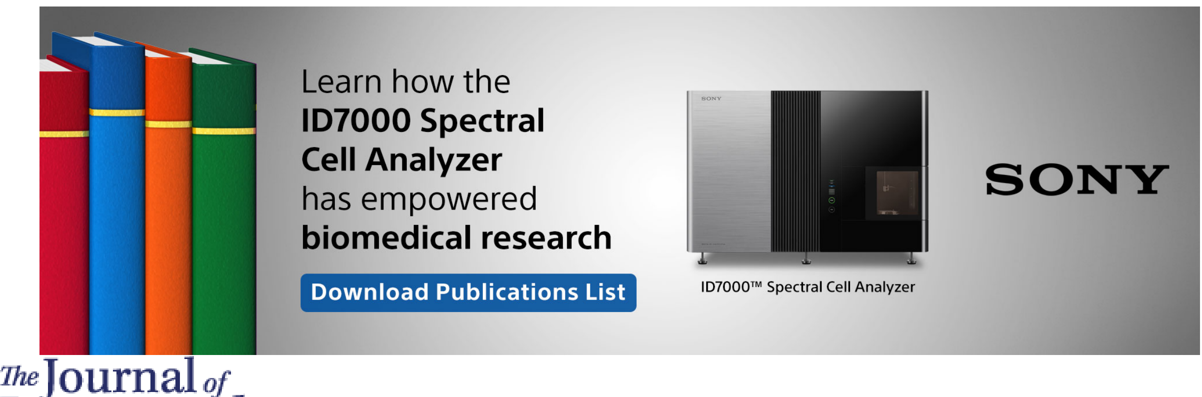


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# RADIOSENSITIVITY OF THE HELPER CELL FUNCTION[1]

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**The helper function of T cells primed and irradiated *in vivo* was tested *in vitro* by the Mishell-Dutton technique. Spleen cells from mice carrier-primed with HRBC and exposed to 50 to 2000 rads of x-radiation were assayed for their ability to help syngeneic normal spleen cells to mount an *in vitro* anti-hapten antibody response after stimulation with the conjugate TNP-HRBC. The anti-TNP response was evaluated by the Jerne technique. The helper activity was titrated by adding graded numbers of carrier-primed spleen cells to a constant number of normal spleen cells. The slope of the initial linear portion of the response-cell dose titration curve was taken as an estimate of the helper activity and found to decrease with increasing the x-ray dose. The curve describing the remaining helper activity as a function of the radiation dose shows the presence of two components, one radiosensitive, the other, radioresistant. This suggests the existence either of helper cells at different stages of activation or of two cell subpopulations participating in the helper function.**

The radiosensitivity of T cell helper function has been studied in a variety of experimental conditions (reviewed in 1). Early studies (2–4) demonstrated that the capacity of unprimed T lymphocytes to promote antibody production against T-dependent antigens was abrogated by x-irradiation. As to primed T cells, their helper activity was found radiation resistant when tested *in vitro* (5–7), but controversial results were obtained when tested *in vivo* (7–13). Indeed, the helper function of primed T cells was found mostly radiosensitive when tested upon adoptive cell transfer (7, 9–11) although radioresistant when assayed *in situ* (7, 9, 11–13). All these data suggest that radiation alters the migration or homing properties of primed T cells (9) rather than damaging the cellular capacity to display helper function. Thus, the antigen-induced generation of helper T cells, which involves cell division (14), seems to be radiosensitive, whereas the helper activity of already activated T cells would be radioresistant.

The aim of the present study was to analyze the radioresistance of the helper function of primed T cells in order to establish a dose-response relationship under conditions excluding any influence of radiation damage on cell migration properties. The helper activity of T cells, primed and irradiated *in vivo*, was tested *in vitro*. Spleen cells from mice carrier-primed and exposed to 50 to 2000 rads were assayed for their ability to help syngeneic normal spleen cells to mount an *in vitro* anti-hapten antibody response after stimulation with the conjugate.

