Radiosensitivity of T and B Lymphocytes

V. Effects of Whole-Body Irradiation on Numbers of Recirculating T Cells and Sensitization to Primary Skin Grafts in Mice

Robert E. Anderson, MD, and Wilbur L. Williams, MD

Whole-body exposure of mice to 50, 100, 300, or 500 rads results in an acute dose-related decrease in the number of viable recirculating T cells. The magnitude of this decrement becomes more pronounced with the passage of time. The dose-response relationship over this range of dosages appears to consist of three components: a steep drop between 0 and 50 rads, a plateau between 50 and 500 rads, and a second drop between 300 and 500 rads. The residual radioresistant cells are able to recognize a histoincompatible skin graft during the initial 5 days after irradiation. Low to moderate doses (50 to 300 rads) abrogate the partial tolerance noted in nonirradiated recipients exposed to the skin graft for 5 days and then regrafted from the same donor source 25 days after complete removal of the primary graft. A large (500 rads) dose results in prolonged graft survival in comparison with the nonirradiated group. It is suggested that the subpopulation of recirculating T cells which develops partial tolerance during a 5-day exposure to a homograft is more radiosensitive than the effector subpopulation which is involved in graft rejection. (Am J Pathol 89:367–378, 1977)

THE RELATIONSHIP between ionizing radiation and allograft rejection has been extensively investigated and forms the basis of several excellent monographs.[1,2] In general, whole-body irradiation prolongs survival of histoincompatible organ transplants, including skin. This relationship has been documented in several species and appears to pertain both when the recipients are grafted immediately after irradiation and when they are grafted days or even weeks later. Thus, Dempster *et al.*[3] demonstrated enhanced survival of skin grafts in rabbits given 250 rads of whole-body radiation 24 hours prior to transplantation, and whole-body exposure of CBA mice to 150, 300, and 600 rads prolonged the survival of A-strain skin homografts applied 3 days later in such a fashion that the reciprocal of the survival time was proportional to the radiation dose.[4] In a comparable study, Tylan and Cole [5] showed prolongation of skin grafts among sensitized and nonsensitized mice irradiated with 670 rads 30 and 61 days prior to transplantation.

From the Department of Pathology, University of New Mexico School of Medicine, Albuquerque, New Mexico.

Supported by Grants CA-13805 and CA-14270 from the National Cancer Institute, National Institutes of Health, and by the Veterans Administration.

Accepted for publication July 11, 1977.

Address reprint requests to Dr. Robert E. Anderson, Department of Pathology, University of New Mexico, School of Medicine, Albuquerque, NM 87131.

Similarly, the extracorporeal irradiation of blood and lymph generally results in prolonged allograft acceptance in several species, including man. Much of the relevant literature has been reviewed by Cronkite, who related enhanced survival to radiation-induced depletion of the recirculating pool of small lymphocytes,[6] which is now known to be composed primarily of thymus-derived (T) cells.

Although circulating antibody may play a role, rejection of first set allografts is thought to be a T-cell function and to involve especially those T cells which constitute the recirculating pool.[7] Most of the studies concerned with the relationship between radiation and graft rejection were performed prior to the definition of subpopulations of T cells and involved large doses of radiation. Many cellular immune responses are now thought to be modulated by subpopulations of lymphocytes.[8] In addition, evidence exists to suggest that at least two of these subpopulations (e.g., suppressor and helper cells) may differ in their response to irradiation, especially at low doses.[9] Therefore, the general concept that whole-body irradiation prolongs graft survival could relate to the large doses employed, doses sufficient to destroy most if not all lymphocyte function.

On this basis, it was decided to reevaluate the relationship between graft rejection and radiation in mice with particular reference to low-dose exposures. Immediately after irradiation, a histoincompatible skin graft was introduced and left in place for varying periods of time. The graft was then removed and the recipient permitted to recover from the acute effects of radiation injury; 30 days after irradiation a second graft from the same donor strain was applied. The time required for second set rejection was determined and correlated with a) radiation dose, b) duration of exposure to the skin graft, and c) number of thoracic duct lymphocytes (TDL) as determined at various times after irradiation.

