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Radioresistance of Carrier-Specific Helper Thymus-Derived Lymphocytes in Mice

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ABSTRACT The effect of x-irradiation on the capacity of carrier-specific mouse T (thymus-derived) lymphocytes to exert a helper function for hapten-specific B (bone marrow-derived) lymphocytes *in vivo* has been investigated again. Helper function is abolished by exposure of such cells to x-irradiation before, or immediately after, transfer to adoptive hosts. On the other hand, when exposure to x-irradiation occurs under circumstances in which the carrier-primed cells are well-localized in the appropriate lymphoid organs, i.e., either in the carrier-primed animal or 24 hr after adoptive transfer to syngeneic recipients, the capacity of T lymphocytes to serve as specific helper cells is clearly radioresistant. Evidence indicates that x-irradiation results in undefined, and subtle, changes in the migratory capacity of mouse T lymphocytes, but not in abrogation of T-cell helper function.

Since the now classic observation by Claman *et al.* (1) of the required participation of both T- and B-lymphocytes for development of effective antibody responses to most antigens, considerable data have accumulated to contribute to our present understanding of the respective functions of these cells (for review, see ref. 2). Among the various properties of T and B cells, such as functional and receptor specificity, it has become apparent that a working knowledge of T- and B-cell functions must also take into account any distinctions between the two cells on the basis of their respective sensitivity or resistance to physical and chemical agents. In this regard, the most extensive analyses have been performed with corticosteroids and x-irradiation (2). While there has been general agreement concerning the functional resistance of differentiated T cells and sensitivity of B cells to corticosteroids (2–8), the same differential pattern with respect to x-irradiation has been somewhat more controversial.

The latter controversy derives from a series of *apparently* contradictory observations, which have been reviewed recently (2) and are the subject of this report. Thus, the earliest observations of Claman and coworkers (9, 10), and Miller and Mitchell (11, 12) indicated that the functional capacity of mouse T lymphocytes was quite sensitive to x-irradiation. A year or so later, we observed that carrier-primed lymphocytes of guinea pigs were highly radioresistant with respect to helper function *in vivo* (13). This observation was quickly confirmed and extended to "educated" helper T cells of the mouse in studies of *in vitro* antibody responses (14–16). The original explanation (13) for these conflicting results was based on the fact that radiosensitivity had been observed in systems (9–12) where, at the time of exposure to irradiation, the specific T lymphocytes were not yet fully primed and, therefore, required a crucial period of proliferation and differentiation in order to develop functional maturity; conversely, in systems (13–16) where exposure to irradiation occurred after T lymphocytes had undergone functional maturation, helper activity consistently appeared to be radioresistant. The validity of this interpretation was generally accepted.

Recently, however, Anderson, Sprent, and Miller (17) reported on the functional effects of x-irradiation on mouse T lymphocytes. Using antigen-activated T cells from spleen or thoracic duct lymph in adoptive transfer experiments, they were consistently able to demonstrate considerable radiosensitivity of primed T-cell helper function in antibody responses (17). Their data left little room for doubt, and the immediate question arose as to whether there could be such a striking fundamental biological difference between guinea pig and mouse T lymphocytes. Moreover, how could the extensive corroboration of mouse T-cell radioresistance in *in vitro* systems be reconciled with the Anderson, Sprent, and Miller findings (17)?

The present paper reports the results of a series of experiments in inbred mice performed specifically to clarify these differences. Our studies have demonstrated that the helper function of antigen-activated mouse T lymphocytes is, indeed, resistant to x-irradiation. The *apparent* abrogation by x-irradiation of helper function observed in adoptive transfer systems in mice reflects rather an undefined influence of x-irradiation on the capacity of such lymphocytes to repopulate the lymphoid organs of the adoptive host in the appropriate fashion. This necessitated the use of appropriate experimental designs in order to demonstrate and/or make use of this property of mature T cells.

MATERIALS AND METHODS

Antigens. Bovine gammaglobulin (BGG) was obtained from Pentex Biochemicals, Kankakee, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Biomarine Supply Co., Venice, Calif. The following 2,4-dinitrophenyl (Dnp) conjugates were prepared as described (18, 19): Dnp_9-KLH, Dnp_{14}-KLH, Dnp_{32}-BGG. Subscripts refer to the average number of moles of Dnp per mole of carrier in the case of Dnp-BGG, whereas for Dnp-KLH subscripts denote average number of moles of Dnp per 100,000 molecular-weight units of KLH.

