CO-PRESIDENTIAL ADDRESS

IN VITRO EVALUATION OF RADIATION-INDUCED AUGMENTATION OF THE IMMUNE RESPONSE

The American Journal of PATHOLOGY

December 1979 • Volume 97, Number 3

In Vitro Evaluation of Radiation-Induced Augmentation of the Immune Response

Robert E. Anderson, MD, and Ivan Lefkovits, PhD

Small doses (5–25 rads) of radiation augment the *in vitro* response of murine spleen cells to sheep red blood cells (SRBCs). Such augmentation appears to result from radiation-induced disruption of a homeostatic component of the response that exerts maximum effect soon after the introduction of antigen. Evidence is presented to support the concept that augmentation is due to injury of an exquisitely radiosensitive subpopulation of T cells with suppressor activity. (Am J Pathol 97:456–472, 1979)

THE IMMUNOSUPPRESSIVE EFFECTS of irradiation have been known for some time and have been studied extensively.[1–3] More recently, radiation-induced augmentation of the immune response has been described.[4–8] This phenomenon has been primarily investigated *in vivo*, where the timing between the introduction of antigen and irradiation appears to be of particular importance. The specific details of these and other variables involved in augmented responses associated with whole-body or local irradiation are reviewed elsewhere.[8] The primary purpose of the present study is to investigate the cellular basis of this phenomenon by the use of *in vitro* responses to select antigens. Specific attention will be devoted to the effects of low doses of radiation because of preliminary evidence that such exposures result in significantly augmented responses both *in vivo*[5] and *in vitro*.[9]

Materials and Methods

Highly inbred, specific pathogen-free (SPF) mice of the following strains were obtained from the Institut für Biologisch Medizinische Forschung AG, Füllinsdorf, Switzerland: C57BL/6, DBA/2, and C3H. Congenitally athymic (nu/nu) female C57BL/6 mice of the

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

Supported in part by Grant CA-13805 from the National Cancer Institute.

Presented in part on April 8, 1979, at the FASEB Meetings, Dallas, Texas: President's Plenary Session, "Radiation and the Immune Response."

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

0002-9440/79/1207-0456$01.00

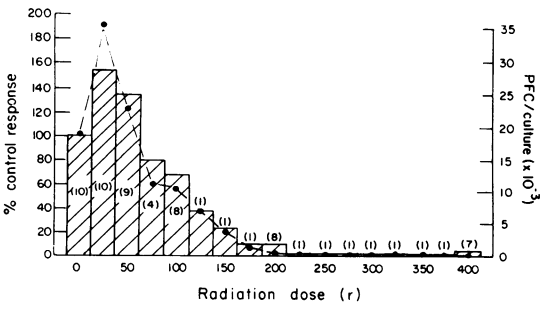
seventh backcross were obtained from Bomholtgaard, Ltd., Ry, Denmark. Donors were sacrificed by cervical dislocation and single-cell suspensions of spleen prepared aseptically by gentle teasing through stainless steel sieves into cold RPMI-1640 medium with added L-glutamine (2 ng per ml). When indicated, T cells were removed by incubation with AKR anti-θ serum plus guinea pig complement as described elsewhere.[10] Similarly, B cells were removed by passage through nylon columns according to the method of Julius et al.[11] Irradiation of cells was accomplished with a Phillips RT 305 x-ray machine operated under previously described conditions.[9] Cell viability was determined by trypan blue exclusion or fluorodiacetate (FDA) incorporation.

Cultures of spleen cells were carried out with the use of 1-ml cultures of the Mishell–Dutton type[12,13] or the 10-μl system of Lefkovits.[14,15] The 1-ml cultures contained 1×10^7 spleen cells, were set up in quadruplicate, and were incubated in standard fashion using a rocker platform. Unless otherwise indicated, these cultures were terminated on both Days 4 and 5, and the number of plaque-forming cells (PFCs) per culture was determined by the Jerne technique as modified by Cunningham.[16] Background PFCs never exceeded 400 per culture. The 10-μl cultures contained 2×10^5 spleen cells and were incubated in plastic trays as described elsewhere.[15] Duplicate trays, each containing 60 wells, were assayed on Day 6. As antigens, sheep red blood cells (SRBCs), horse red blood cells (HRBCs), and trinitrophenyl-Ficoll (TNP-Ficoll) were employed. SRBCs and HRBCs were obtained from a single donor and utilized within 3 weeks of receipt. Immunization of cultures with TNP-Ficoll was accomplished according to the method of Mosier et al.[17]

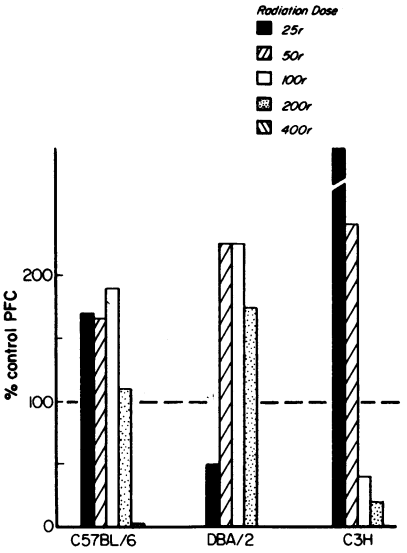
In evaluating the results presented herein, it is important that one pay particular attention to the relationship between variance and mean. In a group of randomly distributed events, the mean is equal to the variance, and thus the square root of the mean equals the standard error (SE). On this basis, for example, in counting 10,000 events the SE equals ± 1% (square root of 10,000). However, if only 1000 events are recorded, the SE equals ± 3%, and with 36 items the SE is ± 20%. For this reason, many of the experiments were repeated on many (up to 10) occasions in order to ensure that we were dealing with highly significant events.

