

Brief Definitive Reports

CELL INTERACTIONS BETWEEN HISTOINCOMPATIBLE T AND B LYMPHOCYTES

VI. COOPERATIVE RESPONSES BETWEEN LYMPHOCYTES DERIVED FROM MOUSE DONOR STRAINS DIFFERING AT GENES IN THE S AND D REGIONS OF THE *H-2* COMPLEX*

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Previous studies from our laboratory have demonstrated a requirement for identities among genes in the major histocompatibility complex for the most effective and efficient cooperative interactions to occur between carrier-specific helper T lymphocytes and hapten-specific B lymphocytes in the development of antibody responses (1-5). Although, these studies have demonstrated the involvement of histocompatibility gene loci in such cell interactions, the precise gene or combination of genes have yet to be elucidated. To accomplish this necessitates the use of lymphocytes from appropriate mixtures of inbred and recombinant strains and a thorough analysis of which gene identities permit and, conversely, which gene differences prevent successful cooperative cell interactions. The experiments presented in this paper establish conditions for performing such analyses in a totally in vitro system in which minimal numbers of donor animals are needed, and, moreover, demonstrate that successful cooperative interactions occur in this system between T and B lymphocytes sharing gene identities in the K and I regions but differing at gene loci in the S and D regions of the *H-2* complex.

Materials and Methods

The proteins, reagents, and preparation of hapten-protein conjugates were described in previous reports (1, 6); 2.1×10^{-7} mol of DNP/mg of *Ascaris suum* (DNP_{2.1}-ASC)¹ and 14 mol of DNP/100,000 daltons of keyhole limpet hemocyanin (DNP₁₄-KLH)² were employed in these studies. The preparation of anti- θ serum, its characterization, and the method of anti- θ serum treatment of spleen cells are described elsewhere (7). Inbred A/J (*H-2^a*),

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² KLH was obtained from the Pacific Bio-Marine Supply Co., Venice, Calif.

congenic B10.A ($H-2^a$), and B10.BR ($H-2^b$) mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. Mice were immunized intraperitoneally at 3–5 mo of age with either 10 μg of DNP-ASC in 4 mg aluminum hydroxide gel (alum) or 10 μg of KLH in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) and then used as spleen cell donors 2–6 mo later.

Spleen cell cultures were established in a modification of the Mishell-Dutton system (8) using microtiter culture plates as described previously from this laboratory (9). Culture wells contained a total vol of 0.3 ml consisting of $1.0\text{--}2.0 \times 10^6$ cells (i.e., final density of $3.3\text{--}6.7 \times 10^6$ cells/ml). Antigen-stimulated cultures received 0.1 μg of either DNP-ASC or DNP-KLH/individual well. Cultures were established in triplicate and assayed after 4 days for IgM and IgG anti-DNP plaque-forming cells (PFC) as previously described (10), using 2,4,6-trinitrophenyl hapten derivatized SRBC (TNP-SRBC) as indicator cells (11).

RESULTS

In order to minimize the possibility of a complicating nonspecific stimulation due to an allogeneic effect (12) and to eliminate any contribution of B cells in the carrier-primed spleen cell population to the antibody response, donors of carrier-primed cells (and normal controls) were irradiated with 700 R 1–2 h before sacrifice. We have previously shown that cells from such carrier-primed mice provide ample specific helper T-cell function *in vitro* while failing to manifest any B-cell activity (1).

Two experiments will be presented in this study: In the first (Fig. 1), DNP-ASC-primed A/J and B10.BR spleen cells were cultured either alone or with irradiated KLH-primed spleen cells from A/J, B10.A, or B10.BR donor mice as shown on the left side of Fig. 1. The gene regions of the $H-2$ complexes are symbolized and the gene region differences among the various combinations are summarized for convenience. Cells were cultured either with no antigen, DNP-ASC, or DNP-KLH. Both the A/J and B10.BR DNP-primed cell populations developed substantial secondary responses to the immunizing antigen, DNP-ASC, and failed to respond to DNP-KLH in the absence of additional KLH-primed helper cells. In contrast, both B-cell populations responded to DNP-KLH when KLH-primed helper cells were added; particularly important is the fact that T and B cells of B10.BR and A origin effectively cooperated despite the fact that these cell combinations differ at genes in the S and D regions of the $H-2$ complex.