Animals and Immunization. Mice of inbred lines BALB/c and A/J were obtained from Jackson Memorial Laboratory, Bar Harbor, Me. All mice were used at 10–16 weeks of age. Cell donors were immunized intraperitoneally with either

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

TABLE 1. *Helper function of carrier-specific BALB/c lymphocytes exposed to x-irradiation before adoptive transfer*

Protocol		Anti-Dnp antibody (μg/ml)	
Group	BGG-primed helper cells (50×10^6)	Secondary challenge	Day 7 after 2° challenge
A	None	Dnp-KLH	1230.4 (1.28)
B	None	Dnp-BGG	0.22 (2.22)
C	Unirradiated	Dnp-BGG	145.0 (1.34)
D	600 R *in vivo*	Dnp-BGG	3.6 (3.46)
E	1000 R *in vivo*	Dnp-BGG	0.87 (2.12)
F	600 R *in vitro*	Dnp-BGG	3.0 (1.95)
G	1000 R *in vitro*	Dnp-BGG	2.2 (1.22)

30×10^6 spleen cells from BALB/c mice primed with Dnp-KLH 78 days earlier were injected intraperitoneally either alone or together with 50×10^6 BGG-primed spleen cells (69 days after priming), as indicated, into syngeneic, irradiated recipients. Secondary challenge with either 10 μg of Dnp-KLH or 100 μg of Dnp-BGG was performed immediately after cell transfer.

The data are expressed as geometric means of serum anti-Dnp antibody titers of groups of five mice 7 days after secondary challenge. Numbers in parentheses represent standard errors. A comparison of geometric mean antibody titers of the various groups gave the following results: Comparison of group C with groups B, E, F, and G yielded P values of $0.001 > P$ in all cases; Comparison of group C with group D yielded a P value of $0.02 > P > 0.01$.

TABLE 2. *Helper function of carrier-specific A/J lymphocytes exposed to x-irradiation before adoptive transfer*

Protocol		Anti-Dnp antibody (μg/ml)	
Group	BGG-primed helper cells (35×10^6)	Secondary challenge	Day 7 after 2° challenge
A	None	Dnp-KLH	713.1 (1.14)
B	None	Dnp-BGG	37.8 (1.67)
C	Unirradiated	Dnp-KLH	549.9 (1.42)
D	Unirradiated	Dnp-BGG	146.1 (1.55)
E	1000 R *in vitro*	Dnp-KLH	345.5 (1.30)
F	1000 R *in vitro*	Dnp-BGG	31.5 (1.43)

40×10^6 spleen cells from A/J mice primed with Dnp-KLH 58 days earlier were injected intraperitoneally either alone or together with 35×10^6 BGG-primed spleen cells (43 days after priming), as indicated, into syngeneic, irradiated recipients. Secondary challenge with either 10 μg of Dnp-KLH or 100 μg of Dnp-BGG was performed immediately after cell transfer.

The data are expressed as geometric means of serum anti-Dnp antibody titers of groups of five mice 7 days after secondary challenge. Numbers in parentheses represent standard errors. A comparison of geometric mean antibody titers of the various groups gave the following results: Comparison of group D with groups B and F yielded P values of $0.01 > P > 0.005$ and $0.05 > P > 0.025$, respectively. Comparison of group C with E yielded a P value of $0.40 > P > 0.30$.

100 μg of Dnp-KLH or 100 μg of BGG emulsified in complete Freund's adjuvant, and were used as donors 6–13 weeks later.

Adoptive Transfer System. Spleen cells obtained from Dnp-KLH-primed or BGG-primed mice were used as hapten-primed cells and carrier-specific helper cells, respectively. Single-cell suspensions in minimum essential medium (Eagle) were prepared from the spleens of these donor mice, washed, and then injected intraperitoneally or intravenously into irradiated, syngeneic mice (30 to 50×10^6 cells per mouse). Recipient mice were then challenged intraperitoneally with either 10–100 μg of Dnp-KLH or with 50–200 μg of Dnp-BGG in saline and bled 7 days later. Modifications of this general adoptive transfer scheme are described below.