Materials and Methods

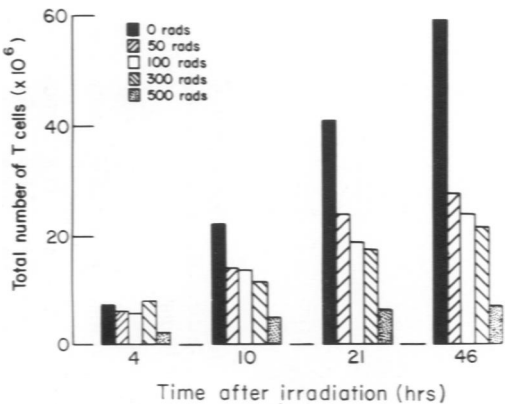
Highly inbred CBA/J mice of indicated ages were exposed in plastic Lucite containers to 0, 50, 100, 300, 500 rads of whole-body radiation generated by a standard General Electric x-ray machine employed under the following conditions: 120 kV peak; 15 mA; HVL, 2.3 mm Cu with a Thoreus filter. The absorbed dose rate was 99 rads/minute at 26 cm. Within 24 hours of irradiation, each mouse received a H-2 histoincompatible skin graft from a 4-month-old DBA/2J donor of the same sex. The method of grafting was that of Billingham and Medawar.[10] The skin grafts were left in place for 3, 5, or 10 days and then were surgically removed with care to preserve the graft bed. Thirty days after irradiation, a second DBA/2J graft from a 4-month-old donor was applied to the contralateral side and observed daily for evidence of rejection according to the approach described by Brent and Medawar.[4] More specifically, the end point of epithelial survival was taken as the time midway between the last day when the graft appeared partially viable and the first day when the graft appeared totally nonviable. Mice with skin grafts intact at 60 days were

recorded as 60-day survivals for statistical purposes. Data obtained from mice dying within 60 days of irradiation were excluded from graft survival calculations.

In a separate series of experiments, 4-month-old female CBA/J mice were irradiated as described. Immediately after irradiation, the thoracic duct was cannulated according to the method described by Sprent.[11] Thoracic duct lymphocytes were collected over ice for 46 hours. Periodically, aliquots were removed for cell counts, and the viability and numbers of thymus-derived (T) and bone marrow-derived (B) cells were determined as described elsewhere.[12]

Results

Table 1 shows the number of TDL recovered by thoracic duct cannulation for various time intervals up to 46 hours after whole-body irradiation with 0, 50, 100, 300, and 500 rads. After the initial 4 hours, a dose-related decrease in the number of TDL is apparent. Text-figure 1 shows the number of T cells recovered during these intervals as a function of radiation dose. With few exceptions, a dose-related decrease in T cells is evident. At the conclusion of the evaluation (46 hours), the most striking differences are between the 0 and 50 rad groups and the 300 and 500 rad groups. Relatively little difference is apparent among the groups exposed to 50, 100, and 300 rads. The latter point is evident in Text-figure 2, in which the dose-response data of Text-figure 1 are replotted in semilogarithmic fashion with the number of residual T cells expressed as a percentage of the corresponding control (0 rad) group. The similarities among the 50, 100, and 300 rad groups are apparent. Text-figure 3 shows a semilogarithmic plot of the number of T cells produced by each radiation group relative to the nonirradiated group for the initial 10, 21, and 46 hours after irradiation. The three curves exhibit similar configurations, with a steep drop between 0 and 50 rads, a plateau between 50 and 300 rads, and a second drop between 300 and 500 rads.

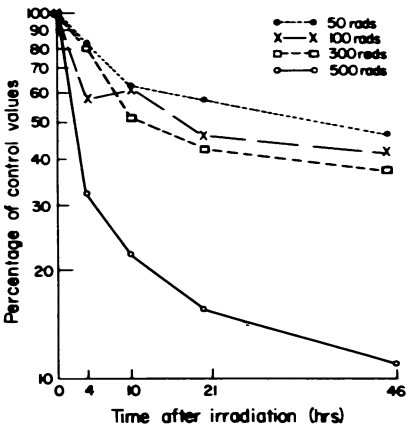


TEXT-FIGURE 1—Cumulative number of viable T cells mobilizable by thoracic duct cannulation as a function of radiation dose and time after exposure. Data derived from Table 1.