Results

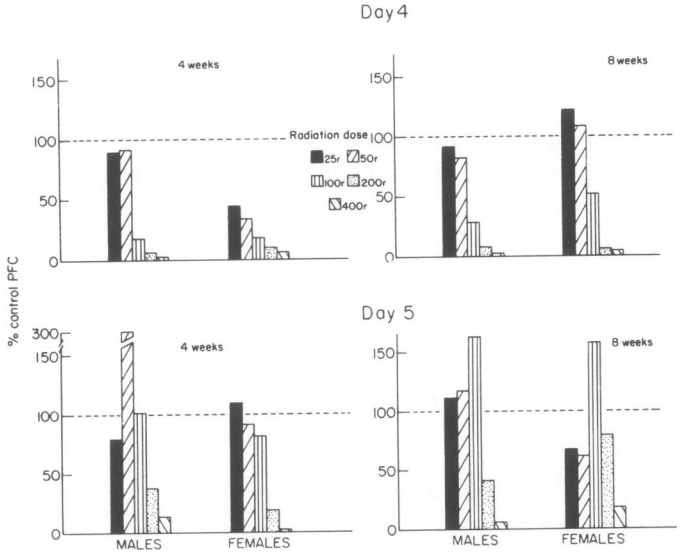
Text-figure 1, adapted from earlier work,[9] summarizes the effect of varying doses of radiation upon the *in vitro* PFC response of C57BL/6



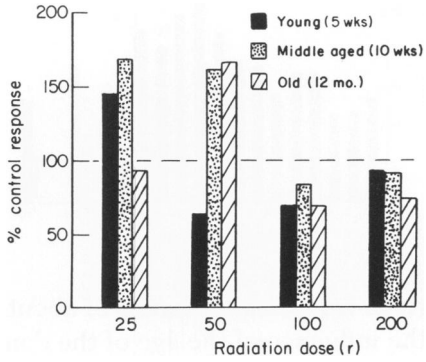
TEXT-FIGURE 1—Effect of irradiation upon anti-SRBC response (adapted from Anderson and Lefkovits[9]). C57BL/6 spleen cells from female donors were exposed to indicated doses of radiation *in vitro* and incubated in 1-ml cultures with SRBCs as antigen. The number of anti-SRBC PFCs was determined on Day 4. The results are expressed as a percentage of the control (0 rad) response. The numbers in parentheses indicate the number of experiments included in the calculation of the indicated value. The actual data from a single experiment (*dotted line*) are included for comparative purposes.



TEXT-FIGURE 2—Influence of source of spleen cells upon radiation-induced augmentation of *in vitro* immune response (adapted from Anderson et al [8]). Spleen cells from indicated sources were exposed to various doses of radiation *in vitro* and incubated in 1-ml cultures with SRBCs as antigen. The results are expressed as a percentage of the control response (*dotted line*).



TEXT-FIGURE 3—Influence of age and sex of spleen cell donor upon radiation-induced augmentation. Spleen cells from indicated sources were exposed to various doses of radiation *in vitro* and incubated in 1-ml cultures with SRBCs as antigen. The results are expressed as a percentage of the control response (*dotted line*) for both Days 4 and 5 of culture.

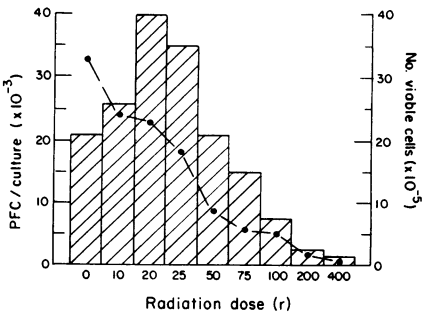


TEXT-FIGURE 4—Influence of age of spleen cell donor upon radiation-induced augmentation. Spleen cells from C57BL/6 female donors of indicated ages were exposed to various doses of radiation *in vitro* and placed in 1-ml cultures with SRBCs as antigen. The results are expressed as a percentage of the Day 5 PFC response (*dotted line*).

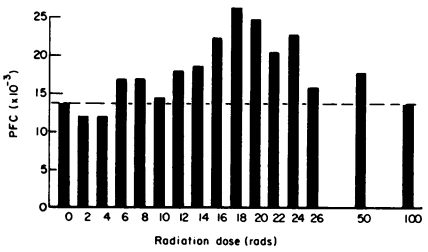
spleen cells to SRBCs in 1-ml cultures. The data were derived from 10 separate individual experiments. Each of the 10 showed varying degrees of augmentation, which tended to be most pronounced with 25 rads. The results are expressed as a percentage of the control (0 rad) response in order to facilitate the combining of the data. The actual numbers of PFCs/culture from a single experiment are also included in the figure for comparative purposes.

