# *Brief Definitive Reports*

## 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*

By DAVID H. KATZ, TOSHIYUKI HAMAOKA, MARTIN E. DORF, PAUL H. MAURER, and BARUJ BENACERRAF

(*From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115 and the Department of Biochemistry, Thomas Jefferson University, Philadelphia, Pennsylvania 19107*)

(Received for publication 21 June 1973)

In previous studies reported in this series of papers, we have shown that histoincompatible mouse carrier-primed T lymphocytes fail to provide the required stimulus for the responses of B cells to hapten-carrier conjugates (1–3). Utilizing a system designed to eliminate nonspecific T cell influences from potential development of an "allogeneic" effect (reviewed in references 4 and 5), mixtures of suitably primed T and B lymphocytes from histoincompatible donors failed to cooperate effectively in developing anti-2,4-dinitrophenyl (DNP) antibody responses either in vivo or in vitro (2, 3). Moreover, experiments carried out in congenic-resistant mouse strains have provided conclusive evidence that a gene or genes present in the H-2 complex control(s) the capacity of antigen-specific T and B cells to effectively interact (3).

In this report, we present results of experiments designed to question the role of the immune response (Ir) genes and their product(s) in the control of physiologic, i.e. antigen-specific, T-B cell cooperative interactions. This has been accomplished by taking advantage of our previous demonstration of highly effective cooperation between reciprocal combinations of parental and $F_1$ hybrid T and B lymphocytes when the carrier molecule employed is one to which both parental strains are genetic responders (2). We now have asked the question of whether $F_1$ carrier-primed T cells can serve as helper cells for either or both parental B cells when (*a*) the carrier molecule employed is under genetic control such that one parental strain is a responder and the other is a nonresponder, and (*b*) the determinant specificity of the parental B cells being assessed is not under genetic control and bears no relationship to the specificity of the carrier molecule. The experimental system utilizes an immune response gene controlling responses to the terpolymer L-glutamic acid-L-lysine-L-tyrosine (GLT) to which A strain mice (*H-2*$^a$) are nonresponders whereas BALB/c (*H-2*$^d$) and (BALB/c × A)$F_1$ hybrids ($CAF_1$) are responders.[1] These studies demonstrate that GLT-primed T cells of $CAF_1$ donors can provide for responder BALB/c.

* This investigation was supported by grants AI 10630, AI 07825, and AI 09920 from the National Institutes of Health, U. S. Public Health Service.

[1] Merryman, C. F., and P. H. Maurer. 1973. Data to be published.

but not for nonresponder A/J, the required stimulus for the anti-DNP responses of DNP-specific B cells of these respective parental strains to the DNP conjugate of GLT.

## *Materials and Methods*

The terpolymer poly-$\alpha$-(L-glutamic acid$^{57}$, L-lysine$^{38}$, L-tyrosine$^{5}$) (GLT) mol wt 50,000 was prepared by polymerization of the $\alpha$-*N*-carboxyanhydrides of the amino acids (6). Before use, the aqueous solution of the polymer was dialyzed free of HBr against distilled water and then lyophilized. All other proteins, hapten-carrier conjugates, animals, methods for determining serum anti-DNP antibody levels, and for depletion of T lymphocytes by anti-$\theta$ serum plus complement have been described (1–3, 7). DNP conjugates employed were: $DNP_{14}$-keyhole limpet hemocyanin (KLH), $DNP_{32}$-bovine gamma globulin (BGG), and $DNP_5$-GLT. $CAF_1$ donors of carrier-primed spleen cells were primed with 100 $\mu$g of GLT or BGG in complete Freund's adjuvant (CFA) 1–2 mo before cell transfer. BALB/c or A/J donors of DNP-primed B cells were similarly primed with DNP-KLH 3–5 mo before cell transfer. Cells were harvested and adoptively transferred (after in vitro anti-$\theta$ treatment in the case of B cells) intravenously to $CAF_1$ recipient mice as previously described (1–3). Anti-DNP antibody determinations were made on sera obtained from recipient mice 7 days after secondary challenge with either 100 $\mu$g of DNP-GLT or 50 $\mu$g of DNP-BGG intraperitoneally in saline.