Table 1—Number of Thoracic Duct Lymphocytes as a Function of Radiation Dose and Time After Irradiation*

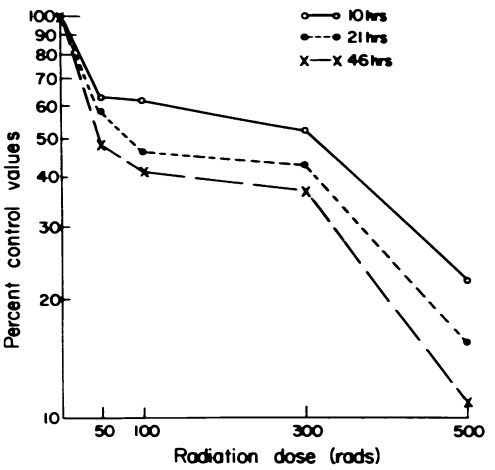
Radiation dose (rads)	No. of mice	4 hours				10 hours				21 hours				46 hours			
		No. of cells	Viability	Percent T	Percent B	No. of cells	Viability	Percent T	Percent B	No. of cells	Viability	Percent T	Percent B	No. of cells	Viability	Percent T	Percent B
0	4	11.8	100	65	33	25.6	98	58	39	33.5	99	56	43	33.8	99	54	48
50	4	9.8	99	63	35	14.5	99	56	44	18.7	99	52	48	7.4	99	53	48
100	8	6.8	99	89	11	8.8	99	90	9	6.1	95	85	11	6.1	95	86	14
300	4	9.5	99	87	9	3.7	96	93	7	6.4	99	93	9	4.6	99	92	6
500	5	3.8	99	59	35	4.8	100	58	40	3.3	100	52	47	1.2	99	55	41

* Four-month-old CBA female mice were exposed in whole-body fashion to indicated dose. Immediately after irradiation the thoracic duct was cannulated, and the number of TDL determined at the indicated times. Only mice which flowed well were employed for data calculations. Results represent cumulative cell numbers.



TEXT-FIGURE 2—Relationship between radiation dose and number of viable thoracic duct T cells for initial 46 hours after exposure. Data derived from Table 1. Number of T cells expressed as a percentage of control values and plotted semilogarithmically.

Table 2 shows the results of a pilot experiment designed to determine the influence of irradiation on the capacity of CBA recipients to recognize DBA/2 skin grafts. The latter were left in place for 3, 5, or 10 days. A control group was not grafted. Among nonirradiated recipients, the duration of survival of the second set graft is inversely related to the length of exposure to the primary graft. Among the irradiated recipients, and with numerous exceptions, a similar trend is apparent. The exceptions especially involve recipients with a 5-day exposure to the primary graft (Group 3). Evaluation of the data of Table 2 by experimental group shows no consistent relationship between radiation dose and graft survival for either male or female recipients of any of the four groups. With one



TEXT-FIGURE 3—Effect of radiation dose on the number of viable thoracic duct T cells 10, 21, and 46 hours after irradiation. Data derived from Table 1. Number of T cells expressed as a percentage of control values and plotted semilogarithmically.