Text-figure 2 demonstrates the influence of donor strain upon low dose augmentation of the immune response. All three strains show augmentation, although considerable variability among the strains evaluated is evident. Despite the observation that the magnitude of augmentation was less pronounced for C57BL/6 female mice than for the other two strains tested, the former was selected for subsequent experiments because of 1) greater availability and 2) more consistent *in vitro* anti-SRBC responses by nonirradiated cells.

Text-figure 3 shows the influence of the age and sex of the spleen cell donor upon low dose augmentation of the *in vitro* PFC response to SRBCs. In this experiment, as well as in several comparable experiments, the most consistent radiation-induced augmentation is seen with cells de-



TEXT-FIGURE 5—Relationship between cell viability and radiation-induced augmentation. Spleen cell suspensions were irradiated *in vitro* and placed in cultures with SRBCs as antigen. Results represent average of two experiments harvested on Day 4. Similar results (not shown) were obtained with analyses on Day 5.



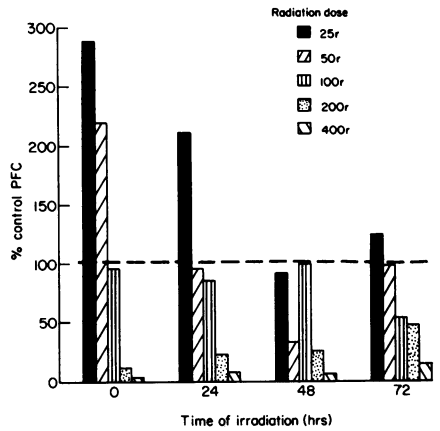
TEXT-FIGURE 6—Influence of small increments of irradiation upon *in vitro* response to SRBCs. Spleen cells were irradiated *in vitro* and placed in 1-ml cultures with SRBCs as antigen. Data represent PFCs on Day 4. Similar results obtained on Day 5 (data not shown).

rived from female donors of about 8 weeks of age. Text-figure 4 shows also the influence of the age of the donor upon the *in vitro* anti-SRBC response of spleen cells from female mice. Cells derived from 10-week donors show an augmented response after exposure to either 25 or 50 rads, while cells from 5-week or 12-month donors show augmentation with only 1 radiation dose. Based upon the above results, and unless otherwise specified, all subsequent data will be derived from experiments utilizing 8–12-week-old C57BL/6 female donors.

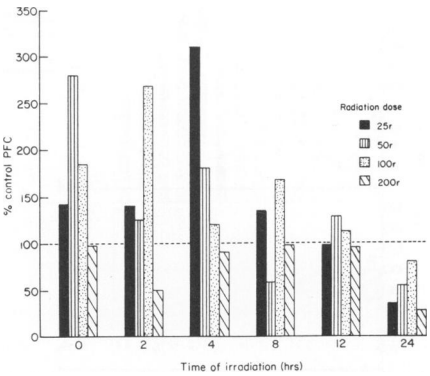
In Text-figure 5, the relationship between cell viability and low dose augmentation is summarized. At the termination of the cultures 1) fewer viable cells remain in the irradiated cultures than in the controls and 2) despite the presence of reduced numbers of cells, cultures exposed to 10 to 25 rads exhibit an augmented response to SRBCs.

Text-figure 6 shows the influence of very small increments of radiation upon the PFC response of spleen cells to SRBCs in 1-ml cultures. In this experiment, maximum augmentation is noted after exposure to 18 rads.

Text-figure 7 demonstrates the influence of timing upon low dose augmentation. One-milliliter cultures were irradiated at various times after the addition of antigen and then harvested at 96 hours for the determina-



TEXT-FIGURE 7—Relationships between timing of exposure and radiation-induced augmentation. Spleen cells were placed in 1-ml cultures with SRBCs as antigen and irradiated at indicated time intervals. Results are expressed as a percentage of the control PFC response *(dotted line)*.

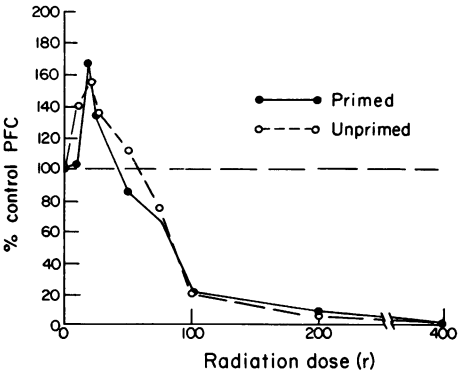


TEXT-FIGURE 8—Relationship between timing of exposure and radiation-induced augmentation. Protocol similar to that shown with respect to Text-figure 7.

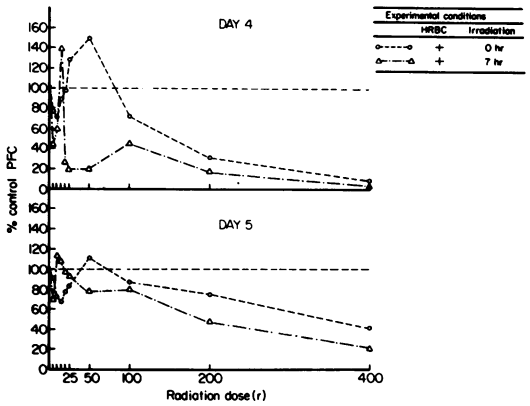
tion of anti-SRBC activity. Irradiation at 0 and 24 hours results in the most pronounced augmentation. Text-figure 8 shows the results of a similar experiment designed to pay particular attention to the initial 24 hours of culture. Here the most striking augmentation is associated with irradiation during the initial 4 hours of culture. Comparable results were obtained with 10-μl cultures (data not shown).

