

CELL INTERACTIONS BETWEEN HISTOINCOMPATIBLE
T AND B LYMPHOCYTES

I. ALLOGENEIC EFFECT BY IRRADIATED HOST T CELLS ON
ADOPTIVELY TRANSFERRED HISTOINCOMPATIBLE
B LYMPHOCYTES*

BY TOSHIYUKI HAMAOKA, DAVID P. OSBORNE, JR.,[‡] AND
DAVID H. KATZ

*(From the Department of Pathology, Harvard Medical School,
Boston, Massachusetts 02115)*

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In the ever-increasing body of experimental data accumulated to date on the nature of cooperative interactions between antigen-specific T and B lymphocytes, there is only limited information on the possibility that such interacting cells need to be histocompatible to effect a physiologic immune response. Indeed, the observations of several investigators (1-4) can be interpreted to indicate a requirement for histocompatibility at the major locus between the two cell types. These studies were done, however, under circumstances that make definitive conclusions difficult, since potential contributions to the results of rejection reactions and/or an allogeneic effect (for review see references 5, 6) cannot be excluded. Quite recently, Kindred and Shreffler (7) reported results of their studies designed specifically to investigate this question in backcrosses of nude and BALB/c mice. Since the nude mouse cannot reject foreign tissue grafts, this complicating feature was theoretically eliminated. In such mice T cell helper effects could be obtained with thymus cells from *H-2*-compatible but not from incompatible donors (7). However, this study may not be considered conclusive because of lack of direct evidence that the transferred histoincompatible cells had not been rejected.

We have felt for some time that the cooperation between carrier-specific T cells and hapten-specific B cells in antihapten humoral responses presents an appropriate model system for investigating the possible importance of histocompatibility in T-B cell cooperation, taking advantage of the *in vivo* radioresistance of primed, carrier-specific T cells. According to this simple model, allogeneic, hapten-specific B cells could presumably be safely transferred to carrier-primed, irradiated recipients to investigate T cell-B cell interactions. Achieving this, however, has not been without considerable technical difficulty for reasons that will become apparent in this and the accompanying report (8). This difficulty has stemmed primarily from the contribution

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of the "allogeneic effect" in nonspecifically enhancing adoptive immune responses in irradiated recipients under quite unexpected circumstances. The allogeneic effect is a phenomenon in which specific B lymphocytes become readily activated by antigen in the absence of antigen-specific helper T cells by virtue of a direct interaction with histoincompatible T lymphocytes recognizing surface antigen differences on such B cells (5, 6).

In this first paper, we will demonstrate that a heavily irradiated mouse (600 R) possesses sufficient numbers of active, residual T cells to exert this effect on a small population of adoptively transferred, histoincompatible, primed B lymphocytes. In order to minimize possible confusion of terminology, the phenomenon of the allogeneic effect has been distinguished from interactions between normally isologous antigen-specific T and B lymphocytes by referring to the latter, for purposes of brevity, as "physiologic" T-B cell cooperation.

Materials and Methods

Proteins and Hapten-Carrier Conjugates.—Bovine gamma globulin (BGG)¹ was obtained from Pentex Biochemical, Kankakee, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Bio-Marine Supply Co., Venice, Calif. The following 2,4-dinitrophenyl (DNP) conjugates were prepared as previously described (9, 10): DNP₃₂-BGG and DNP₁₄-KLH. Subscripts refer to the average number of moles of DNP per mole of carrier for BGG and to the number of moles per 100,000 mol wt units for KLH.

Animals.—Mice of the inbred lines BALB/c, A/J, and (BALB/c × A/J)F₁ hybrids (CAF₁) were obtained from the Jackson Laboratory, Bar Harbor, Maine. All mice were immunized between 8 and 12 wk of age.

Depletion of T Lymphocytes.—The preparation of anti- θ serum, determination of anti- θ serum cytotoxicity, and anti- θ treatment of spleen cells have been previously described (11).

Immunizations and Adoptive Cell Transfers.—Mice used as donors of primed spleen cells were immunized intraperitoneally with 100 μ g of either DNP-KLH or BGG emulsified in complete Freund's adjuvant (CFA). At various times (2-4 mo) thereafter, these mice were killed and their spleens removed. Single-cell suspensions in minimal essential medium (Eagle) were prepared, washed, and transferred intravenously to recipient mice under the varying conditions described in Results. In general, secondary antigen challenge was either 20 μ g of DNP-KLH or 50 μ g 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 anti-DNP antibody levels were determined as described below.