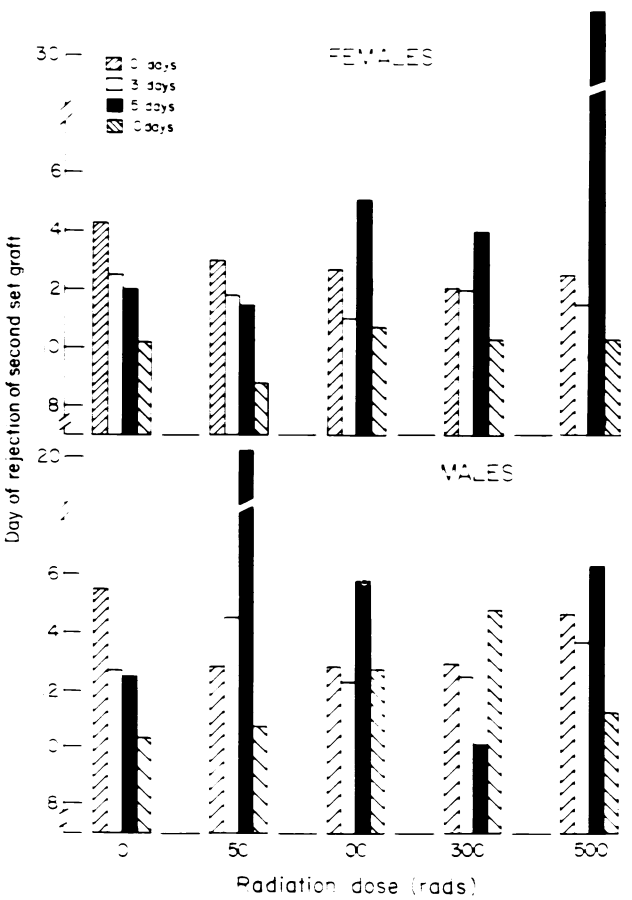
Table 2—Influence of Radiation and Exposure to Primary Skin Grafts for Varying Periods of Time on Second Set Rejection*

Experimental group	Duration of exposure to primary skin graft	Sex of recipients	No. of recipients	Radiation dose				
				0 rads	50 rads	100 rads	300 rads	500 rads
1	0 days	Male	27	15.5 ± 2.0	12.8 ± 1.6	12.8 ± 1.1	12.9 ± 2.6	14.7 ± 3.4
		Female	27	14.3 ± 0.9	13.0 ± 0.9	12.7 ± 2.2	12.1 ± 1.5	12.5 ± 1.4
2	3 days	Male	30	12.7 ± 2.2	14.5 ± 7.2	12.3 ± 2.8	12.5 ± 2.8	13.7 ± 2.8
		Female	29	12.5 ± 4.6	11.8 ± 3.4	11.0 ± 3.2	12.0 ± 2.0	11.5 ± 3.0
3	5 days	Male	30	12.5 ± 4.0	23.4 ± 22.0	15.8 ± 17.8	10.2 ± 3.0	16.3 ± 17.8
		Female	28	12.0 ± 5.6	11.5 ± 2.2	15.1 ± 19.0	14.0 ± 10.4	31.3 ± 27.0
4	10 days	Male	30	10.3 ± 2.8	10.8 ± 1.2	12.7 ± 3.2	14.8 ± 19.0	11.3 ± 3.0
		Female	29	10.2 ± 3.0	8.8 ± 0.8	10.7 ± 0.9	10.3 ± 3.6	10.3 ± 4.4

* Four-month-old CBA mice were irradiated with indicated dose in whole-body fashion. Within the 24 hours, the irradiated recipients were transplanted with DBA/2 skin grafts which were left in place for 3, 5, or 10 days. A control group was not grafted. Thirty days after irradiation, a second DBA/2 skin graft was applied and observed daily for evidence of rejection. Results expressed as mean time of rejection ± 2 SD.

exception (female recipients irradiated with 50 rads), the irradiated subgroups of Group 4 show a longer graft survival than the nonirradiated subgroups. The involved differences, however, are small and are not statistically significant. In general, and with exceptions, the reverse situation is noted with Groups 1, 2, and 3, i.e., the irradiated subgroups show a more rapid graft rejection than the control subgroups.