The experiment summarized in Text-figure 9 was designed to determine whether low dose augmentation is a property of primed as well as unprimed cells. Irradiated spleen cells from mice primed *in vivo* 40 days previously with SRBCs were compared with cells obtained from contemporaries given injections of saline. As shown in Text-figure 9, low dose augmentation of the *in vitro* anti-SRBC PFC response is a property of primed as well as unprimed spleen cells.

Next the effect of *in vitro* priming upon low dose augmentation was investigated. HRBCs were selected as the priming antigen 1) because the response it elicited could be determined independently of the anti-SRBC response and 2) because of known cross-reactivity with SRBCs. A prelimi-



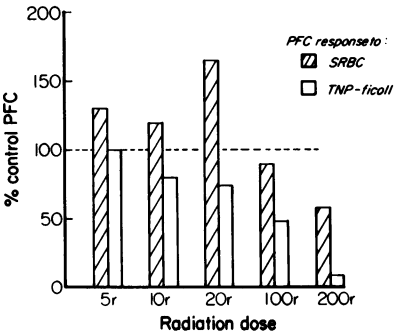
TEXT-FIGURE 9—Influence of irradiation upon primed versus unprimed cells (adapted from Anderson and Lefkovits [9]). Mice were injected with SRBCs or saline 40 days prior to sacrifice. Spleen cell suspensions were exposed to indicated doses of radiation *in vitro* and placed in 1-ml cultures. Results expressed as a percentage of the control response.



TEXT-FIGURE 10—Effect of *in vitro* priming upon radiation-induced augmentation. Spleen cell suspensions were primed with HRBCs at time 0 and with SRBCs 7 hours later. The cultures were irradiated either at time 0 or after 7 hours of culture. The results are expressed as a percentage of the control response (*dotted line*).

nary experiment was carried out to determine the influence of timing between the addition of HRBCs and SRBCs and the maximum numbers of HRBCs which could be added without significant interference with the anti-SRBC response. These results are shown in Table 1. From the data in the table, it appears 1) that of the variables evaluated, 7 hours represents the maximum time between the addition of antigens which is associated with a reasonable PFC response to SRBCs and 2) that as much as 50 λ/ml of a 1% suspension of HRBCs does not block the anti-SRBC response.

Table 2 and Text-figure 10 show the effect in *in vitro* priming with HRBCs upon low dose augmentation of the anti-SRBC response when the latter is added 7 hours after the HRBCs. When the cultures are irradiated at time 0, low dose augmentation is evident with cultures harvested on Day 4, although the character of the dose-response curve is altered, especially at very low doses of irradiation. Postponement of irradiation until the time of the addition of SRBCs, or 7 hours after the initiation of the cultures, results in a markedly altered dose-response curve. The difference in the influence of irradiation at 0 and 7 hours suggests that one action of



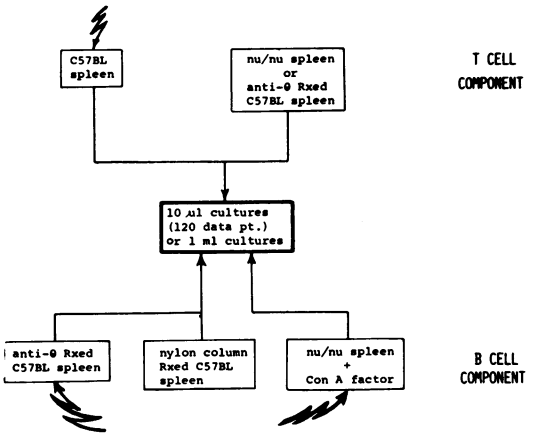
TEXT-FIGURE 11—Influence of irradiation upon T-dependent (SRBCs) and T-independent (TNP-Ficoll) antigen (adapted from Anderson et al [8]). One-milliliter cultures were primed with SRBCs or TNP-Ficoll and irradiated. The results are expressed as a percentage of the control response (*dotted line*).

Table 1—*In Vitro* Response to SRBCs and HRBCs as a Function of Time Between Addition of Antigens and Amount of HRBCs

Group	Number of cultures	Time of addition of SRBCs (1%)	% HRBCs (added at time 0)	PFC					
				Day 4		Day 5		Day 6	
				SRBCs	HRBCs	SRBCs	HRBCs	SRBCs	HRBCs
I	32	0 hr	1	3200	940	7200	1000	6100	920
			0.1	2700	820	3500	400	3900	700
			0.01	3100	700	6300	900	3100	260
			0.001	4000	480	5300	300	1200	140
II	32	7 hr	1	3600	1540	5200	900	2400	1080
			0.1	3700	1260	4500	800	2700	460
			0.01	3800	1200	1700	900	3100	280
			0.001	2200	520	2300	400	2000	200
III	32	24 hr	1	1300	1140	840	1300	440	600
			0.1	780	1400	800	700	360	400
			0.01	400	660	960	800	140	220
			0.001	410	380	360	300	260	180
IV	4	0 hr	—	4400	0	8000	0	ND	ND
V	4	7 hr	—	3600	0	6100	0	ND	ND
VI	4	24 hr	—	ND	ND	160	0	120	0
VII	16	—	1	0	1600	180	2600	ND	ND
		—	0.1	0	1400	160	1100	ND	ND
		—	0.01	0	1140	100	700	ND	ND
		—	0.001	0	720	0	500	ND	ND