Irradiation. Mice were exposed to total body irradiation in a Lucite box by use of a General Electric Maximar 250 III x-ray machine. The dose given was 450–600 rads to mid-point with the machine operated under conditions of 250 kV and 15 mA with 0.5-mm Al and 0.5-mm Cu filters. The focal target distance was 56 cm and the absorbed dose rate was 40 R/min.

BGG-primed cells were irradiated either *in vivo* or *in vitro*. *In vivo* irradiation was either by whole-body irradiation (600–1000 rads) of BGG-primed donor mice immediately before removal of the spleen, or by whole-body irradiation (600 rads) of recipient mice that had been injected intravenously with unirradiated BGG-primed cells.

In vitro irradiation of BGG-primed cells was performed under the following conditions: Suspensions of BGG-primed spleen cells were placed into glass-beakers, held in ice, and irradiated at a focal target distance of 38 cm. The absorbed dose rate was 100 R/min. Portions of BGG-primed spleen cells from the same starting pool that were not irradiated were, nevertheless, subjected to identical conditions of handling used for cell portions receiving irradiation. Subsequent to all manipulations, cell viability was identical (89%) for both irradiated and unirradiated cell populations, as shown by trypan blue exclusion.

The integrated radiation-absorption dose was measured by dosimeter in all the experiments.

Measurement of Anti-Dnp Antibodies. Serum anti-Dnp antibody titers were determined by a modified Farr technique (20) with [^{3}H]Dnp-ϵ-amino-*N*-caproic acid (21). With standard curves constructed for individual mouse strains in a manner identical to that described for inbred guinea pigs (19), percentage of binding was converted into amount of anti-Dnp antibody in μg/ml of serum.

Statistical Analysis. Serum antibody titers were logarithmically transformed, and means and standard errors were calculated. Group comparisons were made by Student's t test. In BALB/c and A/J mice, in which no specific antigen binding could be detected in the serum, a value of 0.1 μg/ml and 0.01 μg/ml, respectively, was arbitrarily assigned to allow logarithmic transformation of the data.

RESULTS

Helper Function of Carrier-Specific Lymphocytes Exposed to X-Irradiation before Adoptive Transfer. The initial experiments were designed to test the effects of x-irradiation on the capacity of carrier-primed spleen cells to serve as helper cells for syngeneic hapten-primed lymphocytes in a classical, double-adoptive transfer system. To rule out possible gross

strain differences, experiments were performed in both BALB/c and A/J mice as follows: Spleen cells from Dnp-KLH-primed donor mice were injected either alone or together with spleen cells from syngeneic BGG-primed donors intraperitoneally into irradiated (500 R), syngeneic recipient mice. BGG-primed cell suspensions consisted of unirradiated lymphocytes or lymphocytes that had been exposed to x-irradiation (600–1000 R) either *in vivo* immediately before the donor was killed or *in vitro*. Secondary challenge with either 10 μg of Dnp-KLH or 100 μg of Dnp-BGG in saline, intraperitoneally, was performed immediately after cell transfer, and all mice were bled 7 days later.

The protocols and results of two such experiments are summarized in Tables 1 and 2. In both strains of mice, control animals, which received only Dnp-KLH-primed cells and were then challenged with the homologous conjugate, Dnp-KLH, developed good adoptive secondary anti-Dnp antibody responses, while little or no response was obtained with Dnp-BGG. On the other hand, mice that received unirradiated BGG-primed cells together with Dnp-KLH-primed cells made significantly greater responses to the heterologous conjugate, Dnp-BGG. The capacity of BGG-primed spleen cells to enhance the adoptive secondary response to Dnp-BGG was completely abrogated, however, by exposure of such cells to X-irradiation before transfer. This was true irrespective of the dose of irradiation or whether cells were irradiated *in vivo* in the donor or *in vitro**.

Helper Function of Carrier-Specific Lymphocytes Exposed to X-Irradiation In Situ. The preceding experiments demonstrated quite clearly that the capacity of mouse T lymphocytes to perform their helper role in antibody responses could be abolished by x-irradiation of such cells before adoptive transfer, in agreement with the results of Anderson *et al.* (17). A possible explanation for these findings, however, is that x-irradiation causes subtle changes in the capacity of mouse T lymphocytes to migrate normally to appropriate lymphoid organs for optimal interaction with B lymphocytes. We assessed this possibility by several experimental designs described below.

