Immune Response in Irradiated Mice with Peyer's Patch Shielding.* (26974)

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We reported(1) that the capacity to produce antibodies to an injected particulate antigen was retained in the rabbit if the spleen or appendix were shielded while the rest of the body received an LD_{50} dose of x-radiation. We were able to demonstrate that this capacity was retained if these shielded tissues were removed 24 hours, or even as soon as 3 hours, after irradiation(2). These observations have been confirmed by Taliaferro and others(3,4). We also showed that a more rapid regeneration of the lymphatic tissues occurred in rabbits which had the appendix or spleen shielded during irradiation than in control animals. These studies were conducted using dosages of irradiation in the LD_{50} range, thus permitting the bone marrow of survivors to regenerate, though not as rapidly as the lymphatic tissues(5). Recently Süssdorf(6) has repeated our original study and found an essentially similar course of events.

One of our most interesting findings was that no granulocytopoiesis, megakaryocytopoiesis or erythropoiesis was observed in the shielded appendix, suggesting that under the conditions of the experiment these lymphatic cells did not exhibit a multipotential capacity as far as local production of other blood elements was concerned. In the light of present knowledge concerning cellular colonization(7,8,9), the rapid proliferation of lymphatic tissues following appendix- or spleen-shielding is presumably the result of migration and subsequent colonization of cells from the protected lymphatic tissues.

It seemed of interest, therefore, to repeat the experiment of protecting a pure source of lymphoid cells during total-body exposure to a supralethal dose of x-radiation, and to study 1) lymphocyte migration, recolonization, and potential for the production of other cell types; 2) modifications of the immune response that might result; and 3) value of the technique as an assay of the control of lymphopoiesis.

A patch of Peyer in the small intestine of the mouse measuring about 2.5 mm in width and 1 mm in depth and weighing about 5 mg was selected for these studies. Approximately 5 million fixed and free lymphatic cells are contained in such a patch as estimated by geometric calculations.

Adult female CF No. 1 and (C3H $\times$ 101)F_1 mice approximately 14 weeks old, were used as recipients in most of these studies. Donor tissues were obtained from female A/Tex mice and from male Holtzman rats.

The shield used to protect the Peyer's patch during irradiation was constructed of ¼ inch lead, 11 mm wide, with a groove 4 mm in diameter to house the intestine. Under Nembutal anesthesia, the small intestine was brought out through a small incision into the peritoneal cavity and a single Peyer's patch was shielded during total-body irradiation with 950 r. In control groups, either an equivalent portion of the intestine having no Peyer's patch was shielded, or a sham operation was performed without any shielding. Following irradiation the intestine was returned to the peritoneal cavity and the incision sutured with silk.

Since 100×10^6 rat bone marrow or 10×10^6 mouse bone marrow cells afford good initial protection in lethally irradiated mice, these cell concentrations were used in the experiment. The technique for preparation of the various suspensions has been described (10). The suspensions were made in iso-

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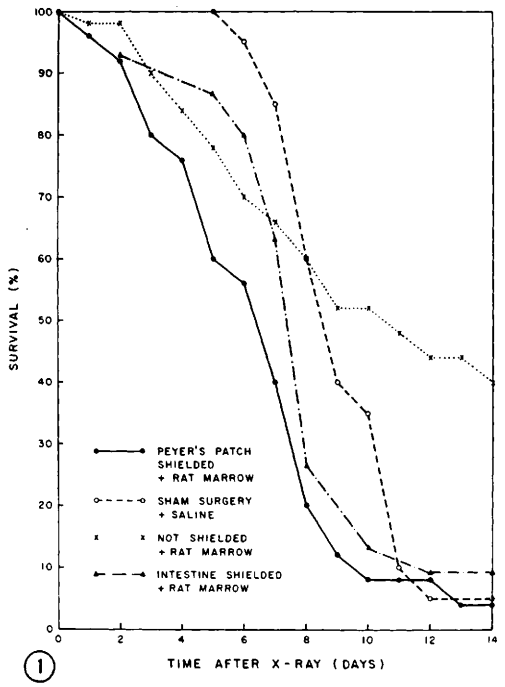