Measurement of Antibody-DNP Antibodies.—Serum anti-DNP antibody levels were determined by a modified Farr technique (12, 13) using [³H]DNP- ϵ -amino-*N*-caproic acid (9). Using standard curves constructed for individual mouse strains in a manner previously described for inbred guinea pigs (9), percentage of binding was converted into amount of anti-DNP antibody in micrograms per milliliter of serum.

Statistical Analysis.—Serum antibody levels were logarithmically transformed, and means and standard errors were calculated. Group comparisons were made employing Student's *t*

¹ Abbreviations used in this paper: BGG, bovine gamma globulin; CAF₁, (BALB/c × A/J)F₁ hybrid mice; CFA, complete Freund's adjuvant; DNP, 2,4-dinitrophenyl; KLH, keyhole limpet hemocyanin.

test. In those mice in which no specific antigen binding could be detected in the serum, a value of 0.10 $\mu\text{g/ml}$ was arbitrarily assigned to allow logarithmic transformation of the data.

RESULTS

Stimulatory Influence of Allogeneic Host Environment on Histoincompatible, DNP-Primed Lymphoid Cells.—

When the allogeneic host has been previously primed with carrier: Since it was our design to use the property of radioresistance of activated T lymphocytes (6, 14–16) to permit development of a system to explore physiologic T-B cell cooperation across major histocompatibility barriers, the initial experiments were carried out under the conscious assumption that irradiated, carrier-primed mice could serve as appropriate hosts for allogeneic, DNP-primed spleen cells to verify whether they were able to exert a physiologic cooperative effect. Irradiated, unprimed mice could, we reasoned, serve as appropriate negative control hosts for the DNP-primed, allogeneic donor cells.

In the first experiments (Table I) 25 or 50 $\times 10^6$ spleen cells from DNP-KLH-primed BALB/c donor mice were injected intravenously into allogeneic, A/J, irradiated (600 R) recipients. Certain groups of recipients (C and F, Table I) had been primed with BGG in CFA approximately 1 mo before X irradiation and cell transfer, whereas the other groups consisted of unprimed recipients.

TABLE I
Stimulatory Influence of Irradiated, Carrier-Primed, Allogeneic Host Environment on Histoincompatible, DNP-Primed Lymphoid Cells

Group	Protocol*		Secondary challenge	Anti-DNP antibody on day 7 after secondary challenge
	BALB/c DNP-KLH-primed cells	A/J irradiated recipients		
A	25 $\times 10^6$	Unprimed	DNP-KLH	1,658.4 (1.16)
B		Unprimed	DNP-BGG	199.7 (1.31)
C		BGG-primed	DNP-BGG	74.8 (1.43)
D	50 $\times 10^6$	Unprimed	DNP-KLH	3,582.0 (1.32)
E		Unprimed	DNP-BGG	946.6 (1.39)
F		BGG-primed	DNP-BGG	216.4 (1.41)

* 25 or 50 $\times 10^6$ spleen cells from DNP-KLH-primed BALB/c donor mice were injected intravenously into allogeneic, A/J, irradiated (600 R) recipients that were either unprimed or primed with BGG in CFA as indicated. Recipients were secondarily challenged with either 10 μg of DNP-KLH or 50 μg of DNP-BGG intraperitoneally in saline as indicated, and bled 7 days later.

† The data are expressed as geometric means of anti-DNP antibody levels of groups of five mice. Numbers in parentheses represent standard errors. A comparison of geometric means of antibody levels gave the following results: comparisons of group B with group C and group E with group F yielded $0.10 > P > 0.05$ in both cases.

Recipients were secondarily challenged with either 10 μg of DNP-KLH or 50 μg of DNP-BGG immediately after cell transfer and bled 7 days later.

