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

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(Received for publication 20 February 1973)

In the preceding paper (1), experimental data were presented that demonstrated the capacity of a heavily irradiated host to exert an active allogeneic effect (2, 3) on adoptively transferred, histoincompatible, DNP-specific B lymphocytes. This finding places great restrictions on model systems used to study the possibility of cooperative interactions between carrier-primed T and hapten-specific B lymphocytes of differing histocompatibilities. For brevity, we will again refer to antigen-specific cell interactions as "physiologic" T-B cell cooperation, contrasting with the allogeneic effect.

In this report, we describe appropriate in vivo and in vitro experimental protocols designed to study physiologic cooperation between histoincompatible T and B cells under conditions that avoid the allogeneic effect. These results provide unequivocal proof that totally histoincompatible carrier-primed T lymphocytes fail to provide the required stimulus for the responses of B cells to hapten-carrier conjugates, whereas semiallogeneic T and B cells are capable of interacting effectively in the normal manner, and effect physiologic cooperation in this system.

Materials and Methods

The proteins, hapten-carrier conjugates, animals, immunization schedules, antibody determinations, and statistical analyses were identical with those described in the preceding paper (1).

Adoptive Transfer System.—The basic methodology used for preparation of spleen cell suspensions and for treatment of such cells with anti-μ serum and complement are described in the preceding paper (1). The protocol followed for double adoptive transfers in the present set of experiments is detailed in the Results section and Fig. 1.

Spleen Cell Cultures.—The Mishell-Dutton culture system was used; reagents and conditions were previously described (4). Cultures were established at spleen cell densities of 10^6-
20 × 10⁶ cells/ml. After 4 days of incubation, cells from individual dishes of triplicate cultures were assayed for IgM and IgG anti-DNP, antibody-producing plaque-forming cells (PFC)¹ using a modification of the hemolytic plaque technique (4-6). Sheep erythrocytes (SRBC) lightly conjugated with 2,4,6-trinitrobenzenesulfonic acid (TNP) were used as indicator (7). IgG PFC were developed using a rabbit antimouse immunoglobulin-facilitating serum.

**RESULTS**

**Failure of Physiologic Cooperative Interactions to Occur between Histoincompatible T and B Lymphocytes.—**

*In vivo:* The experiments presented in detail in the preceding paper (1) clearly demonstrated a major drawback that must be recognized and dealt with in any attempts to ask the question concerning physiologic T-B cell interactions across major histocompatibility specificities. The surprising finding that a heavily irradiated host can exert an allogeneic effect on adoptively transferred, histoincompatible, primed B lymphocytes made it mandatory to find a way to circumvent this obstacle. We approached this problem by establishing conditions for obtaining physiologic cooperation between syngeneic T and B lymphocytes in a semiallogeneic, irradiated recipient genetically incapable of reacting against histocompatibility specificities of either parental strain lymphocytes.

The protocol is schematically illustrated in Fig. 1. 50 × 10⁶ spleen cells from either bovine gamma globulin (BGG)-primed or normal parental donor mice were injected intravenously into nonirradiated, unprimed, (BALB/c × A/J) F₁ (CAF₁) hybrid recipients. 24 h later, when the transferred cells had migrated to the lymphoid organs, these mice were irradiated (600 R) and then injected intravenously with a second cell inoculum consisting of 20 × 10⁶ DNP-keyhole limpet hemocyanin (KLH)-primed anti-θ serum plus complement-treated spleen cells (i.e., B lymphocytes) derived from the same or the other parental strain. Immediately thereafter secondary challenge was performed with 50 μg of DNP-BGG intraperitoneally in saline and the mice were bled 7 days later. This experiment is always performed in a simultaneously symmetrical fashion to alleviate potential variability between different pools and strain origins of carrier-primed and DNP-primed donor cells.