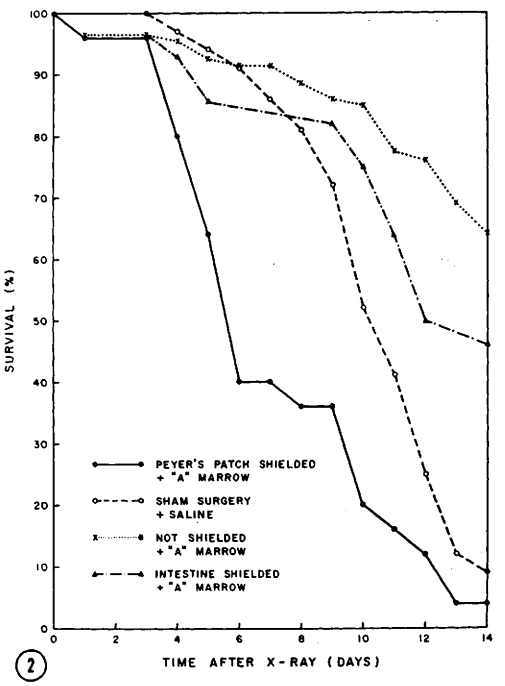

FIG. 1. Survival of CF No. 1 mice receiving 950 r and treatments as shown.

FIG. 2. Survival of (C3H × 101)F_1 mice receiving 950 r and treatments as shown.

tonic saline and 0.5 ml injections were given via the tail vein.

Dosimetry. The x-rays were generated in a 250 kv G.E. Maxitron operating at 30 Ma and using 0.25 mm copper and a 1-mm aluminum filter. The half-value layer in copper of the filtered beam was 1.02 mm. The exposures were measured with a Victoreen condenser meter equipped with a 250 r chamber. Measurements were made in air at a position estimated to be the center of the animal's body. The dose rate averaged 120 r per minute at 57 cm.

Results. Survival. As can be seen in Fig. 1, 40% (20 of 50) CF No. 1 mice in the control group given rat bone marrow following total-body irradiation without shielding were alive at 14 days, whereas only 4% (1 of 25) survived in the group given rat bone marrow following protection of one Peyer's patch. Furthermore, the survival pattern shows that death in such Peyer's patch protected mice occurred significantly earlier ($P<0.001$) than in sham operated controls that were lethally irradiated without shielding and without cellular injection.

In an additional control group, an 11-mm portion of intestine that contained no Peyer's patch was protected during exposure to 950 r, and these mice were also injected with rat bone marrow cells. Death also occurred significantly earlier in this group ($P<0.02$) than in irradiation-only controls, but the results were not as dramatic as in the Peyer's patch group. By the 14th day, however, only 9% (2 of 22) were alive.

In a companion series of experiments using homologous strains, when (C3H × 101)F_1 hybrid mice were given bone marrow from Strain A/Tex mice following Peyer's patch shielding during irradiation with 950 r, an early death pattern also resulted (Fig. 2). In this strain combination, however, protection of 11-mm of intestine lacking a Peyer's patch did not produce an equally severe reaction, and 48% (14 of 29) were alive at 14 days. This may be interpreted as indicating that fewer lymphoid cells are required to re-

TABLE I. Comparative Effect of Varying X-ray Dosages Delivered to Shielded Peyer's Patch and Intestine on Lethally Irradiated (950 r) CF No. 1 Mice Injected with Rat Bone Marrow.

X-ray dosage to Peyer's patch (r)	No. of mice used	14-day no.	Survival (%)	X-ray dosage to 1.1 mm intestine	No. of mice used	14-day no.	Survival (%)
None	25	1	4	None	15	1	6.6
200	10	0	0	200	10	0	0
300	10	0	0	300	10	0	0
400	10	0	0	400	10	2	20
600	10	0	0	600	10	1	10
650	10	3	33.3	650	10	4	40
700	10	6	60.0	700	10	9	90
950	50	20	40.0	950	50	20	40

ject a heterologous graft than to reject an homologous graft.