Two points are noteworthy about the data in Table I. First, there is a highly significant response to the DNP conjugate of a heterologous carrier, DNP-BGG, in all groups so challenged. (An additional control group consisting of 50×10^6 DNP-KLH-primed BALB/c cells transferred to unprimed, syngeneic, irradiated recipients failed, as expected, to develop secondary anti-DNP antibody responses to DNP-BGG [less than 5.0 $\mu\text{g}/\text{ml}$].) Secondly, the magnitude of the secondary response to DNP-BGG is actually appreciably lower in the allogeneic BGG-primed (groups C and F) than in the unprimed recipients (groups B and E).

The latter point has been a consistent observation in many such experiments in our hands and probably reflects the binding of available DNP-BGG to some extent by circulating anti-BGG antibodies in BGG-primed animals. The correctness of this interpretation is strongly indicated by the results of experiments in which the contribution of circulating anti-BGG antibodies has been obviated.

When the allogeneic host is unprimed but serves as a passive recipient of syngeneic, carrier-primed cells: A second approach took advantage of our recently described model of exposing carrier-primed mouse T lymphocytes to X irradiation *in situ* after transfer to an unprimed, syngeneic recipient (16). This model has two advantages in that it permits careful control of the numbers of carrier-primed T cells involved in the response, and removes the responding cells from the potential influence(s) of circulating anticarrier antibodies.

Since this scheme tends to be somewhat confusing and, more importantly, since it will be employed in all of the subsequent experiments described in this and the accompanying paper (8), a detailed diagram of the protocol is depicted in Fig. 1. Briefly, a fixed number (usually 50×10^6) of BGG-primed or normal spleen cells are injected intravenously into nonirradiated, unprimed, syngeneic recipients. 24 h later, when the transferred cells have migrated to the lymphoid organs, these mice are irradiated with 600 R and then injected intravenously with a second cell inoculum consisting of $20\text{--}25 \times 10^6$ DNP-KLH-primed cells derived from either syngeneic or allogeneic donors. Secondary challenge is performed with 20 μg of DNP-KLH or 50 μg of DNP-BGG immediately thereafter and the mice are bled 7 days later.

The results obtained after DNP-BGG challenge in a representative experiment of this type are depicted in Fig. 2. The experiment has been performed in symmetrical fashion in A/J (groups I-IV) and BALB/c (groups V-VIII) recipient mice. Comparison of groups I and II and groups V and VI illustrates clearly the requirement for, and radioresistance of, carrier-specific, helper T cell function in eliciting secondary responses to DNP-BGG under syngeneic conditions. In contrast, DNP-KLH-primed cells developed significant responses to DNP-BGG when transferred to allogeneic, irradiated recipients whether or not the latter had also received BGG-primed helper T cells (groups III, IV, VII, and VIII).

Demonstration of the Capacity of Irradiated Hosts to Exert an Active Allogeneic Effect on Adoptively Transferred, Histo incompatible, DNP-Primed, Lymphoid Cells.—In the preceding experiments, obviation of the requirement for carrier-specific helper T cells in the secondary responses of DNP-primed cells that had been adoptively transferred to allogeneic recipients indicates the existence of an allogeneic effect (5, 6). There are two conceivable ways, one more obvious than the other, in which this may occur. The more obvious manner is that the T cells of the DNP-KLH-primed donor inoculum react against foreign histocompatibility antigens of the irradiated host and, in turn, exert a facilitating effect on their isologous DNP-primed B lymphocytes. This type of “bystander” allogeneic effect was found not to occur *in vivo* in earlier published experiments from this laboratory (5, 17–19). The alternative explanation, which in fact seemed at first more improbable, is that sufficient radioresistant T lymphocytes remain in a (600 R) irradiated host to exert a considerable allogeneic effect directly on the primed B cells in the transferred donor inoculum. The following experiments were designed to distinguish between these two alternatives.

