CELL-TO-CELL INTERACTION IN THE IMMUNE RESPONSE

VIII. RADIOSENSITIVITY OF THYMUS-_DERIVED LYMPHOCYTES*

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Noncycling small lymphocytes are generally acknowledged to undergo interphase death after exposure to ionizing radiation (1). Recently, however, several studies have suggested marked radioresistance of the helper function of thymus-derived (T)
lymphocytes in systems requiring collaboration between these cells and nonthymus-derived (B) lymphocytes (2, 3). Among the possible explanations for this resistance may be listed the following. (a) The T cell pool contains a subpopulation of radioresistant cells which mediate helper functions. (b) Antigen-activated helper T cells (ATC [4]) are protected from interphase death by their heightened metabolic activities which serve to repair or prevent radiation damage (e.g., by stabilizing cell membranes). (c) Helper functions may require passive rather than active lymphocyte participation. Injured cells, or perhaps even subcellular components released at the time of cell death, might be effective.

The following experiments were performed to test the above possibilities and to investigate further the radiosensitivity of the helper function of T cells in antibody responses.

Materials and Methods

Mice.—Highly inbred CBA, C57BL, and AKR strains and F₁ hybrids from crosses between CBA and C57BL were used (5).

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Abbreviations used in this paper: ATC, activated thymus cells; B, nonthymus-derived; F\textsubscript{yG}, fowl immunoglobulin G; NNP, 4-hydroxy-3,5-dinitrophenylacetie acid; OVA, ovalbumin; PFC, plaque-forming cells; 7S PPC, indirect plaque-forming cells; T, thymus-derived; TDL, thoracic duct lymphocytes; T\textsuperscript{3}H, tritiated thymidine.
Cell Suspensions.—Single cell suspensions of thymus and spleen, and thoracic duct lymphocytes (TDL) were obtained as previously described (5).

Antigens.—Fowl immunoglobulin G (FyG) and 4-hydroxy-3,5-dinitrophenylacetic acid (NPN) were prepared as described elsewhere (6, 7). Crystalline ovalbumin (OVA) was obtained from Calbiochem, Los Angeles, Calif. NNP-azide was conjugated onto the proteins FyG or OVA (7); the substitution ratios were generally 10 hapten groups per molecule of protein.

Immunizations.—100–500 μg of alum-precipitated protein or hapten-protein conjugates were injected together with 2 × 10^{9} killed pertussis organisms\(^2\) intraperitoneally for immunization (5). In some cases 100 μg of fluid antigen was injected intraperitoneally as a challenge.

Detection of Antibody-Forming Cells.—Plaque-forming cells (PFC) in spleen cell suspensions were detected according to the methods of Cunningham and Szenberg (8), Miller and Warner (6), and Pasanen and Miikeli (9).

Anti-θ Serum.—Antiserum against θ-C3H was raised in AKR mice (10) and used according to conditions detailed elsewhere (5).

Irradiation.—Details of the irradiation technique for mice have been given before (5). Cell suspensions were briefly aerated in a Lucite chamber and then irradiated under conditions similar to those employed for mice but at a lower dose rate: 122 R/min instead of 170 R/min. They were injected intravenously within 10 min after irradiation.

Radioisotopic Techniques.—\(^{51}\text{Cr}\) as Na\(^{51}\text{CrO}_{4}\) in isotonic saline (specific activity 150 μCi/mg Cr) and tritiated thymidine (\(^{3}\text{H}\text{tdT}\)) in sterile aqueous solution (specific activity 156.3 μCi/mg) were obtained from the Radiochemical Centre, Amersham, England. TDL were labeled with \(^{51}\text{Cr}\) and their localization was determined at serial intervals, as previously described (11). Levels of radioactivity in the spleens of irradiated mice pulsed with \(^{3}\text{H}\text{tdT}\) were determined as described elsewhere (4).

Statistical Analyses.—Calculations of the geometric means, upper and lower limits of the SE and \(F\) values according to the randomization test (12), were performed using an IBM 7044 computer. In comparison of the means of any two groups of observations, a significance level of 0.05 was chosen.