Furthermore, it must be kept in mind that in addition to the fact that the genetic relationship in this experiment is closer than in the rat-to-mouse combination (thus diminishing the antigenic differences), only 10×10^6 mouse bone marrow cells were injected while 100×10^6 rat cells were used. In a preliminary experiment, (C3H $\times$ 101)F_1 mice that received larger numbers of A/Tex bone marrow cells (20 to 40 $\times$ 10^6) following the shielding of 11 mm of the small intestine did not survive.

These results led us to investigate whether the shielding effect could be reproduced by intravenous injection of isologous lymphocytes or spleen cells into mice treated with rat bone marrow following exposure of the entire body to lethal irradiation. Lymphocytes from the mesenteric lymph node and spleen cells from adult CF No. 1 donors were injected into CF No. 1 mice 4 hours after the irradiation bone-marrow treatment. Results agree with those of van Bekkum and Vos(11) and indicate that in the CF No. 1 mouse also, fewer than one million cells from lymphatic tissue or spleen of its own strain, given by intravenous injection, hasten the death of the recipient.

Hybrid (C3H $\times$ 101)F_1 mice that received A/Tex bone marrow (10×10^6 cells) or rat bone marrow (100×10^6 cells) after total body x-irradiation and were subsequently injected with 10×10^6 cells from the mesenteric lymph node of their own strain responded in the same manner, and all mice died by the 14th day after treatment.

It requires a supralethal dose of whole-body x-irradiation (about 900 r) to suppress the capacity of the mouse to reject a heterologous (rat) bone marrow graft. The amount of x-irradiation required to suppress the capacity of the Peyer's patch or of 11 mm of intestine to reject a heterologous graft, was determined by removing the lid from the shield after a calculated number of roentgen units had been given to the mouse. Thus the protected Peyer's patch or intestine received dosages of from 200 r to 700 r at the same time that the rest of the body received 950 r (Table I). These values were found to be 650 r and 400 r respectively, and suggest that the smaller the lymphoid population, the smaller is the dose of x-irradiation necessary to suppress the total immune response. These findings also indicate how few immunologically competent cells need remain in the body after irradiation successfully to defeat heterotransplantation.

In another series of experiments, the Peyer's patch was protected initially while the body was given 950 r. Within 10 minutes the protected patches were then given 950 r, followed by injection of 100×10^6 rat bone marrow cells. All mice died and the early death pattern was again observed, suggesting that following irradiation stress there is immediate migration of lymphoid cells from the Peyer's patch into the damaged body, where they presumably recolonize and maintain the immune response of the individual.

Histology. Histological examination of mice following Peyer's patch protection shows that before death the shielded patch has accomplished partial or essentially complete re-