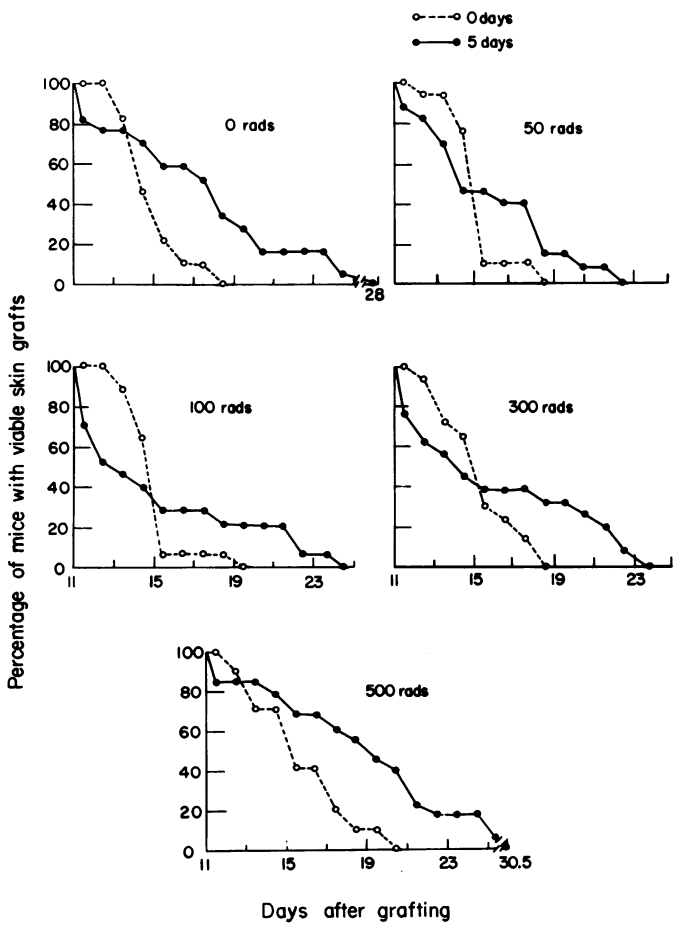
Text-figure 4 shows the effect of the duration of exposure to the primary graft upon second set rejection in female and male recipients for the five radiation doses. In the 0 rad group, increased duration of exposure to the primary graft results in reduced survival of the secondary graft, although the differences between the various subgroups are generally not statistically significant. A similar graduated response is noted in female animals



TEXT-FIGURE 4—Mean day of second set skin graft rejection in female and male recipients as a function of duration of exposure to primary graft and radiation dose. Data derived from Table 2

irradiated with 50 rads but not in the groups receiving other doses of radiation. Particularly among mice exposed to the primary graft for 5 days, the reverse situation often pertains: irradiation often results in prolonged graft survival. In mice, the period 3 to 6 days after grafting appears to be critical in terms of host recognition of H-2 incompatible skin grafts.[13]

On the basis of the above, it was decided to repeat the graft experiment but to concentrate attention on a) female recipients (and donors), b) the influence of recipient age, and c) comparisons between mice not previously grafted (Group 1) and mice exposed to the primary graft for 5 days (Group 3). Text-figure 5 shows the effect of irradiation on graft recognition in these two experimental groups, which include a total of 221

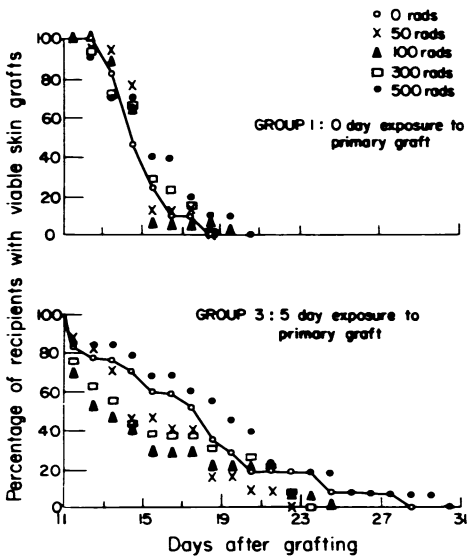


TEXT-FIGURE 5—Comparisons of graft survival in Groups 1 and 3 as a function of radiation dose.

recipients. Examination of the 0 rad data shows that prior exposure of the recipients to the primary graft influences the second set response, with the majority of mice rejecting the second set graft more slowly than their nongrafted contemporaries. However, a minority of the second set recipients show the opposite effect. Prior exposure to moderate amounts (50 to 300 rads) of radiation results in an increased proportion of second set recipients which show a more rapid rejection response. Exposure to 500 rads results in graft survivals very similar to the control (0 rad) situation.