## RESULTS

An initial experiment was carried out to confirm that the genetic control of responses to GLT was not altered by conjugating DNP to the terpolymer. Groups of normal BALB/c and A/J mice were immunized intraperitoneally with 100 $\mu$g of DNP-GLT emulsified in CFA and then bled 10 and 23 days later. The BALB/c mice developed anti-DNP antibody responses by day 10 that progressed in titer with time, whereas none of the A/J mice manifested circulating anti-DNP antibodies at either of the times tested (Table I). Thus, at the level of DNP substitution employed, no major structural alteration occurred to remove responsiveness to GLT from normal genetic control.

The protocol and results of the critical adoptive transfer experiment are depicted in Fig. 1. To reiterate briefly the sequence of transfers in this model (2), $50 \times 10^6$ normal or carrier-primed $CAF_1$ spleen cells are transferred to nonirradiated $CAF_1$ recipients; 24 h later, the recipients are irradiated with 600 R and then given a second transfer consisting of $20 \times 10^6$

TABLE I

*Primary Anti-DNP Antibody Responses of BALB/c and A/J Mice to $DNP_5$-GLT*

| | Anti-DNP Antibody ($\mu$g/ml)* | |
|---|---|---|
| | Day 10 | Day 23 |
| BALB/c | 12.0 (8.4–17.2) | 193.9 (157.6–238.5) |
| A/J | 0.10 | 0.10 |

* The data are expressed as geometric mean antibody levels of groups of five mice on the days indicated after primary immunization with 100 $\mu$g of $DNP_5$-GLT in CFA. Ranges of the mean for BALB/c mice are in parentheses. The values for A/J mice are arbitrarily assigned lower limits in this assay when less than 15% binding activity of neat serum is detected.

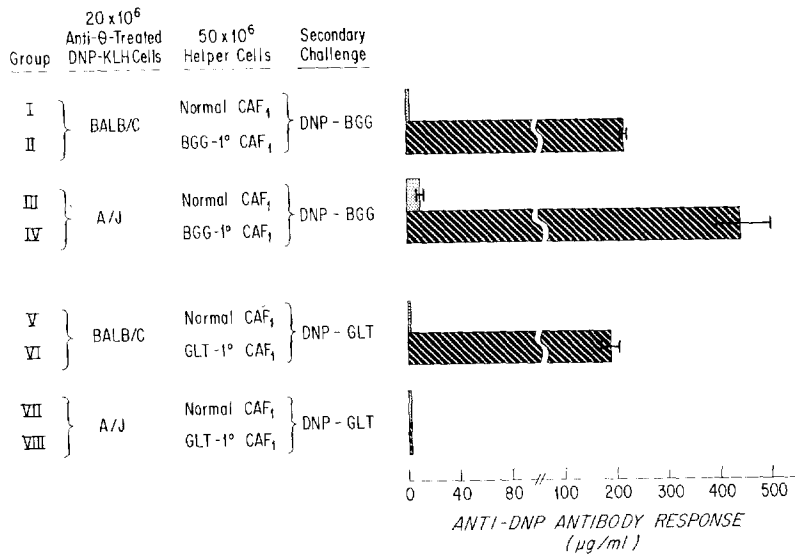


FIG. 1. Involvement of Ir gene in control of T and B lymphocyte interaction. See Results for description of cell transfer scheme. Recipients for all cell combinations were $CAF_1$ hybrids. Combinations, strain origins, and specificities of T and B cells are indicated. Recipients in groups I-IV were secondarily challenged intraperitoneally with 50 $\mu$g of DNP-BGG in saline; groups V–VIII received 100 $\mu$g of DNP-GLT. Mean serum anti-DNP antibody levels of groups of five mice on day 7 after secondary challenge are illustrated. Horizontal bars represent ranges of the standard errors. Statistical comparisons between the various experimental and control groups gave $P$ values of $0.001 > P$ in all cases.

anti-$\theta$-treated DNP-primed B lymphocytes from either BALB/c or A/J donors. Secondary challenge is given immediately thereafter.

