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*

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This series of papers have dealt with studies performed over the past 2 ½ years on the requirement for identities among certain genes in the major histocompatibility complex of the mouse for optimal cooperative interactions to occur between carrier-specific helper T lymphocytes and hapten-specific B lymphocytes in the development of antibody responses (1–5). Heretofore, these studies have enabled us to determine that the relevant genes involved in such interactions were located in the K end of the *H-2* complex, i.e. in the *K* and/or *I* regions (5, 6). The existence of appropriate inbred and recombinant strains of mice differing at known loci of the *H-2* gene complex makes it possible to define more precisely which region(s) and/or subregion(s) of the complex contain(s) the gene or genes controlling T-B-cell interactions. The experiments presented in this paper demonstrate that such genes are located in the I region of the *H-2* complex in that mixtures of cells from strains differing at genes in the *K*, *S*, or *I* regions but sharing gene identities in the *I* region will develop effective cooperative interactions whereas, conversely, mixtures of cells differing at genes in the *I* region fail to interact despite the existence of gene identities elsewhere in the complex.

Materials and Methods

The proteins, reagents, and preparation of hapten-protein conjugates were described in previous reports (1, 7); 2.1×10^{-7} mol of DNP/mg of *Ascaris suum* ($DNP_{2.1}$-ASC, kindly provided by Dr. Kurt J. Bloch, Massachusetts General Hospital, Boston, Mass) and 14 mol of DNP/100,000 daltons of keyhole limpet hemocyanin (DNP_{14}-KLH), Pacific Bio-Marine Supply Co., Venice, Calif. 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 (8). Inbred A/J ($H\text{-}2^a$) mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. The congenic mice A.TL ($H\text{-}2^{a1}$), A.TH ($H\text{-}2^{a2}$), A.AL ($H\text{-}2^{a3}$), A.SW ($H\text{-}2^s$), and (A × A.TH)F_1 ($H\text{-}2^{a/t2}$) have been bred and maintained in our own animal facilities. Mice were immunized intraperitoneally with either 25 μg of

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DNP-ASC or 20 μg of KLH, both administered in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.); the spleen cells were obtained from these donors 4–5 wk after immunization.

Spleen cell cultures were established in a modification of the Mishell-Dutton system (9) using microtiter culture plates as described previously from this laboratory (10). Culture wells contained a total vol of 0.3 ml consisting of 1.0–2.0 $\times$ 10^6 cells (i.e. final density of 3.3–6.7 $\times$ 10^6 cells/ml). Antigen-stimulated cultures received 0.1 μg of either DNP-ASC or DNP-KLH per 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 (11) using 2,4,6-trinitrophenyl hapten-derivatized sheep erythrocytes (TNP-SRBC) as indicator cells (12).

Results

In order to minimize the possibility of a complicating nonspecific stimulation due to an allogeneic effect (13) and to eliminate any contribution of B cells in the carrier-primed spleen cell population to the antibody response, donors of carrier-primed cells 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, 5).

The relevant results from one experiment will be presented here although two experiments were performed independently and at different times yielding identical results. The left side of Fig. 1 depicts the protocol and various combinations of cell mixtures analyzed for cooperative responses to DNP-KLH.

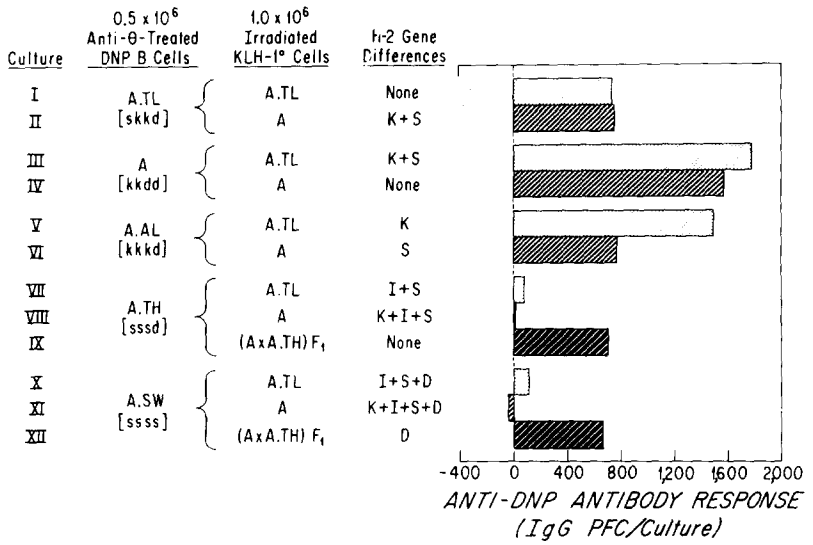

FIG. 1. DNP-ASC-primed spleen cells from A.TL, A, A.AL, A.TH, and A.SW mice were depleted of T cells by in vitro treatment with anti-θ serum plus complement and then cultured with irradiated KLH-primed spleen cells from A.TL, A, or (A $\times$ A.TH)F_1 donors in the combinations indicated. Cells were 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 value depicted in culture XI). IgG (indirect) DNP-specific PFC responses are shown. Responses in the IgM class (not shown) were parallel.

The gene regions of the *H-2* complexes are symbolized and the gene region differences among the various combinations are summarized for convenience. Before discussing the data shown in Fig. 1, it is pertinent to cite the following data from control cultures that were set up in this same experiment but are not shown in the figure: (*a*) All of the DNP-primed spleen cell populations were capable of developing secondary anti-DNP-PFC responses to the immunizing antigen, DNP-ASC, in parallel cultures established with 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 vitro response in the absence of additional carrier-primed cells; (*c*) The capacity of A.TH-primed cells to develop effective syngeneic cooperative interactions was not inhibited or diminished by the addition of irradiated KLH-primed spleen cells from A.TL donors thereby ruling out a possible suppression mechanism, due to e.g. an allogeneic effect, as an explanation for the findings to be described. Thus, in a syngeneic cooperative response A.TH cells developed 1253 IgG anti-DNP PFC in the absence of A.TL cells and 1486 anti-DNP PFC in the presence of 1.0×10^6 irradiated KLH-primed A.TL cells.