Text-figure 6 shows in summary fashion the effect of radiation on graft survival in Groups 1 and 3. In Group 1, irradiation 30 days prior to transplantation has little influence upon graft survival. Most of the data are clustered close to the 0 rad curve, although more data points are to the right of the curve than to the left, indicating a tendency toward slower rejection with irradiation. The effect of irradiation is more pronounced with respect to Group 3, which has less clustering of the data. Also, the vast majority of the data points are located to the left of the 0 rad curve, indicating a tendency toward more rapid rejection with irradiation. In fact, with few exceptions, the only data points to the right of the curve represent 500-rad recipients. In addition, all data points from 500-rad recipients fall to the right of the 0 rad curve.

The influence of recipient age on the relationship between irradiation and graft survival was next evaluated. The results are shown in Table 3. In Group 1, and at each radiation dose except 500 rads, grafts on the older



TEXT-FIGURE 6—Effect of radiation dose on graft recognition in female recipients exposed to primary graft for 0 or 5 days.

Table 3—Influence of Irradiation and Age of Recipients on Survival of Second Set Skin Grafts*

Experimental group	Age of recipients	No. of mice	Radiation dose				
			0 rads	50 rads	100 rads	300 rads	500 rads
1	6 wk	106	16.50 ± 4.8	16.20 ± 6.2	15.65 ± 4.1	16.60 ± 4.2	17.90 ± 5.0
	4 mo	85	15.44 ± 0.8	15.50 ± 0.6	15.26 ± 0.6	15.43 ± 1.0	17.80 ± 1.0
	24 mo	50	17.23 ± 4.0	17.95 ± 3.4	16.80 ± 7.0	16.71 ± 4.4	16.75 ± 4.0
3	6 wk	77	17.40 ± 6.1	15.70 ± 4.8	16.10 ± 7.8	16.84 ± 6.2	19.00 ± 9.9
	4 mo	67	17.85 ± 2.4	15.88 ± 1.6	15.68 ± 2.1	15.91 ± 2.1	19.11 ± 2.4
	24 mo	53	14.65 ± 5.8	15.29 ± 4.2	15.58 ± 3.2	16.60 ± 5.8	IN

IN = Insufficient numbers of survivors for evaluation.

* Six-week-old and 4- and 24-month-old CBA female mice were irradiated with indicated doses in whole-body fashion. Within 24 hours the irradiated recipients were transplanted with DBA/2 skin grafts which were left in place for 5 days (Group 3). A control group was not grafted (Group 1). Thirty days after irradiation, a second DBA/2 skin graft was applied and observed daily for evidence of rejection. Results are expressed as mean time of rejection ± 2 SD.

mice survive longer than those on the corresponding 6-week-old and 4-month-old recipients. These differences are not statistically different. With a single exception (4 months, 300 rads), the reverse situation is seen with respect to Group 3. Again, the differences are not statistically significant.

Discussion

Whole-body exposure to doses as small as 50 rads results in an acute decrease in the pool of viable recirculating T cells. The magnitude of this decrement becomes more pronounced with the passage of time and reaches 47% of control values 46 hours after exposure to 50 rads, the last time tested (Text-figure 2). Exposure to amounts of radiation greater than 50 rads causes a more pronounced decrement, although the differences among the 50, 100, and 300 rad groups are relatively small (Text-figures 2 and 3). The dose-response curves of Text-figure 3 appear to consist of three components: a steep drop between 0 and 50 rads, a plateau between 50 and 300 rads, and a second drop between 300 and 500 rads. One interpretation of these observations is as follows: a) thoracic duct T cells contain two subpopulations which differ in radiosensitivity; b) one subpopulation is very radiosensitive and ceases to traffic normally within 4 hours of whole-body exposure to doses as small as 50 rads; and c) a second subpopulation is relatively insensitive to doses up to 300 rads, at least for the initial 46 hours after irradiation. The skin graft data included herein are consistent with this interpretation.