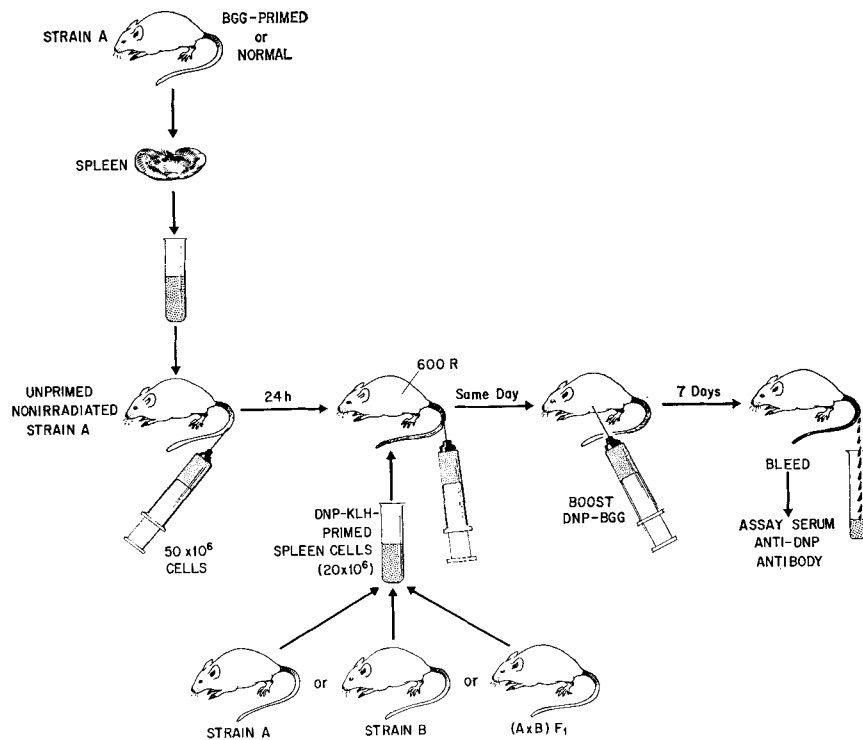


FIG. 1. Stimulatory influence of the allogeneic host environment on histoincompatible, DNP-primed lymphocytes when the allogeneic host serves as a passive recipient of syngeneic, carrier-primed cells exposed to X irradiation *in situ* after transfer. See text of Results for explanation of protocol.

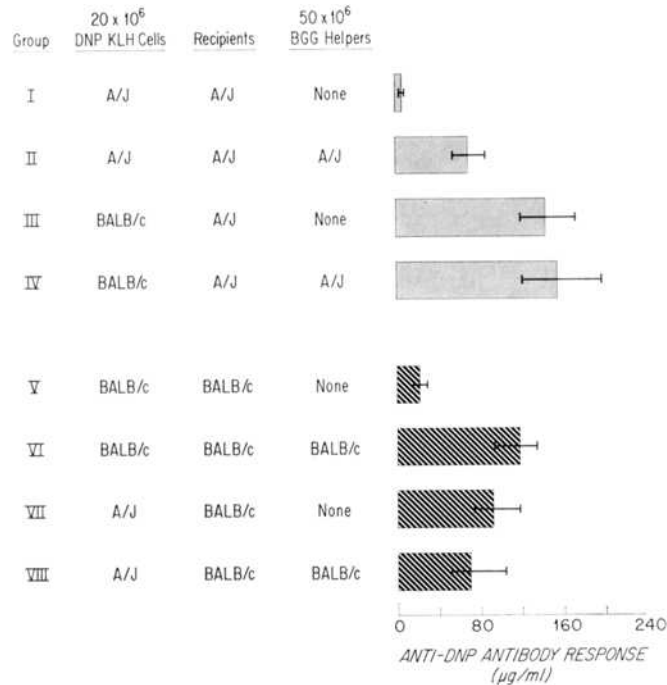


FIG. 2. The sequence of adoptive cell transfers is detailed in Fig. 1. All recipient mice were secondarily challenged with 50 μg of DNP-BGG immediately after transfer of the DNP-KLH-primed cells. Mean serum anti-DNP antibody levels of groups of six mice on day 7 after secondary challenge are illustrated. Vertical bars represent the range of the standard errors. Statistical comparisons of the responses of the various groups gave the following results: groups I and II, $0.001 > P$; groups III and IV, $0.90 > P > 0.80$; groups V and VI, $0.0005 > P > 0.0001$; groups VII and VIII, $0.90 > P > 0.80$.

Allogeneic effect in irradiated parental recipients of adoptively transferred, DNP-primed F₁ hybrid cells: The existence of a bystander allogeneic effect as postulated above can be controlled in two ways; if this interpretation is correct, then one would not expect to observe such an allogeneic effect when the donor cell inoculum consists of DNP-primed F₁ hybrid donor cells that have been transferred into irradiated parental recipients.