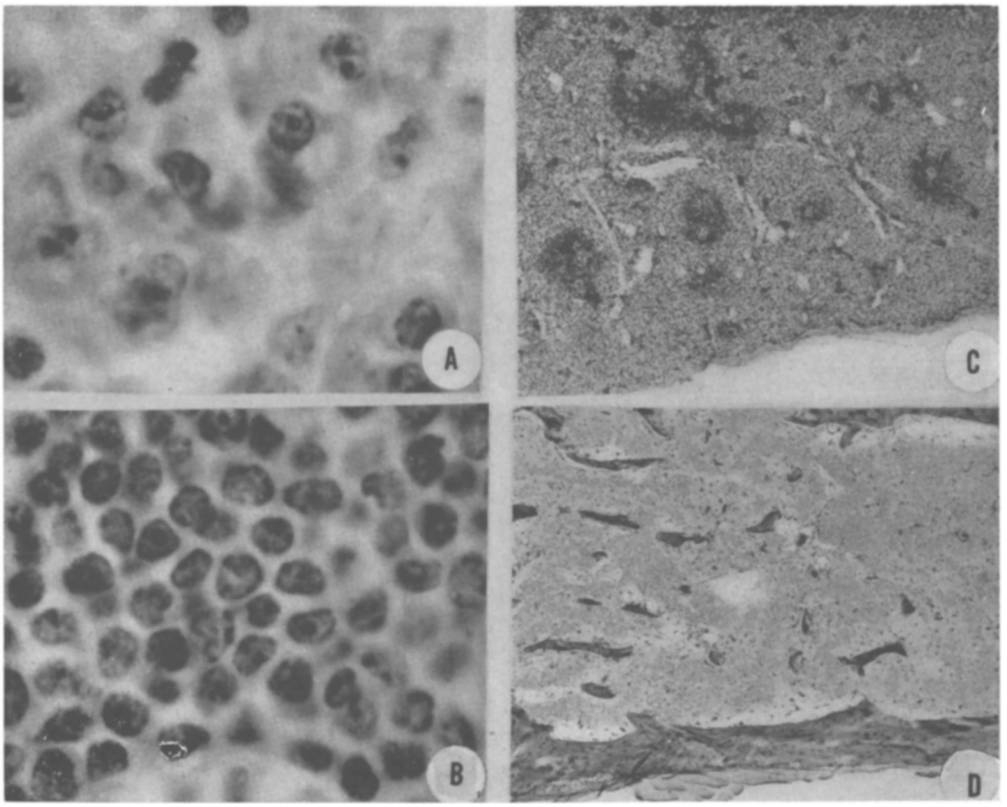

FIG. 3. A—Mesenteric lymph node of CF No. 1 mouse 6 days after 950 r with Peyer's patch shielded, showing plasma-like cells (× 1567). B—Normal mesenteric lymph node of CF No. 1 mouse (× 1567). C—Spleen of CF No. 1 mouse 6 days after 950 r with Peyer's patch shielded, showing foci of lymphoid recolonization (× 36). D—Aplastic bone marrow in CF No. 1 mouse 6 days after 950 r with Peyer's patch shielded, showing absence of any recolonization (× 36).

population of the depleted lymphatic structures, including the thymus. We have sacrificed such animals at daily intervals from 1 through 12 days after the shielding-irradiation procedure. Celloidin-embedded tissues were cut at 6 μ and sections stained with hematoxylin-eosin and azure. In the shielded Peyer's patch itself we observed increased mitotic activity 24 hours after shielding, and this may be seen in other lymphoid structures as repopulation proceeds. In many animals repopulation of the spleen, lymph nodes, and thymus is well along at 6 days, but essentially complete repopulation is not accomplished before 10 to 12 days (Fig. 3). No bone marrow regeneration was observed (Fig. 3-D) nor was there evidence of granulocytic, megakaryocytic, or erythrocytic regeneration elsewhere.

One interesting finding in these mice, for which we have no explanation, is the great prominence of plasma (or plasma-like) cells in the regional mesenteric lymph node during repopulation (Fig. 3A). These plasma cells, which by 3 days constitute about 90% of the free cell population at this site, are found principally in association with the blood vessels. They show marked mitotic activity, and under low power light microscopy the lymph node appears to be composed of virtually hundreds of rosettes of them.

An increased number of such plasma (or plasma-like) cells is also found in the mesenteric node of mice given whole-body x-radiation in the LD_{50} range or above, without Peyer's patch shielding, but in this case not until 6 to 8 days postirradiation. Since these cells are morphologically identical in both experimental models, we made an attempt to determine whether they were also function-

Table II. Effect of Lymph Node Transplantation on Survival of 950 r Irradiated CF No. 1 Mice Treated with Rat Bone Marrow.

Group	Donor mesenteric* lymph nodes	Survival (days) 8	14
1	2 nodes from mice 6 days after 950 r	17/18	14/18
2	2 nodes from mice with shielded Peyer's patch 6 days after 950 r	3/14	2/14
3	One-half normal node	2/24	0/24
	One-fourth " "	7/11	1/11

* Transplantation of spleens instead of lymph nodes from similarly treated mice gave comparable results.

ally identical in both situations, and the following experiment was performed to test the capacity of a mesenteric node transplant to reject a rat bone marrow graft previously given to irradiated mice.