Radioresistance of Carrier-Specific Helper Cells in Primed Mice. Spleen cells from BALB/c and A/J donor mice, which had been primed with Dnp-KLH 62 days earlier, were injected intravenously (50×10^6 cells per recipient) into syngeneic, irradiated (600 R) recipients. One group of recipients of each strain had been primed with BGG in complete Freund's adjuvant 50 days before x-irradiation and cell transfer, while another group of unprimed recipients were used as controls. All recipients were secondarily challenged with Dnp-BGG in saline, intraperitoneally, immediately after cell transfer and bled 7 days later.

The results are depicted graphically in Fig. 1. In both strains, only meager adoptive secondary anti-Dnp antibody responses to the heterologous conjugate, Dnp-BGG, were obtained in irradiated recipients that were not primed to

* The lower anti-Dnp responses of recipient mice in group E, as compared to groups A and C (Table 2), do not reflect a nonspecific toxic effect of irradiated spleen cells on the Dnp-KLH-primed cells with which they were mixed before adoptive transfer. Appropriate control experiments (not shown) have definitely excluded this possibility.

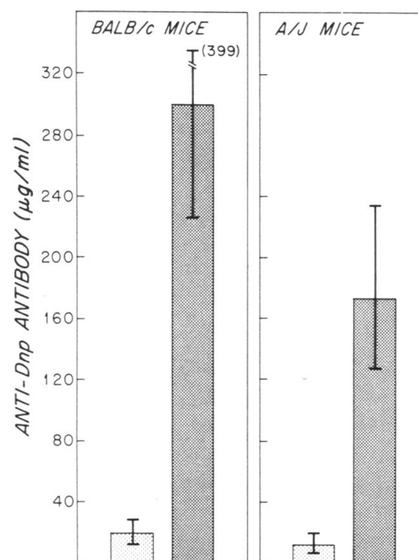

FIG. 1. Radioresistance of carrier-specific helper cells in primed animals. 50×10^6 spleen cells from BALB/c and A/J donor mice immunized with Dnp-KLH 62 days previously were injected intravenously into respective syngeneic, irradiated (600 R) recipients. One group of recipients of each strain had been primed with BGG in complete Freund's adjuvant 50 days before x-irradiation and cell transfer, while another group of unprimed recipients were used as controls. All recipients were boosted with 200 μg of Dnp-BGG intraperitoneally in saline immediately after cell transfer and bled 7 days later. Geometric mean antibody titers of groups of five mice are illustrated. Statistical comparison of BGG-primed and unprimed recipients yielded P values of $0.01 > P > 0.005$ and $0.005 > P > 0.01$ in BALB/c and A/J mice, respectively. ⊡ Irradiated, unprimed recipients; ▩, irradiated, BGG-primed recipients.

BGG. In contrast, highly significant responses to Dnp-BGG were manifested by BALB/c and A/J recipient mice that had been previously primed with the heterologous carrier, BGG. This result, which was consistently reproducible in many such experiments, even when doses of x-irradiation somewhat higher than 600 R were administered, certainly demonstrates that carrier-primed mice are capable of providing helper function for adoptively transferred, hapten-specific lymphocytes, even though such mice have been heavily irradiated.

Function of Carrier-Specific Helper Cells Exposed to x-Irradiation after Adoptive Transfer to Syngeneic Recipients. A difficulty in interpretation of the preceding experiment is raised by the fact that irradiated mice that have been previously primed with BGG in adjuvant have variable quantities of circulating anti-BGG antibodies that could conceivably contribute to the results obtained. For this reason, the experimental design was modified to remove the carrier-primed lymphocytes from the milieu of the native host.

In the initial experiments of this type, spleen cells from BGG-primed BALB/c and A/J donor mice were injected intravenously (50×10^6 cells per recipient) into respective syngeneic recipients. Recipient mice in one group were irradiated (600 R) immediately before transfer of BGG cells, while a second group was subjected to 600 R irradiation 24