## MATERIALS AND METHODS

*Animals, their priming and irradiation.* Female BDF$_1$ (C57BL/10 × DBA/2)F$_1$ mice, raised in our own colony and aged 10 to 12 weeks, were used throughout the present study. For induction of helper cells, mice were carrier-primed by i.v. injection of $2 \times 10^5$ horse red blood cells (HRBC)[2] in 0.2 ml PBS 4 days before sacrifice. Primed mice were total-body irradiated in a Lucite irradiation chamber immediately before sacrifice. The x-ray machine (Siemens Stabilipan) was operated at 250 kV, 15 mA, 0.5 mmCu filtration; dose rate 133.3 rad/min in air, focus distance 50 cm. Each radiation dose, ranging from 50 to 2000 rads was delivered to groups of five mice.

*Cell culture.* Mouse spleen cell suspensions were prepared and cultured in microtissue culture plates (Falcon No. 3040) by the method of Mishell and Dutton (15) as modified by Kettman and Dutton (16) for immunization with TNP-erythrocytes.

*Titration of helper activity.* A modification of the experimental model of Kettman and Dutton (5) was used to assay the level of helper activity in mice carrier-primed and exposed to varied doses of x-irradiation immediately before the sacrifice. Normal spleen cells immunized *in vitro* with TNP-HRBC respond poorly to TNP because of the low concentration of HRBC-specific helper T cells. The addition of spleen cells from HRBC-primed mice enhances their anti-TNP response. In common with others (5, 17), we have found that if graded numbers of carrier-primed spleen cells are added to a constant number of normal spleen cells, the anti-TNP response increases linearly with the number of primed cells in the range where helper activity is limiting. Therefore, the slope of the initial linear portion of the titration curve can be taken as an estimate of the helper T cell activity in the spleen of the primed animal.

In each experiment, pooled spleen cells from 10 to 15 normal, or five unirradiated carrier-primed, or five primed and irradiated mice were used. One volume of the normal spleen cell suspension ($40 \times 10^6$ nucleated cells/ml medium) was added to an equal volume of a TNP-HRBC suspension ($8 \times 10^6$ erythrocytes/ml). Aliquots of this mixture were added to equal volumes of medium alone or containing carrier-primed unirradiated or irradiated spleen cells ranging from 1 to $5 \times 10^6$ cells/ml. Thereafter, 0.1 ml samples of each final suspension, containing $2 \times 10^5$ TNP-HRBC, $1 \times 10^6$ normal cells, and 0.5 to $2.5 \times 10^5$ primed cells, were distributed in at least 12 wells of the culture plates and incubated at 37°C. At days 4, 5, and 6 of culture, the cells harvested from four wells from the same group were pooled, washed, and assayed for the number of anti-TNP direct PFC by using the Jerne technique (18) and TNP-SRBC (19) as test antigen. The response was expressed as number of anti-TNP PFC per culture well. The number of anti-TNP PFC

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[2] Abbreviations used in this paper: HRBC, horse red blood cells.



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produced by normal spleen cells alone was subtracted from each experimental point, and then a straight line was fitted for each titration by a least square method that forces the line through the origin (20).

This titration assay requires that primed cells be limiting and that their content in B cells be irrelevant to the outcome. That normal spleen cells were in excess was shown by the same anti-TNP response obtained when $3 \times 10^5$ primed cells were added to 1 or $1.5 \times 10^6$ normal cells. The irrelevance of the B cell component of primed cells was demonstrated by the same anti-TNP response of $1 \times 10^6$ normal cells cultured with $3 \times 10^5$ primed cells untreated or pretreated with rabbit anti-mouse Ig serum and C. This treatment was carried out as previously described (21) and was shown to be very effective in abrogating the *in vitro* primary response of normal spleen cells to SRBC.

## RESULTS

The level of helper activity in mice preimmunized with HRBC and exposed to x-irradiation was assayed by titrating the ability of their spleen cells to cooperate with normal cells in the humoral response to TNP. In each experiment, titration curves obtained with spleen cells from mice exposed to various doses of x-rays were compared to the titration curve obtained with spleen cells from unirradiated donors.

The results of four experiments are reported in Figure 1. In all experiments the slope of the titration line decreases by increasing the x-ray dose delivered to the primed cell donors, indicating that irradiation impairs the helper activity of primed spleen cells.