CF No. 1 mice were lethally irradiated and treated with 100×10^6 rat bone marrow cells. About 3 hours later, 2 mesenteric lymph nodes taken from mice 6 days after exposure to 950 r were transplanted to the peritoneal cavity of one group of recipients (Table II). Similarly, 2 mesenteric lymph nodes obtained 6 days after lethal irradiation from mice having a shielded Peyer's patch were implanted into another group of rat-treated irradiated mice. As a control procedure, one-fourth to one-half of a normal lymph node was transplanted to a third group of irradiated mice treated with rat bone marrow. Average weights of the mesenteric lymph nodes used were: Normal mice, 110 mg; 950 r x-irradiated mice, 42 mg; irradiated, Peyer's patch shielded mice, 53 mg.

The data in Table II indicate that immunologically competent cells were present in the mesenteric lymph node 6 days after mice were lethally irradiated with the Peyer's patch shielded. The nodes from irradiated mice without such shielding either contained no immunologically competent cells or contained a number insufficient to reject the heterologous graft. Transplantation of a normal lymph node caused rejection of the rat bone marrow group in the control animals.

These results were not unexpected since it is well known(11) that cells from a normal mesenteric lymph node transplant will reject a rat bone marrow graft previously given to lethally irradiated mice. Neither was it unexpected that the mesenteric node from a mouse previously given whole-body x-radiation should fail to cause rejection of a rat bone marrow graft which had been given to an irradiated mouse. However, the mesenteric node from Peyer's patch-shielded mice proved as capable of rejecting a rat bone marrow graft as the normal node. This latter observation indicates that although these 2 groups of nodes are rich in plasma cells that appear to be morphologically alike, they are functionally quite different. To be sure, the mesenteric nodes in both these groups contained perhaps 10% lymphocytes, or lymphocyte-like cells, so one cannot be sure that the plasma cells were responsible for the immune response. It can be stated categorically, however, that even though the population of these 2 experimental groups is morphologically similar, their functional capacities are vastly different.

Discussion. It is now well established that lethal irradiation destroys the ability of the mouse to react against foreign bone marrow cells(7,8,9), and that such injected cells colonize and substantially reduce acute radiation mortality. In Peyer's patch-shielded animals injected with heterologous or homologous bone marrow cells, the grafted cells are rejected by the mouse's own regenerating lymphatic tissues, and since lymphocytes alone cannot support life at 950 r, the mice succumb. Lymphoid repopulation is well established in the spleen and mesenteric lymph node by the sixth day, and this coincides with the early death pattern observed. Histological evidence shows that lymphocytes normally tend to accumulate in the intestinal wall. Even though it is doubtful that as many as 5×10^6 are present in an 11-mm segment, the supply of lymphatic elements made available by shielding is sufficient to elicit rejection of the foreign rat cells. The results obtained when a Peyer's patch is shielded during lethal irradiation and the host is subsequently injected with heterologous cells agree with those of van Bekkum and Vos(11). These investigators treated irradiated mice with rat bone marrow and

subsequently injected them with a small number of isologous lymph node cells, thus preventing the transplant of the rat bone marrow from being successful. Barnes(12) reported similar results in irradiated mice treated with homologous marrow before isologous lymph node cells were injected. We, too, have found that relatively few cells from lymphatic tissues are needed to reject a heterologous or homologous graft.

The lymphocytic tissue in the Peyer's patch of the guinea pig are reportedly at least as active, if not more so, than those in the mesenteric lymph nodes. The germinal centers tend to be larger in the Peyer's patches than in the lymph nodes, while mitoses are especially numerous in the peripheral part of the center(13).

Cole and Garver(14) report that intraperitoneal injection of 0.6 cc of mouse blood (either isologous or homologous) annuls the protective effect of rat bone marrow in LAF_1 mice. These authors indicate that when the blood and marrow are of the same strain (C3H) there is no indication that the C3H bone marrow transplant is rejected.

Goodman and Congdon(15) reported the "killing-effect" in irradiated mice of a mixture of homologous bone marrow and isologous blood. Histologic studies showed evidence of temporary transplantation of bone marrow and then rejection. Irradiation of the blood, or of the donor prevented this reaction. These results support the theory that immunological reactive cells are present in the peripheral blood.