The relevant data is depicted on the right side of Fig. 1. Cultures I–IV demonstrate the capacity of syngeneic mixtures of T and B cells from A.TL and A mice to cooperate in vitro, and reciprocal mixtures of such cells to interact together despite the existence of gene differences in both the *K* and *S* regions of *H-2* in this combination. Likewise, when gene differences are restricted to only one of these respective regions (*K* or *S*) the capacity for effective T-B-cell cooperation remains intact as evidenced by the ability of T cells from both A.TL and A donors to interact with B cells from A.AL donors (cultures V and VI). In marked contrast, however, is the inability of either of these primed and functionally intact T-cell populations from A.TL or A donors to cooperate with B cells from either A.TH or A.SW mice (cultures VII, VIII, X and XI); in the latter combinations gene differences exist in the *I* region as indicated. The failure of either A.TH or A.SW DNP-primed B cells to respond in these cultures is not a reflection of an incapacity of these B cells to function since irradiated KLH-primed cells from (A $\times$ A.TH)F_1 donors were able to provide helper function for A.TH parental cells (culture IX) and for A.SW cells which differ only at genes in the *D* region (culture XII).

Discussion and Summary

The results of this study provide compelling evidence for the existence of the gene or genes controlling optimal T-B-cell cooperative interactions in the designated *I* region of the *H-2* gene complex. Previously, we have speculated that the relevant gene(s) involved may well be located in this region based on several observations from our earlier work in this area (3, 5, 6). Thus, in the preceding paper, we showed that T and B cells from B10.BR and A strain mice developed effective cooperative interactions in vitro to DNP-KLH in a system identical to the one reported herein. Since these mice differ for genes in the *S* and *D* regions of *H-2*

but are identical for *K* and *I* region genes, we were able to localize the critical genes to the K-end of *H-2*.

The experiment presented in Fig. 1 allows a more precise delineation of the location of the relevant gene(s) concerned with T-B-cell interactions. Thus, the capacity of T and B cells of A.TL and A origin to reciprocally interact with one another and, moreover, of T cells from such mice to effectively cooperate with B cells from A.AL mice essentially rules out the possibility that such gene(s) are located in the *K* region; the capacity for interactions to occur across *S* or *D* region gene differences reconfirms previous data on this point (1, 2, 4–6). On the other hand, the failure of T cells from A.TL or A donors to cooperate with B cells of A.TH or A.SW mice demonstrates the necessity for gene identities in the *I* region to permit effective T-B-cell interactions. This point is particulary emphasized by the inability of A.TL to provide helper T-cell function for A.TH or A.SW B cells in which combinations there are gene identities in the *K* region but differences in *I* region genes.

The appropriate controls, as described in the Results section, rule out the possibilities that the failure to observe cooperative interactions between A.TH or A.SW B cells and A.TL T cells reflect either (*a*) an incapacity of either cell population to function properly, or (*b*) the existence of a suppressive allogeneic effect. We are left, therefore, with but one explanation, namely that gene identities must exist in the *I* region in order for effective cooperative lymphocyte interactions to occur.

We originally hypothesized, based on data obtained in this system, that there must exist on the surfaces of lymphocytes (and macrophages) certain molecules coded for by genes in the *H-2* complex responsible for permitting effective cell-cell interactions (1–6). These molecules, which have recently been termed by us as cell-interaction or CI molecules (15), probably are present in varying quantities on T and B lymphocytes and macrophages, and are envisaged by us as interacting by homology during the cooperative interactions between such cells. The possible mechanisms by which such molecular interactions may occur have been elaborated upon at length elsewhere (15). The data presented here together with other recent observations of Armerding et al. (16) that demonstrate the capacity of anti-Ia antiserum to absorb the biologically active molecule(s) from alloantigen-activated T-cell culture supernates (allogeneic effect factor, AEF) demonstrate the CI molecules are products of genes located in the *I* region of *H-2*.

The precise genetic mapping of CI genes will require experiments using mixtures of T and B cells from strain combinations with differences and identities at one or more subregions of the *I* region. The *I* region has been divided into three subregions, *Ir-1A*, *Ir-1B* and *I-C*, on the basis of documented crossovers between definable *Ir* genes and also Ia serological specificities (14). The data presented here and in the preceding paper indicate that the *CI* genes responsible for control of T-B cell interactions in this system are located in the *Ir-1A* and/or *Ir-1B* subregions. This follows from the fact that the A↔B.10.BR combinations in our previous report (5) and the A↔A.TL and A↔A.AL combinations in the present study, all of which are identical for all genes in *Ir-1A* and *Ir-1B*, were capable of effectively interacting despite the existence of gene differences in the *I-C* subregion. However, despite the fact that the *I-C* subregions of these strains

are derived from different paternal haplotypes, they may nevertheless conceivably share genes coded for in the *I-C* subregion which permit effective T-B-cell cooperation. Further definition of these possibilities should be forthcoming in the near future.

Finally, it should be noted that the *Ir-1A* and *Ir-1B* subregions where the *CI* genes appear to be mapped by this study are precisely the subregions where all known immune response or *Ir* genes have been mapped (17). However, as discussed at length elsewhere (15), while this suggests an association between *Ir* and *CI* gene products in lymphocyte function, it does not imply that these are necessarily products of the same gene(s).

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