A Fatal Reaction Caused by Implantation of Adult Parental Spleen Tissue in Irradiated F_1 Mice. (23612)

E. E. SCHWARTZ,* A. C. UPTON AND C. C. CONGDON
Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tenn.

The importance of splenic and lymphoid tissue in antibody production, and the ability of these cells to confer immunity when grafted into other animals is well documented(1-5). Billingham, Brent, and Medawar(6) introduced the expression, "adoptive transfer," to describe the transfer of immunity by immunologically activated tissue. Mitchison detected the presence of implanted donor spleen cells in the lymph nodes and spleen of irradiated recipients(7). He also noted that the antibody titer after administration of preimmunized spleen is maintained considerably longer in irradiated than in nonirradiated recipients(8) and that production of antibody is more active in the former(7). The influence of the internal environment of the implanted cells on their transfer of the antibody response is seen in the inability of the neonatal rabbit to produce antibodies after transfer of sensitized adult rabbit lymphoid cells unless normal adult lymphocytes have been injected previously(9,10), whereas in the irradiated adult recipient, neonatal lymphoid cells are effective and preinoculation of normal lymphocytes is not required.

In the investigation of possible applications of these immunogenetic principles in the radiotherapy of mouse leukemia, we attempted to transfer to leukemic animals splenic cells from a tumor-resistant donor. Mice of the (C3H x 101) F_1 strain harboring a serially transferred, indigenous leukemia, were inoculated, after supralethal irradiation, with adult spleen tissue from strain-101 donor mice that had been preimmunized against the leukemia. Unexpectedly, these animals died before the untreated leukemic controls. Subsequently, groups of leukemia-free mice were similarly treated; and they, too, died in the second and third week after irradiation, regardless of whether (C3H x 101) F_1 or parent strain (101) bone marrow was administered concomitantly, with the parent spleen tissue, to enhance recovery of hemopoietic tissues from radiation injury. The fatal reaction to the implantation of parent spleen is the subject of this report.

Methods. Eight- to 16-week-old (C3H x 101) F_1 male mice were exposed to a single LD_{100}/30-day dose of total-body radiation of 800 to 1000 r. The mice were irradiated in a lucite drum divided into 12 compartments separately housing each animal, which was continuously revolved in the treatment field at approximately 7 rpm. The physical factors were 250 kvp, 30 ma, 3 mm of Al added filtration, 0.40 mm of Cu, 82 cm target-to-mouse distance, and dose rate 98 r/minute. The spleens from 5 to 10 adult 101 or (C3H x 101) F_1 mice were pooled, minced, ground, and then suspended in Tyrode's solution by a Teflon homogenizer. The tissue was chilled throughout the procedure. Counts revealed the presence of approximately 100 x 10^6 grossly intact, nucleated cells per spleen. Different groups of (C3H x 101) F_1 mice received the equivalent of one-fourth to one spleen (25 x 10^6 to 100 x 10^6 cells) intraperitoneally 3-5 hours after X-irradiation, the implanted tissue being administered 1 hour after sacrifice of the donor. One femoral equivalent from an isologous or parent strain (101) donor was also injected into each recipient via tail vein on the day after irradiation, except in the controls, which received the bone marrow inoculum alone 3-5 hours after irradiation. Bone marrow cell suspensions in Tyrode's solution were prepared in the manner described by Urso and Congdon(11). The parent (101) spleen and bone marrow donors were 14-20 weeks old; in the earliest experiments they were preimmunized 3-7 weeks before transfer by the intraperitoneal adminis-

* Public Health Service Postdoctoral Research Fellow of National Cancer Inst.

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TABLE I. Survival of F_1 Hybrid Mice Injected with Parental Strain Spleen Cells.

Recipient		Implanted spleen		Recipients surviving 30 days		Median time of death
X-ray dose (r)	Strain	Donor strain	No. cells inj. ($\times 10^6$)	No.*	%	(days)
800–1000	(C3H × 101)F_1	101†	75–100	2/42	5	12
"	"	"	25– 50	13/45	29	16
0	"	"	100	10/10	100	
900–1000	"	101‡	100	2/24	17	12
800– 900	"	"	50	43/49	88	19
0	"	"		9/ 9	100	
800–1000	"	(C3H × 101)F_1	50	16/16	100	
400	"	101†	50	8/10	80	19–24
"	(RF × AKR)F_1	RF†	20	0/ 9	0	17
0	"	" †	20	9/ 9	100	
900	(C57BL × 101)F_1	C57BL‡	100	0/20	0	18
"	"	"	50	2/ 7	29	26
"	"	(C57BL × 101)F_1	50–100	20/20§	100	