The results of this experiment are shown in Fig. 2. Groups I-IV are controls that verify the cooperative functional capacities of the irradiated (*in situ*), BGG-primed and the anti-θ-treated, DNP-primed cells of BALB/c and A/J origin in their respective totally syngeneic combinations (including recipients). Additional controls (not shown) confirmed the efficacy of anti-θ treatment in that such T cell-depleted populations failed to respond to DNP-KLH in unprimed, irradiated recipients. Groups V and VI demonstrate that BALB/c syn-

1¹ Abbreviations used in this paper: BGG, bovine gamma globulin; CAF₁, (BALB/c × A/J) F₁ hybrid mice; CFA, complete Freund’s adjuvant; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TNP, 2,4,6-trinitrobenzenesulfonic acid.
geneic BGG-primed (T) and DNP-primed (B) cell populations cooperate quite effectively within the environs of a CAF₁ irradiated recipient. Similarly, A/J syngeneic T and B lymphocytes interact very well with one another in the F₁ host (groups VII and VIII). In striking contrast, however, is the very obvious failure of the BCG-primed T cells from histoincompatible A/J donor mice to effectively cooperate with B cells derived from BALB/c mice (group X), and vice versa (group XII). The complete absence of a complicating allogeneic effect in this particular system is demonstrated by the lack of responses in control groups IX and XI (receiving normal allogeneic cells before irradiation) that are not significantly different from those in control groups V and VII (receiving normal syngeneic cells before irradiation). The reason for the considerably higher cooperative secondary responses of syngeneic T and B cells in the CAF₁ recipients (groups VI and VIII) than in their respective syngeneic recipients (groups II and IV) is not yet resolved, but may reflect increased general proliferation effects on cells residing in an irradiated, semiallogeneic host.

*In vitro:* The preceding experiment demonstrated clearly a failure of physio-
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Fig. 2. Failure of physiologic cooperative interactions to occur between histoincompatible T and B lymphocytes in vivo. The scheme followed is outlined in Fig. 1. The donor cell-recipient strain combinations are indicated. Mean serum anti-DNP antibody levels of groups of five mice on day 7 after secondary challenge with 50 μg of DNP-BGG are illustrated. Vertical bars represent the range of the standard errors. Statistical comparisons between the various groups gave the following results: groups I and II, groups III and IV, groups V and VI, and groups VII and VIII, 0.005 > P > 0.001 in all cases; groups IX and X, 0.30 > P > 0.20; groups XI and XII, 0.40 > P > 0.30; groups V and IX, 0.70 > P > 0.60; groups VII and XI, 0.70 > P > 0.60; groups II and VI, 0.025 > P > 0.02; groups IV and VIII 0.005 > P > 0.001; groups I and V and groups III and VII, 0.40 > P > 0.30 in both cases.

logic cooperation between histoincompatible T and B lymphocytes in secondary responses in vivo. This observation was confirmed in in vitro secondary responses to soluble, DNP-primed conjugates with a culture system that has been partially characterized in a previous report (8). Two types of experimental approaches have been used. In the first, DNP-KLH-primed BALB/c spleen cells were depleted of T lymphocytes by treatment with anti-θ serum plus complement and then cultured with KLH-primed T lymphocytes from syngeneic (BALB/c) or allogeneic (A/J) donors in the presence of DNP-KLH. The KLH-specific helper T cells were obtained from primed mice (100 μg in complete Freund's adjuvant [CFA] 4 wk earlier) that had been irradiated (800 R) several hours before their spleens were removed. Preliminary experiments established that cells exposed to this dose of X irradiation could function effectively as
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helper cells in vitro and, moreover, that no demonstrable allogeneic effect resulted from their incorporation into cultures with histoincompatible, DNP-primed B cells.