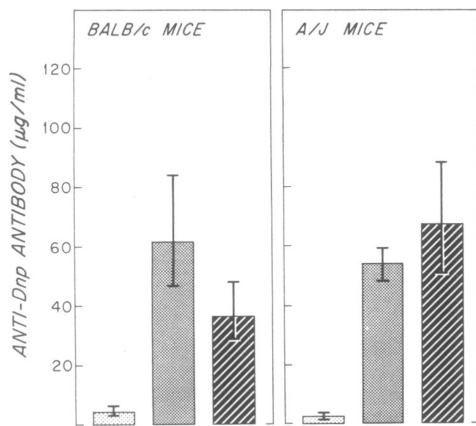

FIG. 2. Radioresistance of carrier-specific helper cells exposed to x-irradiation after transfer into syngeneic recipients. 50×10^6 spleen cells from BALB/c and A/J donor mice, which had been primed with BGG 66 and 39 days earlier, respectively, were injected intravenously into respective syngeneic recipients. Recipient mice in one group were irradiated (600 R) immediately before transfer of BGG cells, while a second group was irradiated 24 hr after the BGG-cell transfer. All recipients were then injected intravenously with 30×10^6 Dnp-KLH-primed spleen cells from respective BALB/c and A/J donor mice 24 hr after the initial BGG-primed cell transfer. Control groups consisted of mice that received Dnp-KLH-primed cells, but had not been injected with BGG-primed lymphocytes. Secondary challenge with 50 μg of Dnp-BGG in saline was administered intraperitoneally. Geometric mean antibody titers of groups of five mice 7 days after secondary challenge are illustrated. Statistical comparisons of recipients of unirradiated BGG cells and BGG cells exposed to irradiation *in situ* 24 hr after adoptive cell transfer yielded P values of $0.30 > P > 0.20$ and $0.50 > P > 0.40$ for BALB/c and A/J mice, respectively. Comparison of experimental and control groups yielded P values of $0.001 > P$ in all cases. ⊡, No BGG cells; ▦, 50×10^6 unirradiated BGG cells; ▨, 50×10^6 BGG cells irradiated *in vivo* 24 hr after *in vivo* adoptive transfer.

hr after receiving BGG-primed lymphocytes. In the latter group, therefore, carrier-primed cells were exposed to irradiation *in vivo* but presumably well after migrating to the lymphoid organs. All recipients were then injected intravenously with 30×10^6 spleen cells from Dnp-KLH-primed donors (of a respective syngeneic strain) 24 hr after the first BGG-primed-cell transfer. Control groups consisted of mice that received Dnp-KLH-primed cells but had not been injected with BGG-primed lymphocytes. Secondary challenge with 50 μg of Dnp-BGG in saline was administered intraperitoneally immediately after transfer of the Dnp-KLH-primed cells.

The anti-Dnp antibody responses 7 days after secondary challenge are depicted in Fig. 2. In both strains, mice in the control groups that did not receive BGG-primed cells failed to respond to Dnp-BGG. In contrast, mice that were injected with BGG-primed lymphocytes developed highly significant, secondary anti-Dnp antibody responses to Dnp-BGG irrespective of whether or not the BGG cells had been exposed to *in vivo* irradiation in the adoptive host. In the experiment illustrated, the responses obtained in BALB/c recipients in whom BGG cells were irradiated after cell transfer were somewhat, though not significantly, lower than those manifested by recipients of unirradiated cells, but this effect was not consistent in other experiments.

It is clearly justifiable to conclude from the preceding experiments that: (*a*) carrier-specific helper activity in hapten-specific antibody responses is functionally radioresistant in carrier-primed animals, and (*b*) helper function of carrier-primed lymphocytes remains intact after exposure to x-irradiation in unprimed recipients of adoptive cell transfer. This is to be contrasted with the results of the first experiments in this report, in which helper function was clearly abrogated by exposure of carrier-specific cells to x-irradiation *before* adoptive transfer. The apparent discrepancy here may be reasonably explained by the induction of a subtle change in the migratory capacity of such cells as a result of x-irradiation.

In order to obtain more convincing evidence for this interpretation, an experiment was designed in which it was possible to demonstrate a differential effect of x-irradiation on the helper function of adoptively transferred, carrier-specific lymphocytes. Spleen cells from BGG-primed, BALB/c donor mice were injected intravenously (50×10^6 cells per recipient) into various groups of syngeneic recipients as follows: Three groups of recipients were irradiated *before* adoptive cell transfer and then injected with: Group 1—no BGG-primed cells; Group 2—unirradiated BGG-primed cells; Group 3—BGG-primed cells that had been exposed to irradiation (600 R) *in vivo* in the donor mice immediately before the mice were killed. Two other groups of unirradiated recipients were in-

