α- and β-genes of the H-2a and H-2b 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 F₁ 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 $\alpha$- and $\beta$-Ir-GL\(\Phi\) 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 $\alpha$- and $\beta$-genes in each respective cell type. Moreover, evidence has been obtained in these studies that indicates a preference for the $\alpha$- and $\beta$-Ir-GL\(\Phi\) 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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