The protocol and data from such an experiment are illustrated in Fig. 3. Secondary challenge in all cases was made with 50 μg of DNP-BGG. Control groups I-IV illustrate again the functional requirement for BGG-specific helper cells in the A/J and CAF₁ syngeneic combinations, respectively. However, when DNP-KLH-primed CAF₁ cells are transferred to irradiated parental A/J recipients, a very good secondary response to DNP-BGG is obtained, whether or not BGG-specific cells have been transferred as well (groups V and VI). Moreover, the magnitude of responses in groups V and VI are considerably higher

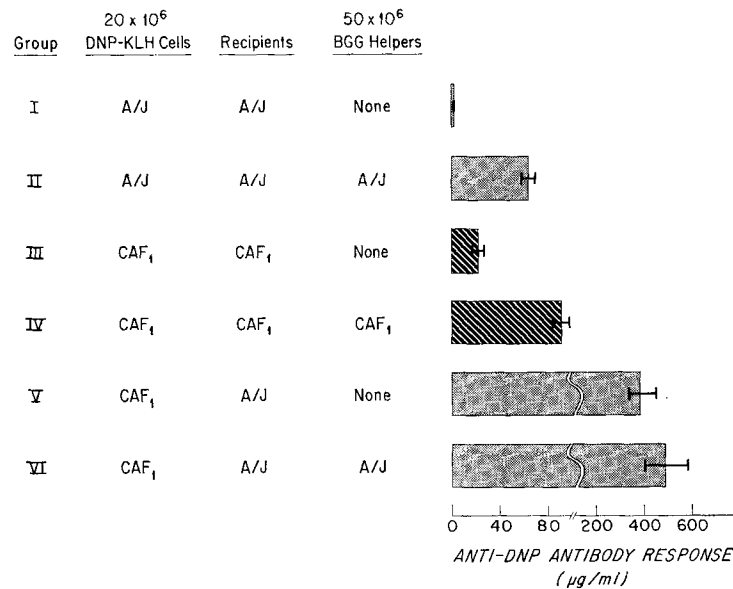


FIG. 3. Allogeneic effect in irradiated parental recipients of adoptively transferred, DNP-primed F_1 hybrid cells. The scheme depicted in Fig. 1 was followed. Donor cell-recipient strain combinations are indicated. Mean serum anti-DNP antibody levels of groups of five mice on day 7 after secondary challenge with 50 μ g of DNP-BGG are illustrated. Vertical bars represent the range of the standard errors. Statistical comparisons of the responses of the various groups gave the following results: comparison of groups I and II, or III and IV, yielded values of $0.001 > P$ in both cases. Comparison of group III with groups V and VI yielded $0.001 > P$ in both cases. Comparison of groups V and VI yielded $0.50 > P > 0.40$.

than those obtained under conditions of syngeneic T-B cell cooperation (group IV). Since the CAF₁ donor cells are genetically incapable of reacting against common histocompatibility antigens on the surface of parental A/J host cells, these results strongly indicate that the irradiated host itself is exerting an allogeneic effect on the primed donor cell population. That this is in fact the case is corroborated by the following experiment using anti- θ -treated donor cells from DNP-KLH-primed allogeneic donors.

Allogeneic effect in irradiated recipients of T cell-depleted, DNP-primed allogeneic cells: BALB/c DNP-KLH-primed spleen cells were depleted of isologous T lymphocytes by treatment with anti- θ serum and complement and then injected into either syngeneic or allogeneic A/J irradiated recipients according to the scheme summarized in Fig. 4. Certain recipient groups had also received BGG-primed spleen cells before irradiation while others had not. All recipients in groups I-VI were challenged with DNP-BGG (50 μ g); control mice in groups VII (recipients of anti- θ -treated cells) and VIII (recipients of untreated cells) were challenged with DNP-KLH to verify the efficacy of T lymphocyte deple-