* No. surviving/No. inj. † Presensitized. ‡ Non-presensitized. § Equal numbers of mice received 50 × 10^6 and 100 × 10^6 cells.

tration of leukemic (C3H x 101) F_1 liver and spleen cell suspensions and in later experiments, by the intravenous injection of normal (C3H x 101) F_1 liver, spleen, and kidney cell suspensions one week before transfer. Additional 8-week-old male (C3H x 101) F_1 and 14- to 15-week-old (RF x AK) F_1 female mice were exposed to sublethal doses of X-radiation (400 r), 3-5 hours and 24 hours, respectively, before they received immunized adult parental spleen tissue intravenously. RF mice that had received normal AK spleen tissue intravenously one week earlier served as preimmunized spleen donors for the (RF x AK) F_1 recipients. Three groups of 12-week-old (C57BL x 101) F_1 female mice exposed to 900 r received nonsensitized adult C57BL spleen cells intravenously. None of the (C3H x 101)F_1 sublethally irradiated, (RF x AKR)F_1 or (C57BL x 101)F_1 mice received bone marrow.

Results. Thirty-day survival. The vast majority of irradiated F_1 mice receiving parental strain spleen, with or without subsequent administration of either F_1 or parental strain bone marrow, died in the second to fourth week after treatment (Table I, Fig. 1). These mice looked well for the first week after inoculation but deteriorated thereafter. Spleen cells of presensitized parental mice caused greater mortality than those of nonsensitized mice, relatively few recipients of the smaller quantities of nonsensitized spleen cells succumbing in the first month (Table I). Of the irradiated F_1 mice (controls) receiving bone marrow without spleen, 19 of 20 recipients of isologous (F_1) and 92 of 98 recipients of parental marrow survived the initial 30 days after exposure. Later deaths among recipients of presensitized parental bone marrow are to be reported in a subsequent communication. Mice receiving parental spleen and bone marrow without prior irradiation and others inoculated with F_1 spleen after X-irradiation suffered no apparent ill effects.

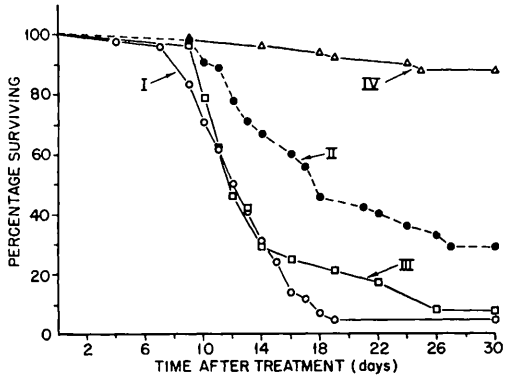

FIG. 1. Survival of irradiated (C3H × 101)F_1 mice inoculated with spleen cells from donor mice of the 101 strain. Curve I, received 75-100 × 10^6 cells from a presensitized donor; curve II, received 25-50 × 10^6 cells from a presensitized donor; curve III, received 100 × 10^6 cells from a non-presensitized donor; curve IV, received 50 × 10^6 cells from a non-presensitized donor.

Each mouse received in addition to spleen cells the equivalent of 1-2 femurs of isologous or 101 strain bone marrow cells, the strain of origin of the marrow having no detectable influence on the 30-day survival.

Autopsy findings. In those mice found dead after exposure to 800-1000 r, there was atrophy of hemopoietic elements in the bone marrow and spleen, with the exception of those in which parental marrow was administered in addition to parental spleen. In the latter, the marrow and red pulp of the spleen were cellular, exhibiting active formation of granulocytes, erythrocytes, and megakaryocytes. The lymphoid follicles were atrophic in all animals, with little or no evidence of lymphopoiesis. Foci of necrosis of varying extent were seen in the liver, and some mice showed extensive bacterial invasion. Focal necrosis of the mucosa of the large intestine, with infection and hemorrhage was also noted. In the sublethally irradiated (RF x AK) F_1 mice, there were, in addition to the previously mentioned findings, reticuloendothelial hyperplasia, with pseudotubercle formation and areas of fibrinoid necrosis in the spleen and lymph nodes.