As shown by the top half of Fig. 1 (groups I–IV), both BALB/c and A/J DNP-specific B cells are effectively "helped" by BGG-specific T cells of $CAF_1$ origin in developing secondary adoptive anti-DNP antibody responses to DNP-BGG. Similarly, GLT-primed $CAF_1$ T cells cooperate very well with B cells from BALB/c donors in response to DNP-GLT (cf. groups V and VI). In marked contrast, however, is the failure of these same GLT-specific $F_1$ T cells to serve as helper cells for DNP-specific B cells from A/J donor mice (cf. groups VII and VIII). There is no possibility that the different results obtained with BALB/c and A/J cells reflect marked functional disparities between them since the same pool of cells of respective origins were used in the cooperating mixtures in groups I–IV in which intact function was manifested in both cases. The same reasoning applies in the case of the GLT-primed $CAF_1$ cells that functioned very well as helpers for BALB/c B cells (group VI), although failing to do so for A/J cells (group VIII). Moreover, these results have been confirmed in a second independent experiment (not shown).

## DISCUSSION

In our previous reports on this issue we have dealt at length with the various possible explanations for the failure of physiologic T-B cell cooperation to occur across the major histocompatibility barrier and have concluded: (*a*) that the genetic restrictions concern the actual nature of the physiologic cooperative interactions between these cells, and (*b*) that the relevant gene or genes involved belong to the major histocompatibility complex (2, 3). The present studies extend these more general earlier conclusions by identifying precisely one genetic region of the H-2 complex, i.e., the Ir gene locus that is directly involved in the control of T-B cell interactions for immune responses controlled by the gene. In previous studies, we have demonstrated that mixtures of T and B cells from BALB/c ($H\text{-}2^d$) and A/J ($H\text{-}2^a$) donors, respectively, fail to cooperate despite the fact that these particular strains are identical at SsSlp and the entire D-end of the H-2 complex (2). We have deduced from these findings that identity, at only the D-end, of the complex is insufficient to permit effective cell cooperation. We can now state, furthermore, that disparity at the Ir gene locus is at least one contributing factor preventing effective T-B cell interaction. It is not possible to conclude, as yet, whether identity at the Ir region alone is sufficient for successful and efficient physiologic cooperation. Experiments currently underway with appropriate recombinant strains should resolve this issue. The possibility clearly exists that: (*a*) identity at the entire H-2 complex is required, or (*b*) identity at the Ir region together with genes coded for at either the K- or D-end of H-2 are sufficient. However, if this is the case, the present experiments establish that insofar as the $CAF_1$ T cell is concerned, the Ir gene cannot function with the possibly required K- or D-end genes when in the *trans* position.

The body of evidence concerned with the expression of H-linked Ir genes in immunocompetent cells has clearly shown that these genes are functionally expressed in T lymphocytes (8). Their necessary expression in B cells as well had been questioned by the observation that humoral responses controlled by Ir genes could be induced in nonresponder animals by providing appropriate T cell activity either with cells specific for an immunogenic carrier or by inducing an allogeneic effect (4, 5, 8). The experiments reported herein indicate, but do not establish, that Ir genes may also be expressed in B cells and demonstrate that their activity is concerned with successful T-B cell interactions in specific immune responses involving carrier recognition. Functionally, however, B cells can be triggered in the absence of specific T cell function under special circumstances, and alternatively, can utilize a wide variety of carrier-specific helper T cells provided there is no disparity between these cells at the Ir region.