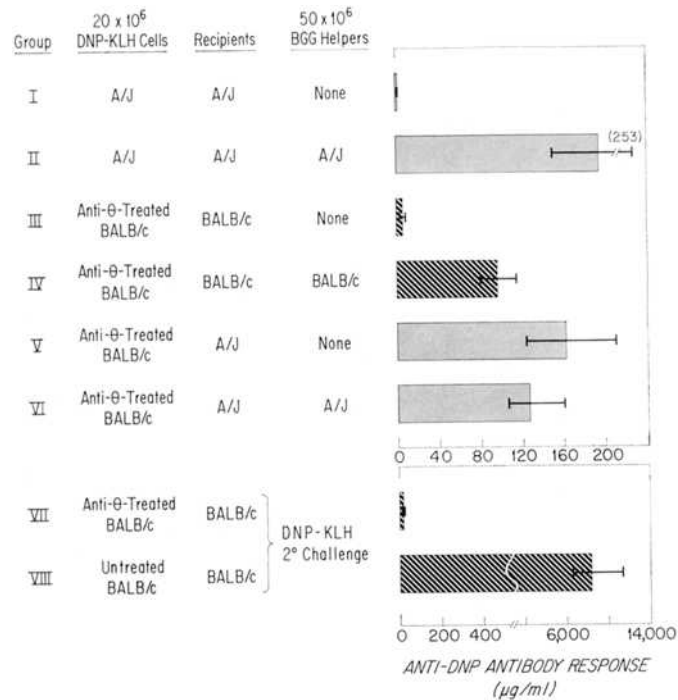


FIG. 4. Allogeneic effect in irradiated recipients of T cell-depleted, DNP-primed allogeneic cells. The scheme depicted in Fig. 1 was followed. Donor cell-recipient strain combinations are indicated. Recipients in groups I-VI were secondarily challenged with DNP-BGG. Groups VII and VIII were challenged with 20 μg of DNP-KLH. Mean serum anti-DNP antibody levels on day 7 after secondary challenge are illustrated. Vertical bars represent the range of the standard errors. Statistical comparisons of the responses of the various groups gave the following results: groups I and II, $0.001 > P$; III and VI, $0.001 > P$; V and IV, $0.60 > P > 0.50$; VII and VIII, $0.001 > P$; III and V, $0.001 > P$.

tion by anti- θ treatment. Comparison of responses in groups I and II (syngeneic A/J combination) and in groups III and IV (syngeneic BALB/c combination) once again confirm physiologic T and B cell cooperative interactions in the syngeneic system. An indisputable allogeneic effect is elicited, however, when anti- θ -treated, BALB/c, DNP-primed lymphocytes are adoptively transferred to allogeneic, irradiated A/J recipients, and once again the presence or absence of BGG-specific cells in such recipients is of no significance.

DISCUSSION

The studies presented here demonstrate that an allogeneic effect (for review, see references 5, 6) on DNP-primed lymphocytes can be obtained after adoptive transfer of such cells into a heavily irradiated, allogeneic host. The existence of an allogeneic effect, as originally defined (17), is supported by the finding that

under such circumstances DNP-KLH-primed cells developed significantly enhanced secondary anti-DNP antibody responses to DNP-BGG, whether or not the irradiated allogeneic host possessed BGG-specific helper cells.

One possible explanation for the occurrence of an allogeneic effect in an irradiated host is that the T lymphocytes of the DNP-KLH-primed donor cell inoculum react against foreign histocompatibility antigens of the host and exert, in turn, a facilitating effect on their isologous, DNP-specific B cells. We have referred to this possible mechanism as a bystander effect because the pertinent B cell is not an integral participant in the relevant cell interaction (5, 18, 19). In fact, in these as well as in many previous attempts to elicit an allogeneic effect in vivo in this manner, we have been unable to do so (5, 17-19). Moreover, this important feature of the in vivo allogeneic effect has been confirmed recently by Rajewsky et al. (20).