The results of this experiment are illustrated in the upper panel of Fig. 3. Untreated BALB/c DNP-KLH cells (group I) developed very good secondary responses to soluble DNP-KLH (1 μg/culture) whereas the exposure of such

![Table](image)

Untreated BALB/c DNP-KLH cells (group I) developed very good secondary responses to soluble DNP-KLH (1 μg/culture) whereas the exposure of such

![Diagram](image)

FIG. 3. Failure of physiologic cooperative interactions to occur between histoincompatible T and B lymphocytes in vitro. See text of Results for details of the protocol. 10 X 10^6 cells of each type were used. Geometric mean IgG anti-DNP PFC responses of three cultures to 1 μg/culture DNP-KLH are illustrated. Vertical bars represent ranges of the standard errors. IgM PFC responses in these cultures (not shown) varied in parallel with the IgG responses. Statistical comparisons of the various cultures gave the following results: groups I and II, and groups II and III, 0.001 > P in both cases; groups III and IV, 0.005 > P > 0.001; groups V and V1 and groups V1 and V1, 0.005 > P > 0.001 in both cases; groups V and VII, 0.90 > P > 0.80.

cells to anti-0 serum plus complement virtually abolished this response (group II). The response of the anti-0-treated cells was adequately restored by the addition of syngeneic, irradiated, KLH-primed helper cells (group III), whereas allogeneic, A/J, KLH-primed cells failed to restore the in vitro response to DNP-KLH (group IV), although the same irradiated, A/J, KLH-primed cells were perfectly capable of restoring responses of syngeneic, A/J, DNP-primed B cells (not shown).

A second approach was to use spleen cells from DNP-BGG-primed A/J donor mice, which ordinarily are unable to develop secondary responses to DNP-KLH in vitro (group V) (as well as in vivo), and to assess the capacity of either syn-
geneic or allogeneic (BALB/c) irradiated, KLH-primed T cells to cooperate effectively with the DNP-BGG lymphocytes in response to DNP-KLH. Once again, as shown in the lower panel of Fig. 3, effective cooperative interactions occurred only between syngeneic T and B cells (group VI) and not between histoincompatible mixtures of such cells (group VII).

Successful Physiologic Cooperative Interactions between Parental T and F<sub>1</sub> Hybrid B Lymphocytes or between F<sub>1</sub> Hybrid T and Parental B Lymphocytes.—The failure of physiologic cooperation to occur between T and B lymphocytes from allogeneic strains may reflect the effect of any of a number of variables in the system. One important question concerns whether the mere presence of a completely foreign histocompatibility antigen on the surface of the cell may itself serve as a "block" of some sort to cell interactions. This question was approached in reciprocal experiments in which, on the one hand, CAF<sub>1</sub> BGG-primed cells were tested for their capacity to help T cell-depleted, DNP-KLH primed parental cells, and, on the other hand, parental helper cells were tested for their capacity to interact with CAF<sub>1</sub>, DNP-primed B cells.

The first of these experiments is shown in Fig. 4. Groups I and II and groups III and IV demonstrate the positive control cooperative responses obtained with CAF<sub>1</sub> and A/J cell populations, respectively, in their totally syngeneic combination. Likewise, group VII illustrates the effectiveness of T cell depletion of the anti-0-treated A/J population as compared with its untreated control (group VIII) in response to DNP-KLH. In the experimental groups, when anti-0-treated, A/J, DNP-primed cells are adoptively transferred to irradiated CAF<sub>1</sub> recipients a good cooperative response to DNP-BGG is obtained provided CAF<sub>1</sub> BGG-primed cells are present (group VI). The possibility of an allogeneic effect is ruled out by the lack of response in group V recipients.