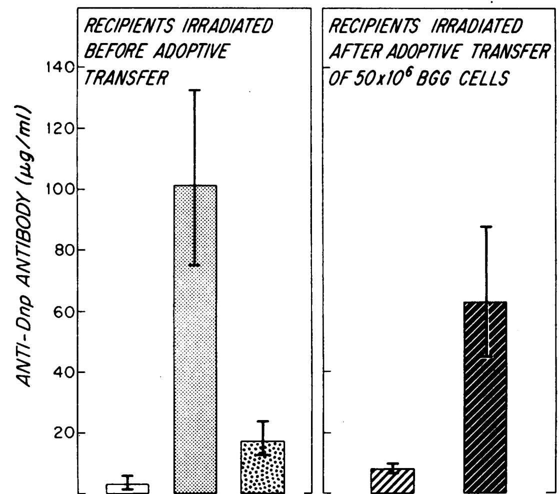

FIG. 3. Function of carrier-specific helper cells exposed to x-irradiation before or after adoptive transfer. 50×10^6 spleen cells from BGG-primed BALB/c donors were injected intravenously into various groups of syngeneic recipients. Three groups of recipients (*left panel*—Groups 1–3) were irradiated (600 R) before adoptive transfer with the cells indicated. Group 1, □, no BGG cells; Group 2, ⊡, 50×10^6 BGG cells unirradiated; Group 3, ▦, 50×10^6 BGG cells irradiated *in vivo* in donor mice. Two other groups of unirradiated recipients (*right panel*) were injected with 50×10^6 BGG-primed cells and then irradiated (600 R) immediately (▨, Group 4) or 24 hr (▨, Group 5) after transfer. All recipients were injected with 30×10^6 Dnp-KLH-primed spleen cells from syngeneic donors 24 hr after the first BGG cell transfer and then challenged with 50 μg of Dnp-BGG. Geometric mean antibody titers of groups of five mice on day 7 after secondary challenge are illustrated. Statistical comparisons of the various groups yielded the following P values: Groups 2 and 3, $0.005 > P > 0.001$; Groups 4 and 5, $0.001 > P$; Groups 2 and 5, $0.40 > P > 0.30$.

jected with unirradiated BGG-primed cells (from the same starting cell suspension used for Group 2), and then subsequently irradiated with 600 R at the following times: Group 4—immediately (less than 10 min) after cell transfer, Group 5—24 hr after cell transfer. All recipients were injected intravenously with Dnp-KLH-primed spleen cells from syngeneic donors 24 hr after the first BGG-cell transfer, then secondarily challenged with 50 μg of Dnp-BGG in saline, intraperitoneally, immediately thereafter.

As shown in the left panel of Fig. 3, recipients of unirradiated BGG-primed cells in Group 2 developed significant secondary anti-Dnp antibody responses by day 7 after challenge with Dnp-BGG, as compared to control mice in Group 1. On the other hand, as shown earlier, recipients of BGG cells irradiated *in vivo* in the original donors (Group 3) manifested significantly lower responses than those obtained in Group 2 recipients. When unirradiated, BGG-primed lymphocytes were exposed to x-irradiation *after* transfer to adoptive hosts, a very different picture emerged, dependent on how soon after transfer x-irradiation was administered (*right panel* of Fig. 3). Thus, exposure of recipients to x-irradiation immediately after injection of BGG cells abolished the capacity to develop a secondary response to Dnp-BGG (Group 4). In contrast, recipients in Group 5 that were not subjected to x-irradiation until 24 hr after the transfer of BGG-primed cells developed significantly higher secondary anti-Dnp antibody responses. This result, which has been consistently obtained in additional experiments of this type in both BALB/c and A/J mice, strongly indicates that x-irradiation exerts an undefined effect on mouse lymphocytes that is independent of the mechanism by which helper-cell function occurs.

DISCUSSION

In the early studies of thymus–bone marrow synergism in response to sheep erythrocyte antigens in mice, the evidence obtained indicated that both T- and B-cell components were sensitive to x-irradiation. Thus, Claman and co-workers (9, 10), and Miller and Mitchell (11, 12) observed that x-irradiation of T cells either *in situ* or *in vitro* abrogated the functional capacity of such cells to collaborate with B cells. However, the experimental protocols used by these investigators (9–12) required a crucial period of proliferation and differentiation by specific T cells in the presence of antigen, as shown by their own data (10) and by studies of others (22), before such cells could effectively facilitate the antigen-induced activity of normal B cells. Under these circumstances, any inhibitory influence on mitotic activity during this crucial period by x-irradiation or antimitotic drugs would understandably abolish the helper function of the T cells.