RESULTS

Parental strain TDL, injected into heavily irradiated (800 rads) F\(_{1}\) hybrid mice, proliferate in the lymphoid tissues as a result of activation by foreign histocompatibility antigens. The magnitude of this response can be quantitated by determining splenic radioactivity after an intravenous pulse of \(^{3}\text{H}\text{tdT}\) given 4 days after TDL transfer and 30 min before killing (4). Table I shows the effect of irradiating the TDL in vitro on their proliferation in vivo. \(^{3}\text{H}\text{tdT}\) incorporation was markedly reduced when the dose was as low as 300 rads and was abolished when it was 1000 rads. This was evident even when large numbers of TDL (6.25 × 10\(^{9}\)) were injected. There is thus no evidence for a subpopulation of radioresistant lymphocytes able to proliferate after exposure to 1000 or 5000 rads.

To determine whether irradiated TDL reached the spleen, \(^{51}\text{Cr}\)-labeled TDL, unirradiated or exposed to 1000 rads, were injected intravenously and their distribution was examined (Table II). Irradiation had minimal effects on cell localization 4 hr after injection. Subsequently, radioactivity levels in the spleen

\(^{2}\) Pertussis vaccine (4 × 10^{9} killed organisms/ml), Commonwealth Serum Laboratories, Melbourne, Australia.
### TABLE I

| Radiation dose (rads) | No. of TDL administered \((X 10^{-6})\) | 1 | 5 | 25 | 62.5 |
|-----------------------|--------------------------------------|---|---|----|-----|
| 0                     | 408,386 ± 63,089                     |   |   |    |     |
| 50                    | 389,159 ± 33,682                     |   |   |    |     |
| 100                   | 303,571 ± 27.699                     |   |   |    |     |
| 200                   | 120,541 ± 8040                       |   |   | 371,227 ± 29,622 |
| 300                   | 36,563 ± 985                         |   |   |     |     |
| 500                   | 22,169 ± 1751                        |   |   | 20,028 ± 1503 |
| 1000                  | 47,711 ± 17,749                      |   |   | 27,167 ± 385 |
| 5000                  | 22,289 ± 1928                        |   |   | 19,680 ± 4877 |

*Results expressed as total disintegrations per minute per F1 spleen 4 days post-TDL injection and 30 min after the mice received an intravenous pulse of 25 μCi TH. Each figure represents the arithmetic mean \((±SE)\) of the activity obtained from four such spleens. Background disintegrations when no TDL were injected = 21,543 ± 140.

### TABLE II

| TDL used | Radiation dose \((rads)\) | Interval between administration of \(^{51}Cr\)-TDL and sacrifice (hr) | Per cent specific \(^{51}Cr\) in: | Liver | Spleen | Mesenteric lymph node | Stomach and small intestine | Spleen lymph node |
|----------|--------------------------|-----------------------------------------------------------------|-------------------------------|------|--------|----------------------|---------------------|-----------------|
| Normal CBA TDL  | 0                      | 4                                                               | 8.0                           | 17.8 | 8.9    | 1.7                  |                     |                 |
| 50        | 24                     | 16.2                                                             | 22.0                          | 16.6 | 5.3    | 0.7                  |                     |                 |
| 100       | 48                     | 13.6                                                             | 13.2                          | 13.6 | 5.7    | 1.0                  |                     |                 |
| 200       | 72                     | 13.4                                                             | 13.4                          | 13.4 | 5.7    | 1.0                  |                     |                 |
| Normal CBA TDL 1000  | 4                      | 7.5                                                             | 27.4                          | 27.4 | 5.7    | 2.2                  |                     |                 |
| 24        | 24                     | 20.8                                                             | 10.6                          | 10.6 | 5.7    | 2.0                  |                     |                 |
| 48        | 48                     | 21.2                                                             | 10.7                          | 10.7 | 5.7    | 2.0                  |                     |                 |
| 72        | 72                     | 16.3                                                             | 10.4                          | 10.4 | 5.7    | 1.6                  |                     |                 |
| BALB/c activated CBA T.TDL*  | 0                      | 4                                                               | 11.4                          | 21.6 | 4.5    |                     |                     |                 |
| BALB/c activated CBA T.TDL* 1000  | 4                      | 13.7                                                             | 22.7                          | 22.7 | 4.5    |                     |                     |                 |