The reciprocal experiment is illustrated in Fig. 5. Once again, the positive control cooperative response to DNP-BGG in the totally syngeneic CAF<sub>1</sub> system is demonstrated by comparing groups I and II. The same is true in the syngeneic A/J combination (groups V and VI). (The rather strong difference in magnitude of anti-DNP antibody production between the CAF<sub>1</sub> and A/J DNP-primed cells reflects the difference in affinity of the respective cell populations used, in part because of time of priming, and in part because CAF<sub>1</sub> mice routinely develop antibodies of relatively higher affinity than A/J mice.) In corroboration of the implications of the preceding experiment (Fig. 4), DNP-KLH-primed CAF<sub>1</sub> cells can develop secondary responses to DNP-BGG with the help of parental, BGG-specific T cells (group IV) that are comparable in magnitude to those obtained with syngeneic helper cells (group II). Moreover, no appreciable allogeneic effect contributes, under these circumstances, to this result as shown by the absence of a response in group III recipients. In other experiments (not presented) physiologic cooperative interaction was also demonstrated between parental BALB/c helper T cells and CAF<sub>1</sub>, DNP-specific B cells in their response to DNP-KLH.
FIG. 4. Demonstration of physiologic cooperative interactions between T and B lymphocytes sharing one major histocompatibility specificity in common and differing by another using F1 hybrid helper T cells with parental B cells. The scheme outlined in Fig. 1 was followed. The donor cell-recipient strain combinations are indicated. Recipients in groups I-VI were secondarily challenged with 50 μg of DNP-BGG. Groups VII and VIII received 20 μg of DNP-KLH. Mean serum anti-DNP antibody levels of groups of six mice on day 7 after secondary challenge are illustrated. Vertical bars represent ranges of the standard errors. Statistical comparisons of the various groups gave the following results: groups I and II, 0.01 > P > 0.005; groups III and IV, 0.001 > P; groups V and VI, 0.005 > P > 0.001; groups VII and VIII, 0.001 > P.

DISCUSSION

In the studies presented in this paper, several approaches have been used to answer the question of physiologic cooperative interactions between histoincompatible T and B lymphocytes in humoral immune responses. The experimental schemes were designed specifically to circumvent the possible contribution to the results of a complicating allogeneic effect (2, 3) based on the unexpected observations presented in the preceding paper (1). This was accomplished for in vivo cell transfer studies by using an F1 hybrid host as the recipient of limited numbers of carrier-primed T lymphocytes from one parent (irradiated in situ after transfer) and DNP-primed B lymphocytes (depleted of T cells by anti-θ treatment) from the opposite parental strain. Since the F1 host is genetic-
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Fig. 5. Demonstration of physiologic cooperative interactions between T and B lymphocytes sharing one major histocompatibility specificity in common and differing by another using parental helper T cells with F1 hybrid B cells. The scheme outlined in Fig. 1 was followed. The donor cell-recipient strain combinations are indicated. All recipients were secondarily challenged with 50 µg of DNP-BGG. Mean serum anti-DNP antibody levels of groups of six mice on day 7 after secondary challenge are illustrated. Vertical bars represent ranges of the standard errors. Statistical comparisons of the various groups gave the following results: groups I and II, groups III and IV, and groups V and VI, 0.005 > P > 0.001 in all cases.

ally incapable of reacting against either parental donor cell population, and since the irradiated carrier-primed parental cells are present in restricted numbers, the allogeneic effect has been avoided. Furthermore, an additional explanation for the absence of allogeneic effects in our experiments, is the fact that the transferred, parental, DNP-specific B cells in the F1 recipient, which would be the potential target for the allogeneic effect, constitute a small proportion of the cells against which the T cells from the second parent can react in the F1 environment.

Under the conditions discussed above, very good T-B cell cooperative interactions occurred between T and B lymphocyte populations derived from syngeneic donors, whereas no cooperative response was obtained when T cells were derived from one parental strain and B cells from the other (Fig. 2). These results confirm and extend the observations in nude mouse backcrosses reported recently by Kindred and Shreffler (9).

The failure of histoincompatible T and B cells to cooperate in vivo was corroborated in a totally in vitro system as well. Thus, DNP-primed B cells from BALB/c (H-24) mice developed good secondary anti-DNP antibody responses in vitro to soluble DNP-KLH when cultured in the presence of irradiated, KLH-primed T cells derived from syngeneic donors but not from allogeneic A/J
This was also found to be the case when a reciprocal combination of B and T cells from the two strains was used. The possibility that the results obtained might reflect an inherent incapability of one or the other cell population to function in vitro was ruled out by testing and verifying the capacity of each reciprocal population to develop cooperative responses with its syngeneic counterpart. It should be pointed out here that the BALB/c H-2^a and the A/J H-2^k strains used in our studies are identical at the Ss, Slp and the entire D-end region of the H-2 complex. However, they display major differences in specificity at the K-end locus.