In contrast, under conditions where T-cell proliferation is not required, because the cell population has already been expanded and differentiated by prior immunization, considerable evidence has been obtained demonstrating that helper function is not affected by x-irradiation. This is well exemplified in our earlier studies in guinea pigs (13), in which the capacity of BGG-primed lymphoid cells to perform a helper function in enhancing secondary anti-Dnp antibody responses of syngeneic recipients was not affected by exposure of such cells to high doses of x-irradiation (1000–5000 R) *in vitro* before cell transfer. The antibody-forming capacity of such cells, on the other hand, was completely abolished by the x-irradiation, thus illustrating a functional distinction between T- and B-lymphocytes with respect to radiation effects. Comparable observations were made in, and the same interpretation applies to, studies of *in vitro* antibody responses (14–16).

As noted in the *Introduction*, markedly different results were obtained in the recent studies of Anderson *et al.* (17) on radiation effects on the capacity of mouse T lymphocytes to collaborate with B cells *in vivo*. In the latter studies, exposure of carrier-primed thoracic-duct lymphocytes, antigen-activated T cells, or spleen cells to 1000 rads *in vitro* before adoptive transfer virtually abolished the capacity of such cells to collaborate with hapten-primed B lymphocytes. On the basis of organ-distribution studies with ^{51}Cr-labeled, normal thoracic-duct lymphocytes, these investigators concluded that no significant difference existed between unirradiated and irradiated cells in the initial localization pattern after adoptive transfer; nevertheless, a difference in the capacity of the respective cells to migrate to other lymphoid organs was detected subsequent to 4 hr after transfer (17).

In the present studies, we have also found that the capacity of carrier-primed mouse lymphoid cells to serve as helpers in the development of anti-Dnp antibody responses can be abolished by exposure of such cells to x-irradiation before adoptive transfer. However, the evidence from our experiments clearly indicates that something *other than* the helper function of primed mouse T cells is predominantly affected by x-irradiation. This interpretation derives from the findings, described herein, that (*a*) carrier-specific helper function remains intact in carrier-primed animals that have been x-irradiated; and (*b*) the capacity of adoptively transferred, carrier-primed lymphocytes to perform a helper role is not abolished by exposure to x-irradiation, provided such exposure is made *in vivo* in the adoptive host at a sufficient time *after* cell transfer. Thus, in the latter situation, helper function is abolished by x-irradiation of the recipient immediately, but *not* 24 hr, after cell transfer. This observation implies that x-irradiation before, or during, the initial period of migration in the adoptive host can profoundly influence the functional expression of mouse T lymphocytes, whereas exposure to irradiation *in situ* after a suitable period of migration has elapsed does not abolish the helper function of such cells.

We feel that these results clarify the existing contradictory observations concerning radiation effects on T lymphocytes. Firstly, the experimental results obtained in the guinea pig studies cited above (13) were remarkably clear and consistent in many such experiments performed in that species. The recent suggestion (17) that perhaps those data reflected the activity of a small number of carrier-primed cells that had escaped from the effects of irradiation appears very unlikely in view of the fact that only a threshold number of cells required to obtain the helper effect in guinea pigs were used. Secondly, the observations on the capacity of radioresistant mouse T lymphocytes to exert helper function *in vitro* (14–16) have been repeatedly confirmed. It is very improbable that these results reflect, as recently suggested (17), the release from irradiated T cells of adjuvant-like substances in view of the fact that appropriate controls in those studies demonstrated specificity requirements for the carrier-primed lymphocytes. Finally, there have been several previous demonstrations of functional radioresistance among T cells

participating in cell-mediated immune reactions in mice (23), guinea pigs (24), and rats (25).

It seems valid, therefore, to conclude that T-cell function, in cellular immunity as well as in antibody production, does not require proliferation once the initial antigen-induced process of differentiation and clonal expansion has occurred. The radiosensitivity observed by Anderson *et al.* (17) appears to be explained by undefined, and subtle, effects of x-irradiation on the migratory capacity of mouse T lymphocytes and not by abrogation of T-cell helper function. Where experimentalists or clinicians wish to make use of the functional difference in sensitivity to x-irradiation of T- and B-cells in transfer or repopulation models, it will be crucial to bear this in mind.

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