* This lymphocyte population consists almost entirely of H-2-activated blast cells (4). For such distribution studies, it was not possible to obtain a population of FγG-activated T cells uncontaminated by other cell types.

and lymph nodes remained virtually unchanged in recipients of irradiated TDL. By contrast, marked reciprocal changes in radioactivity levels in these organs occurred from 4 to 24 hr in mice given normal TDL; by 24 hr and later the levels were as high in the nodes as in the spleen. This implies that irradiated
TABLE III
Effect of In Vitro Irradiation of Carrier-Specific TDL on Antibody Response

| Group | No. and source | Radiation dose (rads) | No. of irradiated recipients* | 7S PFC/spleen† | F values (cf. group 2) |
|-------|----------------|-----------------------|-----------------------------|----------------|-----------------------|
|       |                |                       |                             | FYG            | NNP                   |
| 1     |                |                       | 4                           | 40 (15-10)     | 230 (1820-39)         | <0.005 <0.05 |
| 2     | 10⁶ FYG-primed TDL | 1000                  | 7                           | 18,030 (23,410-13,890) | 88,878 (116,129-68,020) | — —         |
| 3     | 10⁶ FYG-primed TDL | 1000                  | 6                           | 2 (3-1)        | 70 (200-30)           | <0.005 <0.05 |
| 4     | 10⁷ FYG-primed TDL | 1000                  | 7                           | 10 (20-30)     | 50 (100-20)           | <0.005 <0.05 |
| 5     | 5 x 10⁷ FYG-primed TDL | 1000                | 3                           | 9 (1-1)        | 5 (10-2)              | <0.005 <0.05 |

* Given 6 x 10⁶ viable anti-O serum-treated spleen cells from NNP.OVA-primed CBA mice intravenously and 100 µg of fluid NNP.FTG intraperitoneally at time of TDL transfer.
† Geometric means, upper and lower limits of SE.

TABLE IV
Effect of In Vitro Irradiation of Carrier-Specific ATC on Antibody Response

| Group | T cells given | Radiation dose (rads) | No. of irradiated recipients* | 7S PFC/spleen† |
|-------|---------------|-----------------------|-----------------------------|----------------|
|       |               |                       |                             | FYG            | NNP                   |
| 1     |               |                       | 4                           | 4150 (290-63)  | 1650 (649-429)         |
| 2     | 6 x 10⁶ FYG ATC | —                     | 8                           | 2970 (2010-2210) | 26,860 (38,860-17,500) |
| 3     | 6 x 10⁶ FYG ATC | 1000                  | 8                           | 10 (380-30)    | 4650 (4710-4999)       |
| 4     | 10⁷ FYG 6 day primed spleen cells | — | 11,640 (14,440-9390) | 297,230 (339,590-260,150) |
| 5     | 10⁷ FYG 6 day primed spleen cells | 1000 | 7                           | 44,140 (61,590-52,200) | 1120 (2150-583) |
| 6     | 10⁷ FYG 6 day primed spleen cells | — | 4                           | 44,140 (61,590-52,200) | — |
| 7     | 10² FYG 6 day primed spleen cells | 1000 | 5                           | 0              | —                     |

* Mice of groups 1-5 received intraperitoneally, at time of cell transfer, 5 x 10⁶ anti-O serum-treated spleen cells from NNP.OVA-primed CBA mice and 100 µg of fluid NNP.FY. Mice of groups 6 and 7 received only 100 µg of FYG intraperitoneally at time of cell transfer.
† Geometric means, upper and lower limits of SE. Anti-NNP PFC values in group 2 were significantly higher than those in groups 1 (P < 0.05) and 3 (P < 0.005). Anti-FY PFC values in group 2 were significantly higher than those in groups 1 and 3 (P < 0.005). Anti-NNP PFC and anti-FY PFC values in group 4 were significantly higher than those in group 5 (P < 0.005). Anti-NNP PFC values in group 1 were significantly lower than those in group 4 (P < 0.003) but not significantly different from those in groups 3 and 5.

TDL can home normally to the spleen but are less well equipped than normal TDL to migrate subsequently to other lymphoid tissues. Likewise, irradiation of antigen-activated TDL did not impair their spleen-seeking properties.