There are several possible explanations for the failure of physiologic T-B cell cooperation to occur across a major histocompatibility barrier. One trivial possibility is that histoincompatible T or B cells fail to migrate normally to the appropriate sites in the lymphoid organs in vivo where such interactions normally take place. This possibility has been virtually ruled out in the present experiments because: (a) the F_1 hybrid of two parental strains was used as a more or less “neutral” environment in which very good cooperative interactions could be obtained between fully isogeneic cell mixtures, and (b) the failure of physiologic cooperation between allogeneic T and B cells to occur in vitro, as shown herein, could not possibly be reconciled in this way. Moreover, in the experimental designs used the possibility of rejection of one or the other cell type has been eliminated as a contributing factor.

It is conceivable, at the outset, that the presence of a foreign major histocompatibility specificity on the cell surface of one or the other lymphocyte class may in itself serve as a “block” of some sort to cell-cell interaction. This possibility immediately implies the requirement, under normal circumstances, for T and B cells to establish extremely close proximity or even membrane-membrane contact to effect the necessary cooperative response. Whereas, in fact, this may well be the case (see below), evidence has been presented here that argues strongly against the possibility of the mere presence of a foreign cell surface antigen effectively “repelling” either close contact or any other necessary feature involved in the cooperative interaction. Thus, excellent cooperative interactions were obtained between T and B cells derived from parental and F_1 hybrid donors used in reciprocal combinations (Figs. 4 and 5). Since in each such combination the respective donor cells shared one major histocompatibility specificity in common and differed by one other, the circumstances existed whereby a repelling force would be readily manifested by failure of such cells to interact. The fact that they did interact demonstrated that putative cell-cell repulsion is not a likely explanation for the failure of totally histoincompatible cells to interact, and that, likewise, the presence of one major common haplotype is sufficient for effective interaction to occur. The observation of Kindred and Shreffler (9) that F_1 thymocytes restored responses of nude mice to SRBC only in some instances but not others probably reflects an additional undefined variable in their system that is difficult to control.
Another possible explanation for failure of cooperative responses to occur across major histocompatibility barriers concerns the role of the macrophage. One might argue that macrophages, which participate in antigen presentation to either one or both lymphocyte classes, must be totally histocompatible for this effective macrophage-lymphocyte interaction to occur. There is good reason to believe that in mouse systems this possibility is invalid based on the following evidence: (a) The experimental design used in the present studies depended upon the F1 hybrid mouse as a neutral environment for adoptively transferred parental cells. Since the major macrophage component was indeed provided by the irradiated recipient, these cells shared major H-2 antigens in common with both parents. Moreover, the intact cooperative responses obtained in such hosts when isogeneic T and B cells from either parent were transferred verify the intact capacity of F1 macrophages to participate (if necessary) with parental lymphocytes. This, of course, does not exclude the remote possibility that it was parental macrophages from the donor cell inoculum that played the predominant macrophage role. (b) The latter point of whether parental macrophages performed the major role would not seriously influence the argument at any rate. Quite recently, Katz and Unanue (8) provided evidence that, in vitro, macrophages from allogeneic A/J donors were as effective as those from BALB/c donors in presenting DNP-KLH to BALB/c T and B lymphocytes in the elicitation of secondary anti-DNP antibody responses. The same was true in the reciprocal combination (8). Hence, it does not seem possible that any defect in physiologic T-B cell cooperation across H-2 differences can be ascribed to activity of macrophages.