Table 2—Influence of Priming by Cross-Reacting Antigen Upon Radiation-Related Augmentation

Group	Number of cultures	Time of Addition		Time of irradiation	Radiation dose (r)	PFC Day 4		PFC Day 5	
		HRBCs (1%)	SRBCs (1%)			HRBCs	SRBCs	HRBCs	SRBCs
I	60	0 hr	7 hr	0 hr	0	1950	7400	1500	5700
					5	1850	5700	1100	5200
					10	1100	5300	900	4100
					15	750	6600	1700	3800
					20	500	7200	700	4400
					25	1900	9500	950	4700
					50	2650	11,000	1200	6300
					100	1550	5400	300	5100
					200	700	2300	600	4300
					400	100	700	100	2400
II	60	0 hr	7 hr	7 hr	0	1100	6200	1200	6400
					5	1500	2800	900	4800
					10	850	3700	750	7200
					15	1500	8700	1450	7000
					20	700	1800	850	6300
					25	300	1300	1250	6000
					50	1500	1300	1400	4900
					100	1200	2900	550	5200
					200	700	1100	300	3000
					400	100	300	50	1200
III	6	0 hr	—	—	—	1250	0	1300	200
IV	6	—	7 hr	—	—	0	1400	0	6900
V	6	—	0 hr	—	—	50	700	0	6100
VI	6	0 hr	0 hr	—	—	850	3300	900	5700



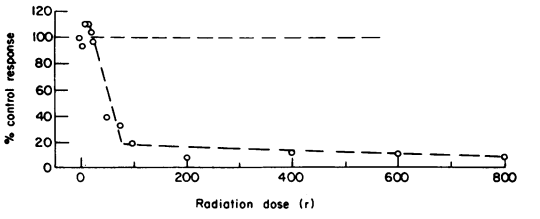
TEXT-FIGURE 12—General experimental approach utilized for irradiation of only the T-cell or the B-cell component of the *in vitro* response to SRBCs.

the HRBCs added at time 0 is to render the subpopulation of cells responsible for augmentation less susceptible to irradiation 7 hours later.

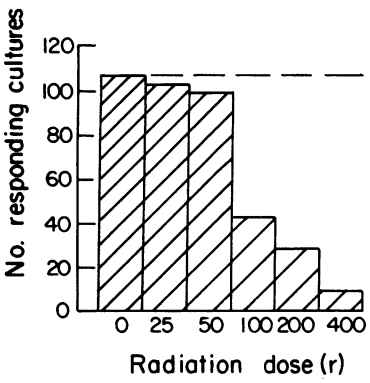
Text-figure 11 shows the effect of low dose irradiation upon a T-dependent (SRBCs) and T-independent (TNP-Ficoll) antigen. The two antigens were administered to duplicate aliquots of the same spleen cell suspension. Low dose augmentation is seen with the T-dependent but not the T-independent antigen.

The next series of experiments was carried out to evaluate the influence of low doses of irradiation upon isolated subpopulations of spleen cells enriched for either B- or T-cell activity. The general experimental approaches are outlined in Text-figure 12.

With respect to suspensions enriched for B cells, Text-figure 13 summarizes the results of two identical experiments in which irradiated anti-θ–treated C57BL/6 spleen cells were combined with saturating numbers of



TEXT-FIGURE 13—Effect of exposure of only the B-cell component upon radiation-induced augmentation (adapted from Anderson and Lefkovits [9]). See Text-figure 12 for the general experimental approach. Anti-θ–treated spleen cells were irradiated and combined with nonexposed nylon-column–treated spleen cells at a T:B cell ratio of 1:3 in 10-μl cultures. The number of microcultures containing demonstrable antibody was determined on Day 6.



TEXT-FIGURE 14—Effect of irradiation upon the capacity of nu/nu spleen cells to respond to SRBCs in the presence of Con A helper factor (adapted from Anderson and Lefkovits[9]). The results are derived from two separate experiments.

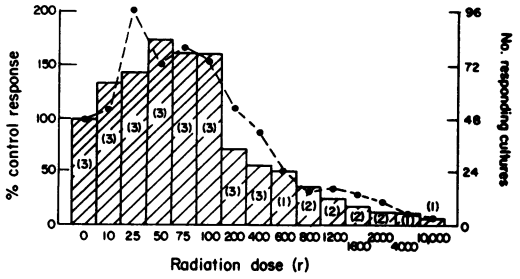
nylon-column–treated spleen cells from the same source. Equivocal augmentation is associated with low (5 to 20 rads) doses.

