Proc. Nat. Acad. Sci. USA
Vol. 70, No. 9, pp. 2624–2628, September 1973

Cell Interactions Between Histoincompatible T and B Lymphocytes. The H-2 Gene Complex Determines Successful Physiologic Lymphocyte Interactions*

(mice/hapten–protein conjugates)

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Contributed by Baruj Benacerraf, June 4, 1973

ABSTRACT **We used congenic-resistant mouse strains to answer questions concerning the respective roles of genes coding for major histocompatibility and background genotypes in T (thymus-derived)–B (bone marrow-derived) lymphocyte cooperative responses to hapten–protein conjugates. These studies demonstrate conclusively that the gene or genes present in the H-2 complex control the capacity of antigen-specific T and B cells to effectively interact. These findings led us to postulate that there exists on the B-lymphocyte surface an "acceptor" molecule for the active T-cell product or for the T cell itself.**

Recently, we have shown that histoincompatible carrier-primed thymus-derived (T) lymphocytes of mice fail to provide the required stimulus for the responses of bone marrow-derived (B) cells to hapten–carrier conjugates (1). Thus, under conditions designed to eliminate nonspecific T-cell influences from potential development of an "allogeneic effect" (2), mixtures of suitably primed T and B lymphocytes from BALB/c and A/J donors, respectively, (or vice versa) failed to cooperate effectively in developing antibody responses either *in vivo* or *in vitro*. Since the strains used in these studies, i.e., BALB/c (H-2^d) and A/J (H-2^a), differed not only at the major histocompatibility locus but for many other polymorphisms as well, we could only speculate that the relevant area of the genome responsible for permitting (or preventing) "physiologic", i.e., antigen-specific, T–B cell cooperative interactions to occur was located in the gene complex coding for the major histocompatibility specificities (1).

In the present report, we used congenic-resistant mouse strains to answer questions concerning the respective roles of genes coding for major histocompatibility and background genotypes in T–B cell cooperation. These studies demonstrate that genes present in the H-2 complex control the capacity of antigen-specific T and B cells to effectively interact.

MATERIALS AND METHODS

Proteins and Hapten–Carrier Conjugates. Bovine gamma globulin (BGG) was obtained from Pentex Biochemical, Kankakee, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Biomarine Supply Co., Venice, Calif. The following 2,4-dinitrophenyl (Dnp) conjugates were prepared (3, 4): Dnp_{32}-BGG and Dnp_{14}-KLH. Subscripts refer to the average number of mol of Dnp per mol of carrier for BGG and to the number of mol per 100,000 molecular weight units for KLH.

The Mice Used in these experiments are listed in Table 1, which presents the relevant genetic differences between them. A/J, C57BL/10 Sn (B10), and congenic-resistant B10.A mice were obtained from Jackson Laboratories, Bar Harbor, Me. Congenic-resistant A.By mice were bred in our own animal facilities, and the (A x B10)F_1 hybrid mice were purchased from Dr. Geoffrey Haughton at the University of North Carolina, Chapel Hill, N.C.

Depletion of T Lymphocytes. Preparation of anti-θ serum, determination of cytotoxicity of anti-θ serum, and anti-θ treatment of spleen cells have been described (5).

Immunizations and Adoptive Cell Transfers. Mice used as donors of primed spleen cells were immunized with 100 μg of either Dnp-KLH or BGG emulsified in complete Freund's adjuvant intraperitoneally. At various times (2–4 months) thereafter, these mice were killed and their spleens were removed. Single-cell suspensions in minimal essential medium (Eagle) were prepared, washed, and transferred intravenously to recipient mice under various conditions (see *Results*). In general, secondary antigen challenge was either 20 μg of Dnp-KLH or 50 μg of Dnp-BGG intraperitoneally in saline immediately after transfer of Dnp-KLH-primed cells. All recipient mice were bled 7 days after secondary challenge from the retroorbital plexus and serum levels of antibody against Dnp were determined.

Measurement of Anti-Dnp Antibodies. Serum anti-Dnp antibody levels were determined by a modified Farr technique (6, 7), with Dnp-[^{3}H]ϵ-amino-*n*-caproic acid (3). From standard curves constructed for individual mouse strains as described for inbred guinea pigs (3), percentage of binding was converted into amount of anti-Dnp antibody in μg/ml of serum.