We must conclude, therefore, that the genetic restrictions for physiologic cooperation between T and B cells in the immune response concern the interaction of these cells with each other. In this respect we must emphasize that what has been precisely demonstrated in this study is the necessity for T and B cells to share one or more genes in common for effective antigen-mediated physiologic cooperation. These findings, together with the observations of Kindred and Shreffler (9) referred to earlier, suggest strongly, but by no means establish, that the gene or genes that condition this cooperation belong to the major histocompatibility system of the mouse. However, since the methodology of studying the genetic requirements for T cell and B cell physiologic interaction has been developed in this study, the use of available mouse strains congenic for the H-2 region and/or recombinant strains for this region should permit a rapid clarification of this issue. If, as suspected, the H-2 region is directly concerned with the phenomenon of T-B cell cooperation, the identification of the genetic region concerned with H-2 (Iγ or K and D) should be a simple matter.

However, we do not need to identify the gene or the gene product concerned with physiologic T-B cell interaction to be able to evaluate the significance of the need for common gene products on T and B cells for physiologic cooperation to be effective in the immune response to hapten-protein conjugates. We must re-examine our understanding of T-B cell interactions in the light of this new find-
ing. The failure of physiologic cooperation to occur when major genetic differences exist in the \textit{H-2} complex must indicate something of critical importance concerning the mechanism by which such interactions occur. We have recently reviewed the substantial evidence supporting the concept that the activated T lymphocyte exerts an active regulatory influence on the B lymphocyte response to antigen (3). One of the ways this might occur is via the release of T cell-produced mediators that act in some undefined way to influence B cell triggering by antigen (3).

This view must now take into account that the most effective T-B cell cooperation, particularly in vivo, requires that the determinants stimulating T and B cells respectively be on the same molecule as in hapten-protein conjugates. In addition, our present understanding indicates that the allogeneic effect, which must be considered one of the most effective ways by which T cells may stimulate B cells to respond to antigen, requires the direct interaction of the allogeneic T cell with the antigen-specific B cell at the time it binds antigen (2, 10). In both of these models, effective triggering of B cells by antigen involves, therefore, the independent but concomitant reaction of antigen with B cells and of T cells with B cells at an extremely close range.

Whether membrane-membrane contact, mediator release, or both are involved, the present data taken collectively with the observations of Kindred and Shreffler (9) provide compelling evidence that a site on the B cell membrane closely related to the histocompatibility specificity is critically involved in physiologic T-B cell cooperation. It is attractive to consider that there exists on the B lymphocyte surface membrane an "acceptor" molecule either for the putative active T cell product or for the T cell itself. The necessity for the T and B cells to possess the same gene or genes for physiologic cooperation requires that the same product be expressed in both cells for this purpose, or alternatively, if two gene products are expressed in the respective cell types, that the genes concerned have remained closely linked.

By this reasoning, the sequence of events surrounding the actual T-B cell interaction could proceed as follows: The antigen-activated T lymphocyte, in close proximity to the appropriate B cell, either engages direct contact at the specific acceptor site(s) on the B cell surface and/or releases active product(s) that have specificity for, and bind to, the specific acceptor sites on the B lymphocyte. The B cell, it would seem, already has antigen bound by its specific surface Ig receptors before the relevant interaction with T cells. Indeed, as we have previously suggested (8), the close proximity demanded may be effected by virtue of B cells presenting antigen to T lymphocytes of corresponding specificity. Subsequent biochemical events concerned with actual triggering of the B lymphocyte follow these surface events. Similarly, suppressive T cell regulatory effects might be explained in the context of this scheme if we assume that saturation of T acceptor sites by an active mediator (i.e., produced in quantitative excess) transiently prevents triggering (but does not specifically tolerize).