The second experiment (Fig. 2) includes relevant controls to rule out any allogeneic effects consisting of: (a) anti- θ serum plus complement-treated DNP-ASC-primed B cells, and (b) irradiated unprimed spleen cells. Again, neither A/J nor B10.BR B cells responded to DNP-KLH unless KLH-primed helper cells were also present and reciprocal combinations of these primed B- and T-cell populations developed good cooperative secondary anti-DNP responses (cultures IV and VIII). The failure to observe responses in reciprocal combinations of DNP-primed B cells and unprimed T cells (cultures III and VII) rules out a nonspecific allogeneic effect.

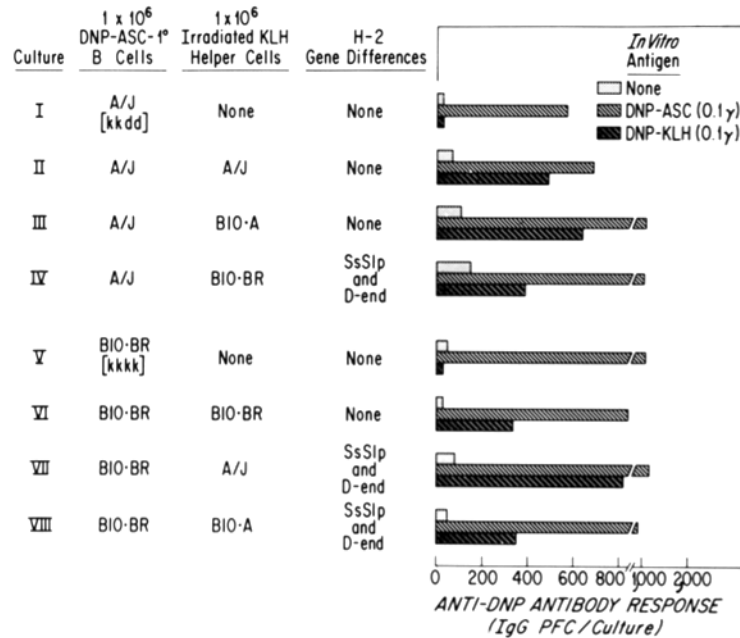


FIG. 1. Spleen cells from A/J (cultures I-IV) and B10.BR (cultures V-VIII) mice primed 2 mo earlier with DNP-ASC in alum were cultured either alone or together with spleen cells from A/J, B10.A, or B10.BR mice primed 2 mo earlier with KLH in CFA as indicated. KLH-primed donors were irradiated with 700 R 1-2 h before sacrifice. Cells or cell combinations were cultured either without antigen or with DNP-ASC or DNP-KLH as shown. IgG (indirect) DNP-specific PFC responses after 4 days in culture are shown. Responses in the IgM class (not shown) were parallel but somewhat lower.

DISCUSSION AND SUMMARY

In our initial studies on the question of histocompatibility requirements in T-B-cell interactions, we found that no cooperation occurred with mixtures of T and B cells from BALB/c ($H-2^d$) and A/J ($H-2^a$) donors, respectively (1). These particular strains are identical for genes in the S and D regions of the $H-2$ complex but possess major differences at the K-end. Many differences are known to exist in the I region as well. Thus, these early data indicated that gene identities only at the D-end are insufficient to permit optimal cooperative interactions to occur under these conditions.

The present experiments were designed to ask the reciprocal question, namely, whether gene identities at only the K and I regions are sufficient to allow effective T-B-cell cooperation. The development of cooperative responses between A/J and B10.BR which differ for genes in S and D regions but are identical for K and I region genes indicate that the critical genes involved in T-B-cell interactions exist in the latter regions. It must be stated, however, that these results should be considered with some degree of caution until fully corroborated