Text-figure 14 shows the effect of irradiation on the anti-SRBC response by nu/nu spleen cells stimulated by Concanavalin A (Con A) helper factor* in 10-μl cultures. The results are expressed as the proportion of the total (120) number of microcultures that responded with antibody production. No augmentation is present. Sequential irradiation demonstrated the most pronounced loss of anti-SRBC activity to be associated with exposure at 12 hours (data not shown).

Text-figure 15 shows the effect of irradiation upon the T-cell component of the response. C57BL/6 spleen cells were exposed to varying doses of radiation and then combined with saturating numbers of anti-θ–treated C57BL/6 spleen cells. Marked augmentation is noted with low-exposure doses. Text-figure 16 summarizes the results of a similar experiment utilizing 1-ml cultures in which nu/nu spleen cells (C57BL background) were employed as a source for saturating numbers of nonirradiated B cells. Again low dose augmentation is evident.

Irradiation of lymphocytes results in an accelerated release of surface glycoproteins,[18] some of which may possess immunologic activity. On this basis, a series of experiments was carried out to determine if a portion of the augmentation described might relate to the release of immunoreactive substances. Spleen cells from 50 mice were divided into five aliquots, suspended in an equal volume of medium, exposed to the indicated doses of radiation, and incubated for 60 minutes at 37 C. The suspensions were then centrifuged and the cell-free supernatants employed at various dilu-

* Con A helper factor is a crude supernatant derived from mouse spleen cells that have been stimulated by Con A.[10]

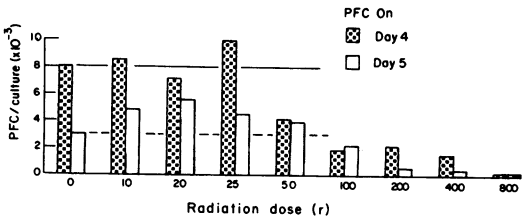


TEXT-FIGURE 15—Effect of exposure of only the T-cell component upon radiation-induced augmentation (adapted from Anderson and Lefkovits [9]). See Text-figure 12 for the general experimental approach. Spleen cells were irradiated and added to saturating numbers of nonirradiated anti-θ–treated spleen cells at an estimated T:B ratio of 1:9 in 10-μl cultures. The number of microcultures containing demonstrable antibody was determined on Day 6. The results are expressed as a percentage of the control response and are derived from three separate experiments. Direct data from one such experiment are included for purposes of comparison.

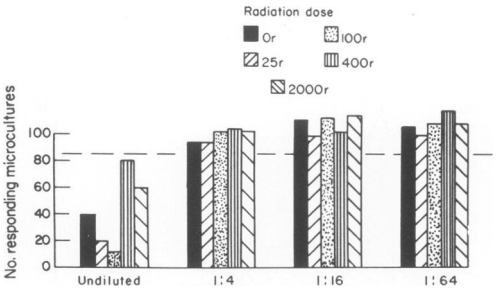
tions as medium in the response of C57BL/6 spleen cells to SRBC in 10-μl cultures. As shown in Text-figure 17, the undiluted supernatant from irradiated cells contains material that suppresses the anti-SRBC response. With dilution, this suppressive effect is negated by material that augments the response. However, no influence of radiation dose is discernible. Text-figure 18 summarizes a similar experiment with different radiation doses in which the incubation period after irradiation was reduced to 30 minutes. Again, the undiluted supernatants are uniformly suppressive, but with dilution there is a suggestion of a dose-related augmentation that is most pronounced in the 100-rad groups and which disappears with dilution.

Discussion

The present studies show that relatively small doses of radiation are accompanied by an augmentation of the *in vitro* response of murine spleen cells to SRBCs. Related studies suggest that the same phenomenon may be

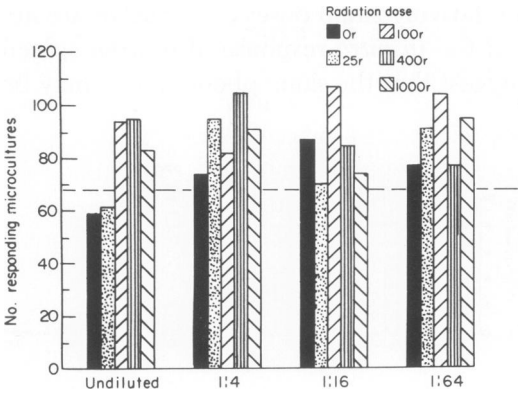


TEXT-FIGURE 16—Effect of exposure of only the T-cell component upon radiation-induced augmentation. See Text-figure 12 for the general experimental approach. C57BL/6 spleen cells were irradiated and added to saturating numbers of nonirradiated nu/nu (C57BL/6 background) spleen cells in 1-ml cultures.