To quantitate the radiosensitivity of primed spleen cells, the ratio of the slope of the titration line for the irradiated group to that for the unirradiated control was calculated for each radiation dose and reported in Table I. These ratios, representing the remaining helper activity after irradiation, are plotted against radiation dose on a semilog scale in Figure 2. As can be seen in Figure 2, there is a fairly rapid decrease of helper activity down to a level of about 40% of control at a dose of 400 rads, followed by a much shallower decrease with doses up to 2000 rads. These data suggest the presence in the primed spleen cell population of at least two components, one radiosensitive, the other very radioresistant.

## DISCUSSION

The present study shows that activated helper T cells are much more radiosensitive than expected (5–7) since the helper activity of carrier-primed spleen cells was progressively impaired to 40% of control by increasing the radiation dose up to 400 rads. Moreover, the helper cell population was found to be

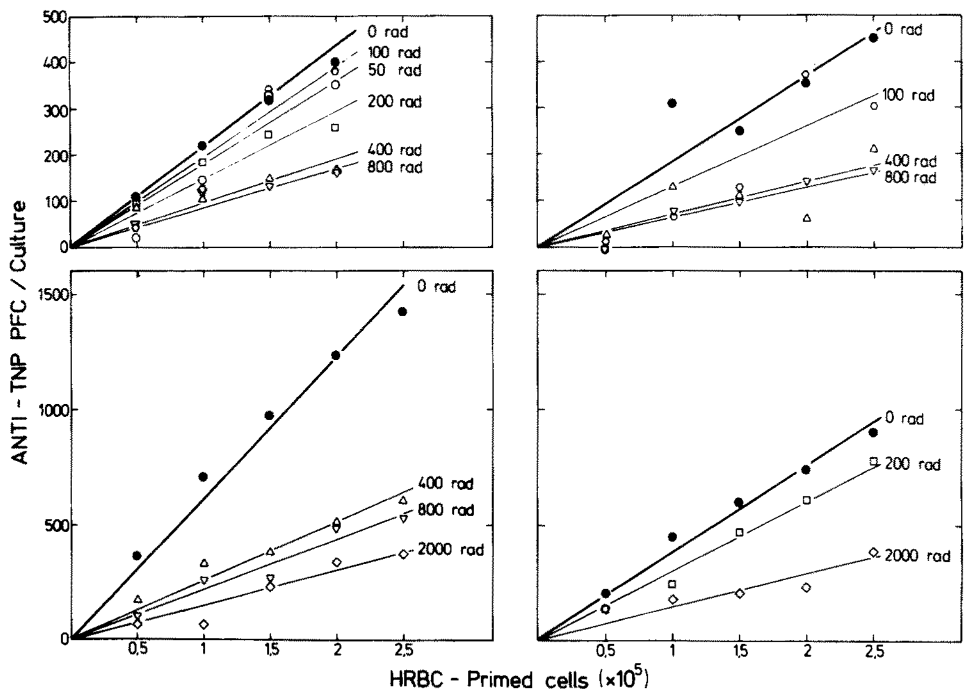


*Figure 1.* Radiation effects on helper activity of spleen cells from carrier-primed mice. Graded numbers of spleen cells from mice HRBC-primed, unirradiated, or irradiated with 50 to 2000 rads were added to $1 \times 10^6$ normal spleen cells and $2 \times 10^5$ TNP-HRBC. Peak responses from four experiments: A (*upper left*) at day 5; B (*upper right*) at day 4; C (*lower left*) at day 4; D (*lower right*) at day 5. Numbers of anti-TNP PFC/culture produced by normal spleen cells alone: 55 in experiment A; 353 in experiment B; 312 in experiment C; 160 in experiment D. Each of these numbers was subtracted from all points of the same experiment before fitting a straight line through the origin.