The second possible explanation, which initially seemed remote, is that a sufficient number of radioresistant T lymphocytes remain in a heavily irradiated host to exert an allogeneic effect directly on the relatively small number of DNP-primed B cells in the adoptively transferred donor inoculum. It was admittedly unexpected that the data would ultimately prove this second possibility to be the case. This conclusion derives from two corroborative experiments presented herein. In the first, an allogeneic effect occurred on DNP-primed CAF₁ spleen cells that had been adoptively transferred to irradiated parental recipients; the second demonstrated the development of an allogeneic effect on anti- θ -treated, DNP-specific donor cells transferred to irradiated, allogeneic hosts. Since T cells capable of reacting with host histocompatibility antigens were lacking in both types of DNP-primed donor cell inocula (for theoretical genetic reasons in the case of CAF₁ donor cells and because anti- θ treatment removed T lymphocytes in the latter case) these experiments clearly establish that the responsibility for the allogeneic effect belongs to an active interaction exerted by irradiated host cells on the donor cell inoculum.

Several points about these findings deserve emphasis. The first relates to radioresistance of lymphocytes per se. We have recently discussed this problem at length (6), and need only reiterate here that radioresistance as it pertains to T or B cells must be considered in the context of function only, since there is no concrete evidence concerning any major differences in the biological consequences of X irradiation on these lymphocyte classes. Within the framework of these limitations, however, there is clear evidence that functional radioresistance is a property predominantly of primed T lymphocytes (6, 14-16). In general, heavily irradiated (600 R) unprimed mice are ideal for adoptive immunity experiments because, it is thought, they have been rendered immunologically unresponsive by X irradiation. The data presented here indicate that this is not entirely the case, since the capacity of host T cells to interact with histoincompatible cells has remained, to some extent, intact. It is impossible to know whether in these conditions T cells have escaped X irradiation or whether X

irradiation did not totally abrogate the function of such cells (i.e., they are functionally radioresistant). If the latter is the case, then it may mean that T cells recognizing alloantigenic specificities are radioresistant in the unprimed state, in contrast to what appears to be the case for T lymphocytes specific for conventional antigens, which are radioresistant only after priming (6).

Whatever the mechanism, we must recognize the potential pitfalls that these findings identify for transfer experiments. Clearly, an allogeneic host is totally unsuitable, despite heavy enough irradiation compatible with transfer experiments, for most adoptive immunity experiments unless one wishes to make use, specifically, of the fact that a very sensitive index of T cell function, namely the allogeneic effect, can be studied very nicely in this way. Moreover, since this effect on transferred B cells was manifested under conditions where no other evidence of residual, irradiated host T cell activity overtly existed, one cannot be safe in assuming usual parameters such as graft rejection, mitogen-induced DNA synthesis, etc., as necessarily valid indicators of intact T cell function. This may be a particularly important consideration, as will be discussed in detail in the following paper (8), in immunologic experimentation carried out in tetraparental (allophenic) mice.

With respect to the original issue under investigation, i.e. the possibility of physiologic cooperation between histoincompatible B and T cells, the data obtained are inconclusive. No such cooperation was observed, but the experiments were affected by the allogeneic effect of T cells in the irradiated host on transferred hapten-primed B cells in a manner that could not be controlled in this system.

These results illustrate, therefore, the extreme caution required in designing experimental approaches to the question of physiologic T-B cell cooperative interactions across major or perhaps even minor histocompatibility barriers. Clearly, appropriate care must be taken under the most unexpected circumstances. Suitable approaches are described in the following paper.

SUMMARY

The adoptive transfer of 2,4-dinitrophenyl(DNP)-keyhole limpet hemocyanin(KLH)-primed lymphocytes into a heavily irradiated allogeneic recipient permits the development of a secondary anti-DNP antibody response to DNP-bovine gamma globulin(BGG) whether or not the irradiated allogeneic host possesses BGG-specific helper T cells. This "allogeneic effect" has been demonstrated to result from the capacity of residual, apparently radioresistant, T cells in the irradiated host to exert an active effect on the transferred histoincompatible B lymphocytes. This conclusion derives from two corroborative experiments. In the first, an allogeneic effect was shown to occur on DNP-primed F₁ spleen cells that had been adoptively transferred to irradiated parental recipients; the second experiment demonstrated the development of an al-

logeneic effect on anti- θ -treated, DNP-specific donor cells transferred to irradiated allogeneic hosts. These results emphasize the extreme caution required in designing and interpreting experiments that may involve adoptive cell transfers into histoincompatible hosts, and illustrate why such models are unsuitable for investigation of the question of physiologic cooperative interactions between T and B lymphocytes. Suitable approaches are described in the accompanying paper.

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