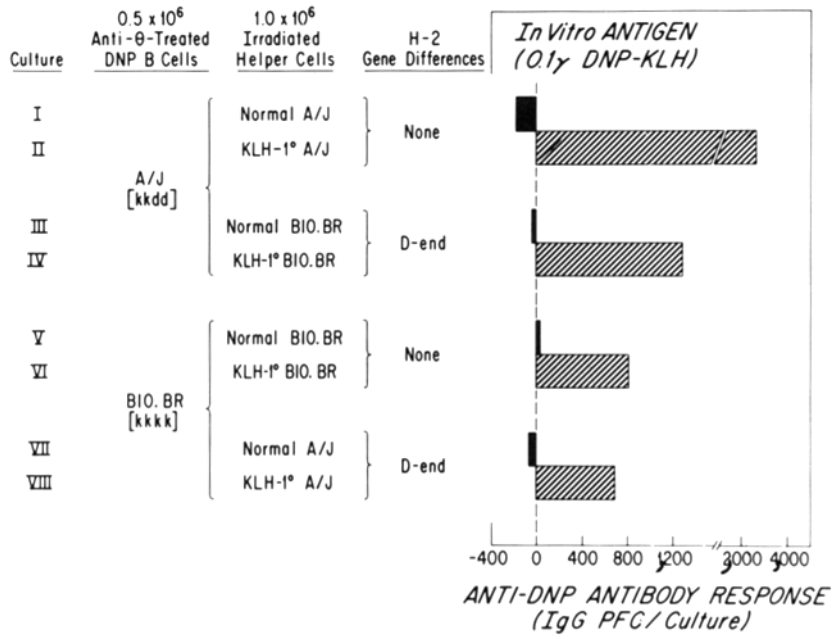


FIG. 2. DNP-ASC-primed spleen cells from A/J and B10.BR donors were depleted of T cells by *in vitro* treatment with anti- θ serum plus complement and then cultured with either irradiated normal or KLH-primed spleen cells in the combinations indicated. Primed donors were immunized with either DNP-ASC in alum or KLH in CFA 6 mo earlier and then boosted with 25 μ g of the respective antigen in saline 1 mo before culture. Cells were cultured with either no antigen (not shown) or DNP-KLH. The background responses of nonstimulated cultures have been subtracted from the numbers of DNP-specific PFC developed in cultures containing DNP-KLH, hence the negative values depicted here. IgG (indirect) DNP-specific PFC responses are shown. Responses in the IgM class (not shown) were parallel.

in an *in vivo* system. Furthermore, these data should not be construed to mean that identities of genes in both the K and I regions are required for effective cell interaction. Thus, we know from previous studies that under certain circumstances a difference in *Ir* genes can prevent effective cooperation when the carrier antigen employed is the one to which the *Ir* gene control is directed (3). However, since the latter studies were, by necessity, performed with combinations of F₁ T cells and parental B cells, it is alternatively possible that only genes other than *Ir* but associated with the responder parental histocompatibility allele are stimulated to participate in the response (5). Similarly, it must be stressed that the response to DNP-KLH employed in the present experiments is not governed by known *Ir* gene differences among the A/J and B10.BR strains. The existence of identities of K region genes in this combination could well be insufficient for effective cooperation to occur under circumstances where the carrier used is one to which the response is controlled by an *Ir* gene that is present in one but not the other. Further definition of these possibilities requires additional experimentation of the type presented here.

The identity at the I region of the *H-2* complex required for physiologic T- and B-cell cooperation may be interpreted to indicate that the surface

molecules on T and B cells responsible for this phenomenon are coded for in this region. The recent finding that alloantisera against lymphocytes prepared between congenic mice which differ at the I region react with B and T cells (13-15) is consistent with the hypothesis that these antisera are specific for surface molecules concerned with cooperative interactions between these cells.

It is essential to emphasize the point that what these series of experiments demonstrate is that certain identities of *H-2* genes are required for the most effective physiologic T-B-cell interactions; such identities may not constitute an absolute requirement for T-B-cell interactions to occur under certain circumstances. Thus, Bechtol et al. (16) have observed that tetraparental mice derived from responder and nonresponder strains may produce antibodies of nonresponder allotype under conditions of hyperimmunization. More recent studies from our own laboratories by Benacerraf et al. (17) have demonstrated that in an *in vitro* system, (responder \times nonresponder) F_1 T cells primed to random synthetic terpolymer of L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT), which fail to provide helper function for nonresponder B cells in response to GAT added to cultures in free soluble form, will "help" such B cells when the GAT is added to the cultures in small quantities attached to macrophages. However, as presented elsewhere (5), DNP derivative of the random synthetic terpolymer of L-glutamic acid⁵⁷-L-lysine³⁸-L-tyrosine⁵ on macrophages failed to elicit a cooperative anti-DNP response between F_1 responder T cells and parental nonresponder B cells *in vivo*. Finally, it should be reiterated that as seen in the allogeneic effect, activated allogeneic T cells provide the necessary stimulus for triggering antigen-activated B cells if the specificity of the T cell is directed against surface alloantigen differences (12).

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