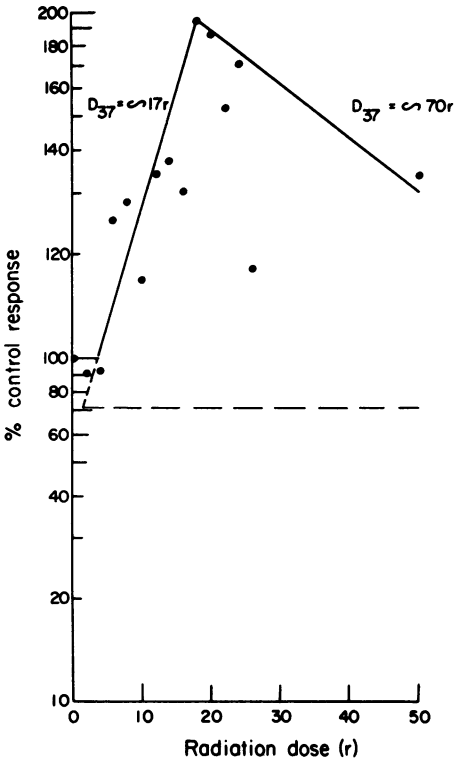


TEXT-FIGURE 17—Release of stimulatory/inhibitory material by irradiated spleen cells. See the text for the experimental approach.

demonstrable with human peripheral blood cells.[19] Augmentation appears to relate to radiation-induced disruption of a homeostatic component of the *in vitro* response that exerts its maximum effect relatively soon after the addition of antigen (Text-figures 7 and 8). The latter observation is consistent with the concept that augmentation is due to radiation-induced interphase death of a subset of lymphocytes. The remarkable radiosensitivity of this phenomenon can be appreciated by replotting the data of Text-figure 6 as a semilog function, as shown in Text-figure 19. With respect to this text-figure, if one assumes that the peak response caused by exposure to 18 rads represents optimal helper activity due to maximum radiation-induced loss of suppressor cell activity, then it is possible to quantitate in very rough fashion the radiosensitivity of the latter cell. In arbitrary fashion, the D_{37} for the suppressor activity of Text-figure 19 is defined as the dose of radiation required to reduce by 63% the maximum help with respect to the "suppressor" or initial component of the dose-response curve. In Text-figure 19, this is the dose required to reduce the PFC response from 195% (maximum response) to 74% and is equal to ap-



TEXT-FIGURE 18—Release of stimulatory/inhibitory material by irradiated spleen cells (adapted from Anderson et al [8]). See the text for the experimental protocol.



TEXT-FIGURE 19—Estimated D_{37} of the suppressor component of the dose-response curve. Data are derived from Text-figure 6.

proximately 17 rads. Even the latter number is probably too large, because small doses of radiation are associated with injury to B cells.[1,9,20-22] The influence of such putative injury to B cells would be to reduce the peak PFC response and thus render the "suppressor" component of the dose-response curve of Text-figure 19 less steep than might otherwise be the case.

The evidence presented herein supports the hypothesis that low dose augmentation of the *in vitro* response to SRBCs is due to radiation-induced injury to a subpopulation of T cells, presumably a subset with a suppressor function. The evidence may be summarized as follows:

1. Augmentation is associated with reduced numbers of viable cells (Text-figure 5). Thus, this phenomenon appears to be associated with a loss of lymphocytes. The possibility that augmentation results from radiation-related enhanced activity on the part of the residual cells cannot be excluded but seems unlikely.

2. Augmentation is found with T-dependent but not T-independent antigens (Text-figure 11).

3. Augmentation is equivocal or absent when irradiated B cells are combined with saturating numbers of nonirradiated T cells or helper factor (Text-figures 13 and 14). Conversely, irradiation of only the T-cell component of the response results in augmented *in vitro* anti-SRBC responses (Text-figures 15 and 16).

Unfortunately, the evidence that links augmentation with radiation-induced injury of a subpopulation of T cells is largely inferential, and therefore current efforts are directed toward the development of direct evidence to support or refute this hypothesis. These efforts may be summarized as follows: 1) the effects, singly and in combination, of low dose irradiation and antiserums directed against the I-J determinant, the latter apparently being a surface property unique to suppressor cells; 2) the effects of irradiation upon varying numbers of Ly 1^-, 2^+, 3^+ cells added to 10-μl cultures in a limiting dilution type of experiment.

Although suppressor T cells are generally considered to be radiosensitive, most reports to date have employed doses considerably in excess of those reported herein. Augmented function *in vivo* or *in vitro* has been associated with exposues of 200 to 3000 rads.[19,23–27] Comparable exposures in the present series of experiments are not followed by augmentation. The basis of this apparent discrepancy is not known but may relate to the following:

1. Specific requirements for various T cell subpopulations in the response under evaluation. In this connection the concept of ratio dominance may be of particular importance. Each type of lymphocyte involved in the *in vitro* immune response appears to regulate, or be regulated by, variable numbers of another cell type. If such regulation is dominant, then the ratio of the two cell types becomes of critical importance, particualrly with respect to the cell type that is limiting for the response. Different doses of radiation would be expected to subtract varying numbers of each of the involved cell types, and thus each exposure level in each experimental model might be expected to result in a unique situation with respect to the interaction of the residual lymphocytes.

2. Discrepancies in the radiosensitivity of different modes of feedback suppression. As reported by Gershon et al,[28,29] different immune responses are associated with dissimilar forms of feedback suppression. The latter may differ in radiosensitivity.

3. Discrepancies in the radiosensitivity of putative subpopulations of suppressor T cells or their precursors. The suppressor T cell involved in tumor immunity is relatively radioresistant, while the precursor of this cell type is more sensitive.[24] In addition, there may be more than one sub-

population of suppressor T cells. To date, each subpopulation of lymphocytes evaluated has differed in radiosensivity.[1] On this basis, it is reasonable to suspect that putative subpopulations of suppressor T cells will vary in their response to irradiation.