TABLE I

*Radiation effects on the regression slope of the anti-TNP response on HRBC-primed cells*

| Radiation Dose (rads) | | 0 | 50 | 100 | 200 | 400 | 800 | 2000 |
|---|---|---|---|---|---|---|---|---|
| Expt. A | b[a] | 216 ± 10 | 181 ± 21 | 194 ± 18 | 148 ± 12 | 96 ± 11 | 92 ± 8 | |
| | R[a] | 1.00 | 0.84 | 0.90 | 0.68 | 0.44 | 0.43 | |
| Expt. B | b | 182 ± 23 | | 126 ± 20 | | 70 ± 14 | 65 ± 9 | |
| | R | 1.00 | | 0.69 | | 0.38 | 0.36 | |
| Expt. C | b | 610 ± 22 | | | | 256 ± 13 | 220 ± 12 | 153 ± 11 |
| | R | 1.00 | | | | 0.42 | 0.36 | 0.25 |
| Expt. D | b | 378 ± 12 | | | 301 ± 10 | | | 144± 13 |
| | R | 1.00 | | | 0.80 | | | 0.39 |

[a] b = regression slope ± standard error; R = b irradiated/b unirradiated.

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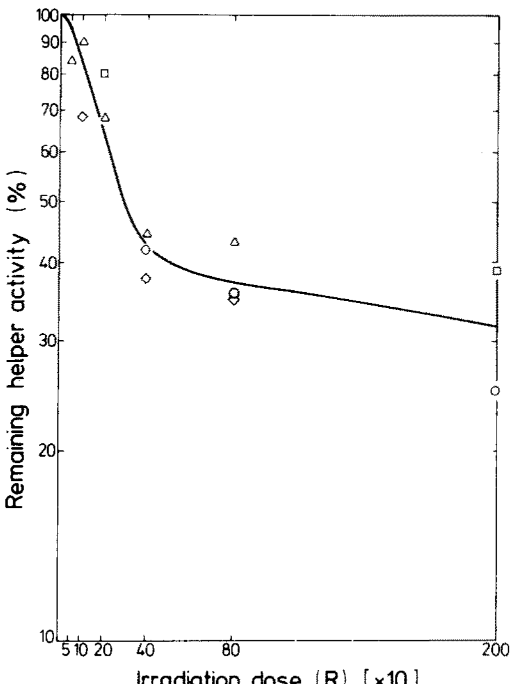


*Figure 2.* Radiosensitivity of helper activity. Each point represents the ratio of the slopes of the titration lines for the irradiated (R stands for rad) groups to that of the unirradiated group, expressed as percentage of the helper activity remaining in the primed spleen cells after each radiation dose. △, experiment A; ◇, experiment B; ○, experiment C; □, experiment D from Figure 1 and Table 1.

heterogeneous in that a sizable subpopulation of it resisted radiation doses up to 2000 rads (Fig. 2).

It has been suggested that activated T cells, in cellular as in humoral immunity, might be protected from interphase death because of their hypermetabolic state (8, 22). Activated cells, however, should not be protected from radiation-induced mitotic death. Thus, our results suggest the presence in the carrier-primed animal of helper cells that are still dividing (radiosensitive fraction) and of helper cells that have already been through proliferation (radioresistant fraction). Hence, helper cells might have different radiosensitivities depending on the time at which they are withdrawn from the carrier-primed donors, similarly to what was reported by Denham *et al.* (23) for effector cells in cell-mediated immunity.

Another explanation is based on the possibility that two different T cells are involved in the helper function, both mature T cells but with different radiosensitivities. One type of T cell (helper) may be required for clone activation, the other (amplifier) for clone expansion. Since the discovery of amplifier T cells (24), the participation of helper and amplifier cells in the response to T-dependent antigens has been supported by several experiments. North and Feinstein (25) observed that both the size and number of splenic foci of antibody-forming cells could be increased by increasing the T cell number. Marrack and Kappler (26) showed the existence of B cell-stimulating factors produced by two different T cell types. Moreover, a T-T interaction in carrier-primed cell populations has been postulated by Janeway (27) to explain the helper cell "premium effect" observed during the in *vivo* anti-hapten antibody response. More recently, the concept of T-T interactions in positive regulation of antibody production has been reinforced by the suggestion that an amplifier factor (TaF) secreted by Ly1 cells could transform Ly1,2,3 cells into helper cells (28).

Whether the differential radiosensitivity of carrier-primed cells reflects the existence of one cell type at different stages of activation or two cell types with different functions is currently under investigation in our laboratory.

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