Statistical Analysis. Serum antibody levels were logarithmically transformed and means and standard errors were calculated. Group comparisons were made with Student's *t*-test. In those mice in which no specific antigen binding could be detected in the serum, a value of 0.10 μg/ml was arbitrarily assigned to allow logarithmic transformation of the data.

Abbreviations: T and B lymphocytes, thymus-derived and bone marrow-derived lymphocytes, respectively; Dnp, 2,4-dinitrophenyl; BGG, bovine gamma globulin; KLH, keyhole limpet hemocyanin.

* This paper is no. 3 in a series. Paper no. 2 is ref. 1.

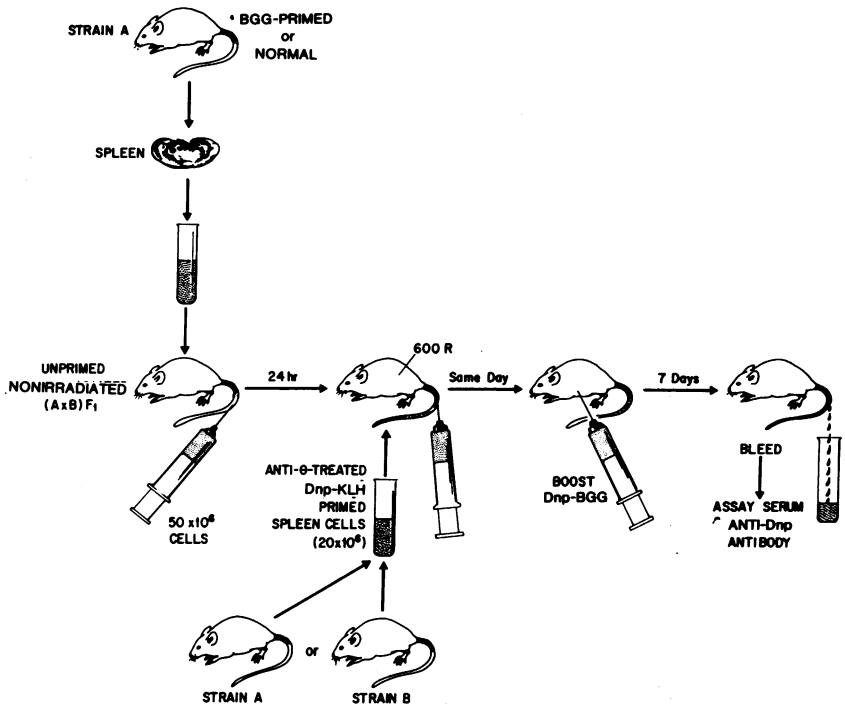

FIG. 1. Protocol for determination of physiologic cooperation between histoincompatible T and B lymphocytes.

RESULTS

The basic protocol followed is schematically illustrated in Fig. 1. This experimental design takes advantage of the fact that: (*i*) primed mature T lymphocytes are relatively radioresistant when subjected to x-irradiation *in situ* (8), and (*ii*) semiallogeneic recipients are genetically incapable of reacting against histocompatibility specificities of either parental strain lymphocytes. The latter point is particularly important since in our earlier studies we found, quite unexpectedly, that irradiated recipient mice were capable of reacting against adoptively transferred histoincompatible B cells, resulting in a clear allogeneic effect on these lymphocytes (9).

As indicated in Fig. 1, 50 × 10^6 spleen cells from either BGG-primed or normal parental donor mice are injected intravenously into nonirradiated, unprimed (A x B10)F_1 hybrid recipients. 24 hr later, when the transferred cells have migrated to the lymphoid organs, these mice are irradiated (600 R) and then injected intravenously with a second cell inoculum consisting of 20 × 10^6 Dnp-KLH-primed, anti-θ serum plus complement-treated spleen cells (i.e., B lymphocytes) derived from the same or another donor strain. Immediately thereafter, secondary challenge is performed with 50 μg of Dnp-BGG intraperitoneally in saline, and the mice are bled 7 days later. This experiment is usually performed in a simultaneously symmetrical fashion to alleviate potential variability between different pools and strain origins of carrier-primed and Dnp-primed donor cells. Indeed, the strain combinations used in the present studies were all tested on the same day with common pools of each donor cell type, thereby allowing for reciprocal inherent controls for their functional capacities.