Recognition of histoincompatible skin grafts appears to involve primarily recirculating T cells.[7] Among young nonirradiated female CBA recipi-

ents, exposure to DBA/2 skin grafts for 5 days results in prolonged acceptance of second set grafts (see Text-figure 5). This prolonged acceptance is partially abrogated by whole body irradiation with 50 to 300 rads (Text-figure 6). Conversely, prior irradiation with 500 rads results in graft acceptance which is more prolonged than that in both nonirradiated recipients exposed to the primary graft for 5 days (Text-figure 6) and irradiated (500 rads) recipients not previously grafted (Text-figure 5). Since there is no evidence of regeneration of T cells during the initial 5 days after whole-body exposures in the 50 to 500 rads dose range,[12] the skin graft results suggest that the homograft response is modulated by at least two subpopulations of T cells which differ in radiosensitivity. Thus, the subpopulation which appears to develop partial tolerance during a 5-day exposure to a primary skin graft appears to be more radiosensitive than the effector subpopulation. This situation is reminiscent of the effects of radiation on the capacity of syngeneic thymus to suppress immunoglobulin production by congenic B cells upon transfer to irradiated recipients. Irradiation of the thymus cell prior to transfer often results in augmented immunoglobulin production, since reduced numbers of effector cells function more effectively in the absence of an even larger proportion of the more radiosensitive suppressor cells.[14]

In summary: Dose-response data on recirculating T cells and the recognition of histioncompatible skin grafts suggests the presence of at least two subpopulations which differ in radiosensitivity. Since recirculating T cells are thought to be involved in the homograft response, it is tempting to relate these observations. If such a correlation exists, it implies that the subpopulation which develops partial tolerance during a 5-day exposure to a histoincompatible skin graft is more radiosensitive than the effector subpopulation which is involved, primarily or secondarily, in graft rejection. The latter appears to be resistant to doses in excess of 300 rads but less than 500 rads.

References

1. Micklem H, Loutit JF: Tissue Grafting and Radiation. New York, Academic Press, Inc., 1966
2. Taliaferro WH, Taliaferro LG, Jaroslow BN: Radiation and Immune Mechanisms. New York, Academic Press, Inc., 1964
3. Dempster WJ, Lennox B, Boag JW: Prolongation of survival of skin homotransplants in the rabbit by irradiation of the host. Br J Exp Pathol 31:670–679, 1950
4. Brent L, Medawar P: Quantitative studies on tissue transplantation immunity. VIII. The effects of irradiation. Proc R Soc Biol Sci 165:413–423, 1966
5. Tyan ML, Cole LJ: Rejection of allogeneic skin grafts and production of isohemagglutinins by sensitized mice after sublethal irradiation. J Immunol 95:945–950, 1965

6. Cronkite EP, Chanana AD, Joel DD, Laissue J: Lymphocyte repopulation and restoration of cell-mediated immunity following whole body radiation and localized irradiation. Interaction of Radiation and Host Immune Defense Mechanisms in Malignancy. Edited by VP Bond, S Hellman, SE Order, HD Suit, HR Withers. Upton, N.Y., Brookhaven National Laboratories Associated University Inc., US Atomic Energy Commission, 1974, pp 181–206
7. Gelfand MC, Paul WE: Prolongation of allograft survival in mice by administration of anti-thy 1 serum. I. Mediation by *in vivo* activation of regulatory T cells. J Immunol 115:1–4, 1975
8. Cantor H, Boyse EA: Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J Exp Med 141:1376–1389, 1975
9. Anderson RE, Warner NL: Ionizing radiation and the immune response. Adv Immunol 24:215–335, 1976
10. Billingham RE, Medawar PB: The technique of free skin grafting in mammals. J Exp Biol 28:385–402, 1951
11. Sprent J: Circulating T and B lymphocytes of the mouse. I. Migratory properties. Cell Immunol 7:10–39, 1973
12. Anderson RE, Olson GB, Autry JR, Howarth JL, Troup GM, Bartels PH: Radiosensitivity of T and B lymphocytes. IV. Effect of whole body irradiation upon various lymphoid tissues and numbers of recirculating lymphocytes. J Immunol 118:1191–1200, 1977
13. McKhann CF, Berrian JH: Transplantation immunity: Some properties of induction and expression. Ann Surg 150:1025–1031, 1959
14. Warner NL, Anderson RE: Helper effect of normal and irradiated thymus cells on transferred immunoglobulin production. Nature 254:604–606, 1975