The number of specific H-linked Ir genes that have been identified and the specific manner in which they permit immune responses to distinct antigens to take place, particularly at the T cell level, has suggested that they are involved

in the specificity of the T cell antigen receptors and may, therefore, be clonally expressed in this class of lymphocytes. The results of our experiments clearly indicate that as far as B cells are concerned, if the Ir gene required for T-B cell interactions is indeed expressed in such cells, their products and functional roles are not clonally restricted.

However, an alternative explanation for our results is that GLT-activated $F_1$ T cells, under control of the relevant Ir gene on one of its H-2 alleles, is limited to effective cooperation only with the B lymphocytes of the parent expressing the histocompatibility specificities coded for by the allele possessing the GLT Ir gene. According to this alternative, there would be no requirement for the expression of the Ir gene product in the B cell, but only a requirement for the Ir gene product from the T cell to govern the interaction with the B cell at the histocompatibility site on the B cell surface. This alternative would, however, require that identity at either K alone or K- and D-end of the H-2 complex exist for successful cooperation to occur. A choice between the two alternatives discussed above would require, as stated earlier, a determination of whether the entire H-2 complex or restricted regions are involved in T-B cell cooperation.

We have previously postulated that B lymphocytes possess a genetically controlled surface membrane site capable of accepting the active product secreted by T lymphocytes specifically activated by antigen (2, 3). The present results indicate the importance of Ir gene products in the mechanism of T and B cell interaction. If, as we suspect, the histocompatibility molecule coded at the K- or D-end of the H-2 gene complex is also involved, the regulation of B cell function by T cells would require an effect on the B cell membrane at or near the site of the surface histocompatibility molecule. This, moreover, would explain the highly efficient action of allogeneic T cells on antigen-activated B cells.

## SUMMARY

In the present study we have asked the question of whether $F_1$ carrier-primed T cells can serve as helper cells for either or both parental B cells when (*a*) the carrier molecule employed is under genetic control such that one parental strain is a responder and the other is a nonresponder, and (*b*) the determinant specificity of the parental B cells being assessed is not under genetic control and bears no relationship to the specificity of the carrier molecule. Utilizing the system of immune response gene control of responses to the terpolymer L-glutamic acid-L-lysine-L-tyrosine (GLT) to which A strain mice ($H\text{-}2^a$) are nonresponders, whereas BALB/c ($H\text{-}2^d$) and (BALB/c × A)$F_1$ hybrids ($CAF_1$) are responders, these studies demonstrate that GLT-primed T cells of $CAF_1$ donors can provide for responder BALB/c, but not for nonresponder A/J, the required stimulus for the anti-DNP responses of DNP-specific B cells of these respective parental strains to the DNP conjugate of

GLT. The implications of these findings for Ir gene function in physiologic T-B cell interactions are discussed in detail.

We are grateful to Ms. Mary Graves, Mr. Michael Moran, and Ms. Melissa Varney for expert technical assistance and to Ms. Candace Maher for excellent secretarial assistance in the preparation of the manuscript.

## REFERENCES

1. Hamaoka, T., D. P. Osborne, Jr., and D. H. Katz. 1973. Cell interactions between histoincompatible T and B lymphocytes. I. Allogeneic effect by irradiated host T cells on adoptively transferred histoincompatible B lymphocytes. *J. Exp. Med.* **137:**1393.
2. Katz, D. H., T. Hamaoka, M. E. Dorf, 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.
3. 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.**
4. Katz, D. H. 1972. The allogeneic effect on immune responses. Model for regulatory influences of T lymphocytes on the immune system. *Transplant Rev.* **12:**141.
5. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15:**1.
6. Katchalski, E., and M. Sela. 1958. Synthesis and chemical properties of poly-$\alpha$-amino acids. *Adv. Protein Chem.* **13:**243.
7. 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.
8. Benacerraf, B., and H. O. McDevitt. 1972. The histocompatibility-linked immune response genes. *Science (Wash. D.C.).* **175:**273.