References

1. Anderson RE, Warner NL: Ionizing radiation and the immune response. Adv Immunol 24:215–335, 1976
2. Micklem HS, Loutit JF: Tissue Grafting and Radiation. New York Academic Press, 1966
3. Taliaferro WH, Taliaferro LG, Jaroslow BN: Radiation and Immune Mechanisms. New York, Academic Press, 1964
4. Taliaferro WH, Taliaferro LG: Effects of radiation on the initial and anamnestic IgM hemolysin responses in rabbits: Antigen injection after x-rays. J Immunol 103:559–569, 1969
5. Taliaferro WH, Taliaferro LG: Effects of irradiation on initial and anamnestic hemolysin responses in rabbits: Antigen injection before x-rays. J Immunol 104:1364–1376, 1970
6. Dixon FJ, McConahey PJ: Enhancement of antibody formation by whole body x-radiation. J Exp Med 117:833–848, 1963
7. Gengozian N, Makinodan T: Relation of primary antigen injection to time of irradiation on antibody production in mice. J Immunol 80:189–197, 1958
8. Anderson RE, Lefkovits I, Troup GM: Radiation induced augmentation of the immune response. Contemp Top Immunobiol (In press)
9. Anderson RE, Lefkovits I: Effects of irradiation on the *in vitro* immune response. Exp Cell Biol (Submitted for publication)
10. Waldmann H, Pope H, Lefkovits I: Limiting dilution analysis of helper T-cell function: II. An approach to the study of the function of single helper T cells. Immunology 31:343–352, 1976
11. Julius MH, Simpson E, Herzenberg LA: A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur J Immunol 3:645–649, 1973
12. Mishell RI, Dutton RW: Immunization of dissociated spleen cell cultures from normal mice. J Exp Med 126:423–442, 1967
13. Schreier MH: *In vitro* immunization of murine spleen cells, Immunological Methods. Edited by I Lefkovits, B Pernis. New York, Academic Press, 1979, pp 327–334
14. Lefkovits I: Induction of antibody-forming cell clones in microcultures. Eur J Immunol 2:360–366, 1972
15. Lefkovits I: Limiting dilution analysis,[13] pp 355–370
16. Cunningham AJ, Szenberg A: Further improvements in the plaque technique for detecting single antibody-forming cells. Immunology 14:599–600, 1968
17. Mosier DE, Johnson BM, Paul WE, McMaster PRB: Cellular requirements for the primary *in vitro* antibody response to DNP-Ficoll. J Exp Med 139:1354–1360, 1974
18. Anderson RE, Standefer JC, Scaletti JV: Radiosensitivity of defined populations of lymphocytes: VI. Functional, structural, and biochemical consequences of *in vitro* irradiation. Cell Immunol 33:45–61, 1977
19. Fauci AS, Pratt KR, Whalen G: Activation of human B lymphocytes: VIII. Differential radiosensitivity of subpopulations of lymphoid cells involved in the polyclonally-induced PFC responses of peripheral blood B lymphocytes. Immunology 35:715–720, 1978
20. Janeway CA Jr: Cellular cooperation during *in vivo* antihapten antibody responses:

II. The effect of *in vivo* and *in vitro* x-irradiation on T and B cells. J Immunol 114:1402–1407, 1975
21. Anderson RE, Warner NL: Radiosensitivity of T and B lymphocytes: III. Effect of radiation on immunoglobulin production by B cells. J Immunol 115:161–169, 1975
22. 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 varius lymphoid tissues and numbers of recirculating lymphocytes. J Immunol 118:1191–1200, 1977
23. Moretta L, Webb SR, Grossi CE, Lydyard PM, Cooper MD: Functional analysis of two human T-cell subpopulations: Help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. J Exp Med 146:184–200, 1977
24. Hellström KE, Hellström I, Kant JA, Tamerius JD: Regression and inhibition of sarcoma growth by interference with a radiosensitive T-cell population. J Exp Med 148:799–804, 1978
25. Dutton RW: Suppressor T cells, Transpl Rev 26:39–55, 1975
26. Lawrence DA, Eastman A, Weigle WO: Murine T-cell preparations: Radiosensitivity of helper activity. Cell Immunol 36:97–114, 1978
27. Benacerraf B, Kapp JA, Debré P, Pierce CW, De La Croix F: The stimulation of specific suppressor T-cells in genetic non-responder mice by linear random copolymers of L-amino acids. Transplant Rev 26:21–38, 1975
28. Cantor H, Hugenberger J, McVay-Boudreau L, Eardley DD, Kemp J, Shen FW, Gershon RK: Immunoregulatory circuits among T-cell sets. Identification of a subpopulation of T-helper cells that induces feedback inhibition. J Exp Med 148:871–877, 1978
29. Eardley DD, Hugenberger J, McVay-Boudreau L, Shen FW, Gershon RK, Cantor H: Immunoregulatory circuits among T-cell sets: I. T-helper cells induce other T-cell sets to exert feedback inhibition. J Exp Med 147:1106–1115, 1978