Discussion. In this investigation, the implantation of adult parental strain spleen cells prevented the survival of irradiated F_1 hybrids, even when the recipients received a sublethal dose of X-radiation (400 r = $LD_0/30$ days) or subsequent inoculation of isologous F_1 hybrid marrow cells. Since this effect was not observed when isologous F_1 hybrid spleen cells were injected, the reaction is believed to have resulted from immunogenetic differences between hybrid and parent tissues. Because tissues of the F_1 hybrid are antigenic to mice of the parent strain but not vice versa, and because of the capacity of transplanted spleen cells to confer "adoptive immunity," we inferred that the fatal reaction described represents the effects of production by the grafted parental spleen cells of antibodies against the tissues of the F_1 recipient. In all experiments, the dose of parent spleen cells was correlated with the median time of death of the lethally irradiated host (Table I). Although relatively few recipients of less than 75 million adult parental spleen cells died within the first 30 days, many others succumbed in the second month. After compilation of these results, Makinodan *et al.*(12) observed a similar fatal reaction in lethally irradiated (C3H x 101) F_1 mice injected with spleen cells obtained from homologous adult mice of the LAF_1 strain. Injection of spleen cells from newborn homologous donors apparently does not produce the early death(13) caused by adult parental strain and adult homologous spleen cells, but the effect of spleen cells from newborn donors of the parental strains has not been tested.

The absence of this reaction in nonirradiated F_1 recipients may have been caused by lack of proliferation of the implanted spleen cells, owing to the normal hemopoietic status of such recipients and to the lack of need for cell proliferation such as is required to repair the radiation injury in the irradiated recipients. Immature animals obviously have a natural stimulus for cell proliferation; thus, situations possibly similar to this one have been reported by Billingham and Brent(14), who noted death in newborn rats after inoculation of homologous antibody-forming cells, and by Simonsen(15), who found hemolytic anemias in newborn chicks inoculated with adult spleen cells prior to hatching.

The pathologic findings and time of death indicate that some of the mice receiving parental spleen died of bone marrow aplasia; in other animals, however, the bone marrow was cellular and the immediate cause of death cannot be given. The granuloma-like reactions in the spleen and lymph nodes of sublethally irradiated F_1 mice receiving immunized parental spleen further suggest that these tissue changes occur as part of, or in response to, an immunologic reaction.

It is interesting that the marrow cavity of mice receiving parental marrow cells and spleen cells was cellular, whereas the marrow of mice receiving *isologous* (F_1) marrow cells along with parental spleen cells, or in that of mice receiving parental spleen cells alone, was usually atrophic. This is in keeping with the interpretation that the grafted spleen cells react against antigens in the F_1 recipient that are inherited from the homologous parent and that, of course, tissue antigenically identical with the graft is unaffected.

Uphoff(16), Koller(17), and Trentin(18) invoked a similar mechanism to account for delayed mortality in irradiated F_1 mice of strains other than those used in this study

after treatment with bone marrow of parental strain donors. It is noteworthy, however, that in the strains used in this investigation, as in the work of van Bekkum and Vos(19), there was little or no delayed mortality in irradiated F_1 recipients of parental bone marrow; *i.e.*, only 11 of 59 irradiated (C3H x 101) F_1 mice given marrow from donors of the 101 strain died within 90 days after irradiation, whereas the injection of spleen cells from 101-strain donors into irradiated (C3H x 101) F_1 recipients caused extensive and prompt mortality, as described. These observations suggest that factors other than the histoincompatibility of the parental strains influence the probability of the occurrence of delayed reactions in F_1 recipients of parental marrow. One such factor is the immunologic status of the bone marrow donor, since on injection of marrow alone from donors of the 101 strain presensitized to (C3H x 101) F_1 tissues, there was appreciable delayed mortality of irradiated (C3H x 101) F_1 recipients (20); as noted, this effect is not observed with marrow from nonpresensitized 101-strain donors. It seems, therefore, that in the pathogenesis of the delayed parent bone marrow reaction, as in the parent spleen reaction, the number, type, rate of growth, and immunologic status of the implanted cells affect the severity of the reaction, as does the relative degree of immunogenetic incompatibility of the donor and recipient(21,16).

Summary. 1) Implantation into irradiated F_1 hybrids of viable spleen cells from nonirradiated adult mice of the parental strains caused death within 2-4 weeks. 2) The fatal reaction occurred even after sublethal doses of radiation or when the recipients were also injected with marrow cells from isologous hybrids or from mice of the same strain as that from which the parent-strain spleen tissue was obtained. The deaths are ascribed to the formation by the implanted cells of antibodies against the recipient.

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