The effects of irradiating carrier-primed T cells on their capacity to collaborate with hapten-primed B cells in vivo in response to the appropriate hapten-carrier conjugate was investigated. TDL from CBA mice, primed to FYG 5
months before, were exposed to 1000 rads in vitro and various cell numbers injected intravenously into irradiated (800 rads) recipients together with anti-\(\theta\) serum-treated spleen cells from CBA mice primed to NNP.OVA 6 wk before. Anti-\(\theta\) serum was used to abrogate any possible overriding of the carrier effect, a phenomenon previously shown to be dependent on the existence of T cells in the spleens of the hapten-primed mice (13). The irradiated recipients were challenged with NNP.F\(\gamma\)G and their PFC responses to both F\(\gamma\)G and NNP were determined 7 days later. As shown in Table III, the helper function of carrier-primed TDL was abolished by exposure to 1000 rads in vitro. This dose also inhibited any anti-F\(\gamma\)G antibody-forming capacity of the primed TDL population.

The radiosensitivity of ATC was next investigated. Irradiated CBA mice were given \(2 \times 10^8\) thymus cells (\(1/2\) intraperitoneally and \(1/2\) intravenously) and 100 \(\mu\)g of alum-precipitated F\(\gamma\)G. Six days later their spleens were used as a source of ATC. These were left untreated or exposed in vitro to 1000 rads, and then injected intravenously together with anti-\(\theta\) serum–treated spleen cells from NNP.OVA-primed mice into irradiated (800 rads) CBA mice which were challenged with NNP.F\(\gamma\)G. The NNP and F\(\gamma\)G PFC responses obtained 7 days later are shown in Table IV. It is evident that the capacity of ATC to collaborate was abolished by irradiation. Similar results were obtained if, instead of ATC, spleen cells from normal CBA mice recently primed to F\(\gamma\)G (6 days before) were provided.

DISCUSSION

The radiation dose used in the present experiments inhibited T cell proliferation (resulting from H-2 activation [4]), abrogated helper functions, but did not impair spleen homing properties. These findings are in general agreement with our previous results on mitomycin C sensitivity of T cells from unprimed or primed mice (13). They must, however, be reconciled with reports that heavily irradiated lymphoid cells retained helper activity in vivo (2) or in vitro (3, 14, 15). In the in vivo experiments, primed guinea pig lymphoid cells were given 5000 rads and transferred; antibody-forming capacity was lost but helper activity retained. Since very large numbers of cells were used (10\(^9\)), it is conceivable that some escaped from the effects of irradiation. In fact, recent observations suggest that primed cells, incubated with radioactive antigen, lost their helper activity but only when the cell dose used was close to the minimal number of cells required for cooperation (16). We found no evidence, however, that even large numbers of TDL given 1000 rads could synthesize DNA in response to antigen. The ability of irradiated thymus cells to restore the in vitro anti-sheep erythrocyte antibody-forming capacity of spleen cells from thymus-deprived mice (14, 15) may be attributed to the release from irradiated thymocytes of substances with properties similar to polynucleotides (poly-adenylic-polyuridylic acid complex, poly-AU). It has indeed been shown that
poly-AU enhances cooperation mediated by small numbers of residual T cells in the spleens of thymus-deprived mice (17). This does not apply in our system since T cells were virtually eliminated, the host being lethally irradiated and the hapten-primed spleen cells pretreated with anti-θ serum. Irradiation also interfered with the activity of ATC and of spleen cells from recently primed mice. This again contrasts with in vitro work in which recently primed cells or ATC were radioresistant (3) or mitomycin C resistant (18). Three explanations may be offered for these discrepancies. First, irradiation may suppress ATC function in vivo by preventing migration within the spleen to areas where interaction between T and B cells occur. Second, continued proliferation of ATC may be required for effective collaboration with B cells in vivo, not in vitro. Third, since different antibody responses are measured in vitro (19S) and in vivo (7S), T cell division may be mandatory for 7S responses, not for 19S responses.

SUMMARY

The helper function of carrier-primed T cells was found to be radiosensitive in vivo. The results could not be attributed to interference with the spleen-seeking properties of the irradiated cells. It is suggested that T cell division is essential for the induction of 7S antibody responses in vivo.

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