TABLE 1. *Relevant genotypic features of strains used*

Strain	H-2 genotype	Background genotype
A	H-2^a	A/J
B10.A	H-2^a	C57BL/10 Sn
A.By	H-2^b	A/WySn
B10	H-2^b	C57BL/10 Sn
(A x B10)F_1	H-2^a/H-2^b	A/WySn and C57BL/10 Sn

The protocol and results of one series of combinations of T and B cells in which the Dnp-primed B cells were derived from A/J (H-2^a) donor mice are shown in Fig. 2. For convenience, the relevant genetic similarities and/or differences are listed for each combination. Groups I and II demonstrate the intact cooperative functional capacities of the irradiated (*in situ*) BGG-primed and the anti-θ-treated Dnp-primed cells of syngeneic A/J origin within the environs of (A x B10)F_1 irradiated recipients (Group II) as compared to control recipients of normal cells (Group I). Similarly, BGG-primed T cells derived from congenic-resistant B10.A donors, which are identical with A/J at the major H-2^a locus but dissimilar with respect to background genotypes, are capable of exerting a clear helper effect in cooperating with A/J B cells (Groups III and IV). In sharp contrast, T cells from A.By or B10 donors, which are both H-2^b, fail to cooperatively interact with A/J B lymphocytes (Groups V–VIII). This is true irrespective of whether or not the genetic background other than H-2 is identical, such as in A.By donor cells (Group VI). Groups IX and X serve as controls for the efficacy of anti-θ serum treatment in that such T cell-depleted populations failed to respond to Dnp-KLH in unprimed irradiated recipients.

A comparable experiment is illustrated in Fig. 3 in which the Dnp-primed B cells were obtained from B10.A (H-2^a) donors. Once again, BGG-primed T cells from histocompatible donors, either B10.A (Group II) or A/J (Group IV), were perfectly good helpers in development of secondary anti-

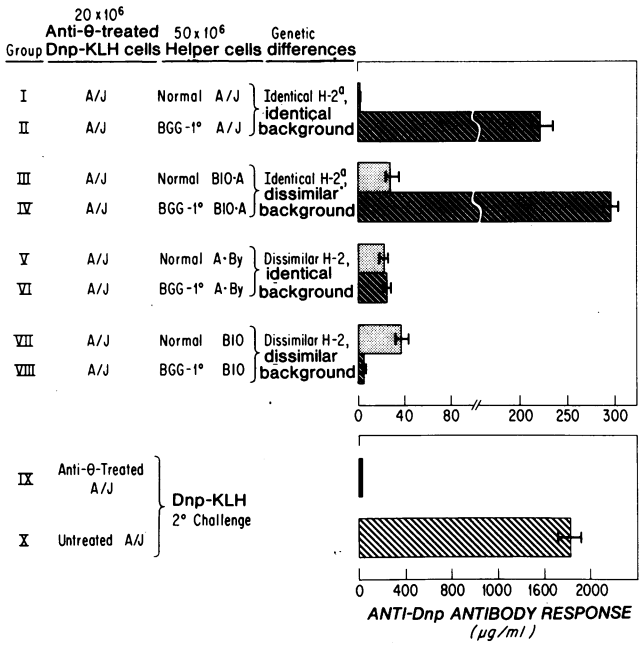

FIG. 2. Failure of physiologic cooperative interactions to occur between T and B lymphocytes differing at the major histocompatibility locus. The scheme followed is outlined in Fig. 1. Recipients for all cell combinations were (A x B10)F_1 hybrids. Combinations and strain origins of T and B cells and the relevant genetic differences are indicated. Recipients in Groups I–VIII were secondarily challenged with 50 μg of Dnp-BGG; Groups IX and X received 20 μg of Dnp-KLH. Mean serum levels of anti-Dnp antibody 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 groups gave the following results: Groups I and II, Groups III and IV, and Groups IX and X—$0.001 > P$ in all cases; Groups V and VI—$0.98 > P > 0.95$; Groups VII and VIII—$0.80 > P > 0.70$.