Santos and Cole(16) have also shown that injection of isologous lymphoid tissue from the spleen or lymph thymus elicits early rejection of rat marrow transplants in irradiated mice.

Under the experimental conditions described, the lymphoid cells of the Peyer's patch, although capable of repopulating other lymph structures, cannot serve as precursors of other cell types of the blood-forming tissues.

It is interesting to note that an x-ray dose of 650 r or more is required before the capacity of the lymphatic tissue in the Peyer's patch or intestine is impaired sufficiently to permit retention of a heterologous graft.

This model, which involves survival of tissue foci *in vivo*, provides us with an opportunity of studying the factors involved in normal control of growth and differentiation of lymphoid tissue, as well as of evaluating the substances which will enhance or inhibit immune responses.

Summary. 1. Shielding one Peyer's patch of a mouse during lethal x-irradiation protects a sufficient number of cells to cause rejection of a heterologous or homologous bone marrow graft. 2. Shielding 11 mm of the small intestine results in a similar but less violent reaction. 3. A dosage of 650 r or more to the protected Peyer's patch is necessary to impair the capacity of the lymphatic tissue to reject the graft. 4. Regeneration of lymphatic tissue is evident in the spleen, mesenteric lymph node and thymus in Peyer's patch-shielded mice 6 days post-irradiation. On the other hand no bone marrow regeneration was observed nor was there evidence of granulocytic, megakaryocytic, or erythrocytic regeneration elsewhere.

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Effect of Phosphate Loading on Leukocyte Labelling with Inorganic P^{32}.* (26975)

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Deoxyribonucleic acid (DNA) labelling with inorganic radiophosphorus (P^{32}) has been used to study leukocyte kinetics (1-11); the usefulness and reliability of this leukocyte label for appraising cell kinetics has been considered previously(12, 13). It has been shown that isotopic labelling of DNA occurs only during mitotic interphase when new DNA is synthesized(1,2,14-16) and that DNA retains label introduced during synthesis until cell death ensues(1,3,4,16-18).

Previous studies in rabbits(13) demonstrated that significant levels of plasma phosphate activity persist for at least 7 days after a single intravenous injection of only 2 μc of inorganic P^{32}/kg. Ultimate distribution of P^{32} to reach a common level of specific activity of the phosphate in the crude acid soluble, phospholipid, RNA, and DNA fractions of marrow cells was observed to occur by 72 hours. At this time the curve of regression of plasma phosphate activity, comparable in level to that of cell fraction phosphate, was noted to be quite flat. It was concluded that significant DNA labelling must continue for several days following a single intravenous dose of P^{32}. In an effort to shorten the exposure time of proliferating cells to high levels of activity in the extracellular phosphate pool, a load of non-isotopic inorganic phosphate was given intravenously and its effect on plasma clearance of P^{32} and on cell labelling evaluated.

Materials and methods. These studies were done in 3-5 kg rabbits each of which received intravenously 2.0 μc of P^{32}/kg of body weight. Technical and analytical procedures have been described(12,13). Serial blood samples to evaluate plasma clearance following intravenous P^{32} were obtained from the superior vena caval system via a size PE-50 polyethylene catheter inserted through a marginal ear vein. Samples showing visible hemolysis were discarded. Plasma was treated with an equal volume of 10% trichloroacetic acid and the supernate obtained after centrifugation was wet ashed in concentrated sulfuric acid prior to phosphorus determination.

Leukocytes were obtained from heparinized blood by the method of Athens, *et al.*(19). Red cells were sedimented in dextran and those remaining in the leukocyte containing supernate were hemolyzed with gramicidin-lysolecithin. Leukocytes were washed in saline and platelets removed by differential centrifugation. The remaining leukocyte suspension was then fractionated by a modification of the Schmidt-Thannhauser procedure, quantitative phosphorus analysis performed by an adaptation of the method of Berenblum and Chain, and radioactivity determined by scintillation counting(12).

Non-isotopic inorganic phosphate was given as a sterile, pyrogen-free solution containing approximately 100 millimols of phosphate/liter. This was prepared by dissolving 128.7

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