The scheme described above is consistent with all of the known phenomen-
ology of in vivo T and B cell interactions (3). While certain inconsistencies exist in terms of recent in vitro phenomenology (2, 11–13), we believe that the relative significance of these latter observations to what in fact occurs in vivo has yet to be proven. One immediate question can be raised about how the in vivo allogeneic effect fits into this framework since the T and B cells involved in this phenomenon are necessarily histoincompatible. The point here is that by very reason of their histoincompatibility these cells must be brought together into intimate contact, thus fulfilling the seemingly obligatory second signal for B cell triggering. Lacking this mechanism for recognition of surface antigen differences, isogeneic, antigen-specific T and B cells must be brought to close proximity by antigen itself (either on macrophages, the B cells, or both) whereupon the T cell and/or its product can act on the appropriately exposed B cell acceptor site. The significance of the need for T and B cell determinants on the same molecule for effective cooperation in vivo is thereby adequately explained. However, the failure of physiologic cooperation between histoincompatible T and B cells demonstrates once more that the role of T lymphocytes is not limited to one of antigen presentation to B cells.

Regardless of the precise mechanism that restricts physiologic cooperative T-B cell interactions to syngeneic or semiallogeneic systems, this essential requirement, particularly as illustrated in F1 recipients of T and B cells, is incompatible with the hypothesis proposed by Feldman (11) for the function of helper T cells in humoral immune responses: i.e., the secretion after stimulation of T cells by antigen of an antibody cytophilic for macrophages. This hypothesis does not require close T-B cell contact and would predict effective cooperation between allogeneic T and B cells, which, as shown by the present study, is clearly not operative.

Finally, our data, which show inability of allogeneic T and B cells to cooperatively interact, indicate the degree of caution that must be exercised in interpreting results of studies carried out in tetraparental mice derived from genetic responder and nonresponder strains (14, 15). Although the majority of such mice develop antibody to poly-L(Tyr,Glu)-poly-D,L-Ala-poly-L-Lys of responder Ig allotype (14, 15), a small proportion of nonresponder allotype antibody has been observed. Since the responder T cells and nonresponder B cells in such animals differ at a major H-2 locus, it is not at all inconceivable that the production of nonresponder allotype antibody reflects an allogeneic effect within these mice rather than physiologic cooperation between their histoincompatible responder T and nonresponder B lymphocytes.

SUMMARY

Several experimental approaches, designed specifically to circumvent the possible contribution of a complicating “allogeneic effect,” have been success-

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fully used to answer the question of physiologic cooperative interactions between histoincompatible T and B lymphocytes in antibody responses to hapten-protein conjugates. This was accomplished for in vivo cell transfer studies by using an F1 hybrid host as the recipient of irradiated, carrier-primed T lymphocytes from one parent and 2,4-dinitrophenyl (DNP)-primed B lymphocytes from the opposite strain. Under these conditions, very good T-B cell cooperative interactions were observed to occur between T and B lymphocyte populations derived from syngeneic donors, whereas no cooperative response was obtained when T cells were derived from one parental strain and B cells from the other. Corroborative experiments were performed in a totally in vitro system in which DNP-primed B cells developed good secondary anti-DNP antibody responses in vitro to soluble DNP-keyhole limpet hemocyanin (KLH) when cultured in the presence of irradiated KLH-primed T cells derived from syngeneic donors but not from allogeneic donors.

The failure of histoincompatible T and B lymphocytes to effect physiologic cooperative interactions has important implications for our understanding of how such interactions normally occur. The possibility that these results reflect the existence of a “block” of some sort to cell-cell interaction by virtue of the presence of a foreign major histocompatibility antigen on the surface of either cell has been definitively ruled out in the present studies. These observations demonstrate that the gene(s) that conditions the capability for physiologic T-B cell cooperation must be shared in common by the respective cell types, and suggest, furthermore, that this gene (or genes) belongs to the major histocompatibility system of the mouse. These findings, together with other relevant phenomena described previously, have led us to postulate that there exists on the B lymphocyte surface an “acceptor” molecule either for the putative active T cell product or for the T cell itself. The important genetic considerations and the possible sequence of events surrounding the actual T-B cell interaction implied by these postulates are discussed in detail.

The authors thank Miss Mary Graves, Mr. Michael Moran, and Miss Melissa Varney for expert technical assistance, and Miss Theodora Polihrom for excellent secretarial assistance in the preparation of the manuscript.

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