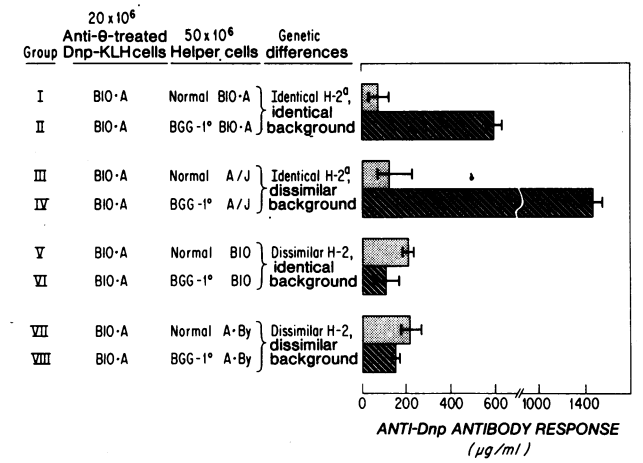

FIG. 3. Failure of physiologic cooperative interactions to occur between T and B lymphocytes differing at the major histocompatibility locus. Legend same as in Fig. 2 except all recipient groups were secondarily challenged with 50 μg of Dnp-BGG. Statistical comparisons between the various groups gave the following results: Groups I and II—$0.001 > P$; Groups III and IV—$0.01 > P > 0.005$; Groups V and VI and Groups VII and VIII—$0.80 > P > 0.70$ in both cases.

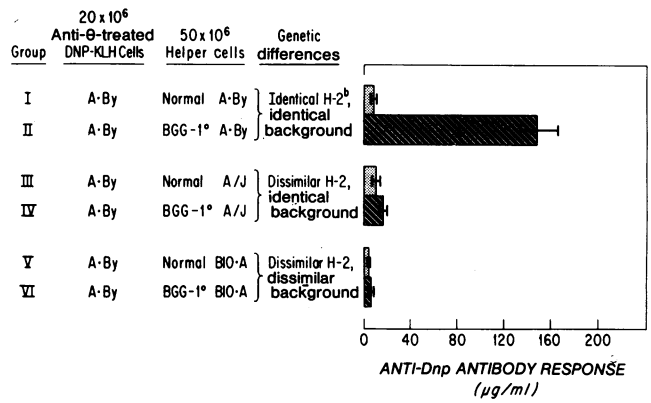

FIG. 4. Legend same as in Fig. 3. Statistical comparisons between the various groups gave the following results: Groups I and II—$0.001 > P$; Groups III and IV and Groups V and VI—$0.95 > P > 0.90$ in both cases.

Dnp responses to Dnp-BGG, whereas T cells from donor mice differing at H-2, i.e., B10 (Group VI) or A.By (Group VIII), failed to do so irrespective of background genotype.

The final experiment (Fig. 4) reiterates the preceding observations in recipients of B cells derived from A.By (H-2^b) donors. Hence, although very good cooperative responses occur between syngeneic A.By T and B lymphocytes (Groups I and II) BGG-primed cells from H-2^a donors, i.e., A/J or B10.A, fail to serve as effective helper cells for the A.By B cells (Groups III–VI).

DISCUSSION

Accumulated experimental data on the nature of cooperative interactions between antigen-specific T and B cells provides relatively limited information on the genetic restrictions to effect a physiologic immune response (9–13). Recently, we have reported results of studies using several approaches designed specifically to answer the question of physiologic cooperative interactions between histoincompatible T and B lymphocytes in humoral immune responses to hapten–carrier conjugates (1, 9). The experimental schemes were developed to circumvent a complicating allogeneic effect based on the unexpected demonstration of this phenomenon when Dnp-primed B cells were adoptively transferred into heavily irradiated allogeneic recipients (9). This was accomplished for *in vivo* cell transfer studies in our previous report (1) and also in the experiments presented herein by use of an F_1 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 from animals possessing the opposite parental-strain major histocompatibility specificity. The latter cells are depleted of T lymphocytes by treatment with anti-θ serum and complement before transfer to eliminate development of a fatal graft-versus-host reaction in the irradiated F_1 recipient. This scheme absolutely avoids the allogeneic effect for several reasons described in detail in our previous reports (1, 9).

Under these conditions, using BALB/c (H-2^d), A/J (H-2^a), and CAF_1 (H-2^d/H-2^a) mouse strains, we previously found that 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 (1). These findings demonstrated the necessity

TABLE 2. *Capacity of T and B lymphocytes of various strain origins to physiologically interact*

B Cells	T Cells	Genetic differences	Cooperative response
A ($H\text{-}2^a$)	A $(H\text{-}2)^a$	None	Yes
A ($H\text{-}2^a$)	B10.A ($H\text{-}2^a$)	Background	Yes
B10.A ($H\text{-}2^a$)	B10.A ($H\text{-}2^a$)	None	Yes
B10.A ($H\text{-}2^a$)	A ($H\text{-}2^a$)	Background	Yes
A.By ($H\text{-}2^b$)	A.By ($H\text{-}2^b$)	None	Yes
A ($H\text{-}2^a$)	A.By ($H\text{-}2^b$)	H-2 alone	No
A ($H\text{-}2^a$)	B10 ($H\text{-}2^b$)	H-2 and Background	No
B10.A ($H\text{-}2^a$)	B10 ($H\text{-}2^b$)	H-2 alone	No
B10.A ($H\text{-}2^a$)	A.By ($H\text{-}2^b$)	H-2 and Background	No
A.By ($H\text{-}2^b$)	A ($H\text{-}2^a$)	H-2 alone	No
A.By ($H\text{-}2^b$)	B10.A ($H\text{-}2^a$)	H-2 and Background	No

for T and B cells to share one or more genes in common for effective antigen-mediated physiologic cooperation and strongly suggested, but failed to establish definitively, that the gene or genes that condition this cooperation belong to the major histocompatibility system of mice (1).

The present experiments confirm and, more importantly, extend the observations cited above by providing definitive proof that the gene or genes restricting physiologic T and B cell cooperation do indeed belong to the major H-2 gene complex. This conclusion derives from the results obtained when mixtures of T and B lymphocytes originating from donor mice differing either at the H-2 region or at multiple non-H-2 loci were assessed in cooperative antibody responses to Dnp–protein conjugates. For convenience, the results obtained with the various strain combinations used are summarized in Table 2. Thus, Dnp-primed B cells from $H\text{-}2^a$ or $H\text{-}2^b$ donors developed adoptive secondary anti-Dnp antibody responses in appropriate F_1 recipients to Dnp-BGG when mixed with BGG-primed T cells from donor mice that were identical at H-2. This finding was true in combinations where the respective cell populations were identical only at H-2 and exhibited several polymorphisms in their "background" genes. Conversely, the same B cells that developed responses when mixed with histocompatible T cells failed to respond when the carrier-primed T cells were derived from donors that differed at H-2 irrespective of whether or not the remainder of the genome was identical (Table 2). Since the strain combinations used in these experiments were all tested on the same day with common pools of donor cells, there is no question of their respective functional capabilities complicating the conclusions drawn from these data.

There are several possible explanations for the failure of physiologic T–B cell cooperation to occur across the major histocompatibility barrier. Certain of these possibilities, which we have dealt with previously (1) and appear to be quite unlikely, include the following:

(*i*) Failure of transferred T and B cells to migrate to appropriate sites in the lymphoid organs *in vivo*, and/or rejection of one or the other cell type. These possibilities have been eliminated by use of the F_1 host as a neutral environment in which very good cooperative interactions could be obtained between H-2 identical cell mixtures and, moreover, by corroboration of these data in a fully *in vitro* system (1).

(*ii*) A "block" of some sort to cell–cell interaction by the presence of a foreign major histocompatibility specificity on the cell surface of one or the other of the lymphocyte classes. This was ruled out in our previous studies by experiments demonstrating highly effective cooperation between reciprocal combinations of parental and F_1 hybrid T and B lymphocytes (1). These previous findings demonstrated, moreover, that the existence of one common major H-2 haplotype is sufficient for effective interaction to occur between two cell populations even though the F_1 cells also possess a set of foreign H-2 specificities.

(*iii*) Ineffective or inefficient macrophage–lymphocyte interaction due to major histocompatibility differences. This possibility plays little, if any, significant role in these data since the major macrophage component is most likely provided by the irradiated F_1 host. The latter not only share a common haplotype with both parental H-2 specificities, but also support good cooperative responses between adoptively transferred isogeneic T and B cells. Furthermore, other studies from our laboratories have provided evidence that, in *in vitro* mouse spleen cell cultures, antigen-bearing macrophages from allogeneic donors are as effective as those from syngeneic donors in presenting Dnp-KLH to T and B cells in the elicitation of secondary anti-Dnp antibody responses (14).

This reasoning has led us to conclude, therefore, that the genetic restrictions for physiologic cooperation between T and B cells in the immune response concern the physiologic cooperation between these cells. The present studies provide clear evidence that the relevant gene or genes involved belong to the major histocompatibility complex. It is now essential to identify more precisely the genetic region concerned with H-2 primarily involved. In our previous studies, no cooperation occurred with mixtures of T and B cells from BALB/c ($H\text{-}2^d$) and A/J ($H\text{-}2^a$) donors, respectively (1). These particular strains are identical at SsSlp and the entire D-end of the H-2 complex but possess major differences at the K-end. Many differences exist in the Ir region as well. The present studies have involved strain combinations with major differences in all regions of the H-2 complex. It will be most important to determine whether differences at the K-end alone, or at either K- or D-ends are sufficient to restrict physiologic T–B cell cooperation from occurring. Perhaps most intriguing is the possibility that identities at the Ir gene region, either alone or together with K- or D-end identities, are the critical determinants for successful T–B cell interaction.

The significance of the requirement for a common gene product on T and B cells for physiologic cooperation to occur clearly suggests something of critical importance relevant to 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 (15). One of the ways by which this was proposed to occur is by release of T cell-produced mediators that act in some way in influencing B-cell triggering by antigen (15). This view must now take into account the genetic restrictions apparently conditioning such lymphocyte interactions. We have recently proposed that these genetic considerations provide evidence for existence on the surface membrane of B lymphocytes of a site closely related to the histocompatibility specificity that is critically involved in physiologic T–B cell interaction (1). We envisage this relevant site as an "acceptor" molecule either for the active T-cell product or the T cell itself. The necessity for the T and B cells to possess the same gene or

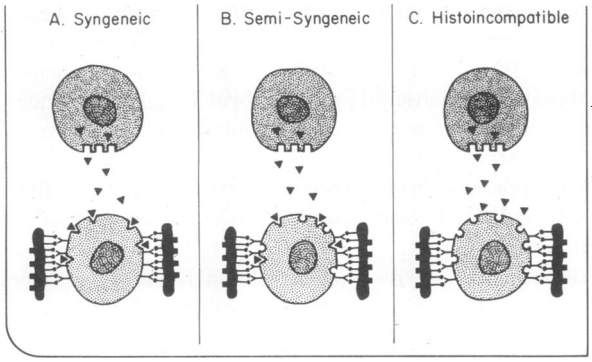

FIG. 5. Genetic requirements for physiologic cooperative interactions between T and B lymphocytes. *Upper cell* is the T lymphocyte, while *lower cell* is the B lymphocyte in all cases.

genes for physiologic cooperation requires that the same gene product is expressed on both cells or, alternatively, if two gene products are expressed in the respective cell types, the genes concerned have remained closely linked.

Taking into consideration the above reasoning, the sequence of events surrounding the actual T–B cell interaction could proceed as indicated schematically in Fig. 5. 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 products that have specificity for, and bind to, the specific "acceptor" sites on the B lymphocyte. We believe that the B cell already has antigen bound by its specific surface Ig receptors before the relevant interaction with T cells. As depicted in Fig. 5, when the reacting T and B cells are syngeneic (*A*) or semi-syngeneic (*B*), the B cell "acceptor" site can recognize and bind the T-cell product. Note that in semi-syngeneic combinations (*B*) an additional "acceptor" site with specificity corresponding to the foreign haplotype also exists on the B cell; the reciprocal possibility, i.e., when the T cell bears two different H-2 specificities and the B cell only one, is that the T lymphocyte secretes two molecularly distinct products of which only one binds to the corresponding B cell. In contrast, in the completely histoincompatible combination (Fig. 5*C*), the T-cell product secreted after antigen activation is incapable of binding to, and subsequently acting upon, the B cell since the latter lacks an appropriately specific "acceptor" site.

These surface events are followed by the crucial biochemical events concerned with actual triggering of the B lymphocyte. Similarly, suppressive T-cell regulatory effects might be explained in the context of this scheme if we assume that saturation of T "acceptor" sites on the B cell by active mediator produced in quantitative excess transiently prevents triggering (but does not specifically make tolerant).

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 for the very reason of their histoincompatibility these cells must be brought together into intimate contact, thus fulfilling the seemingly obligate second signal for B-cell triggering. Active T-cell products participating in this type of interaction and perhaps in analogous *in vitro* phenomena may not be genetically restricted in their range of activity. 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, B cells or both (14)] whereupon the T cell and/or its active product can act on the appropriately exposed B cell "acceptor" site.

We thank Ms. Mary Graves, Mr. Michael Moran, and Ms. Melissa Varney for expert technical assistance and Ms. Candace Maher for excellent secretarial assistance in preparation of the manuscript. This investigation was supported by Grants AI-10630 and AI-09920 from the National Institutes of Health.

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