The Effect of Homologous Marrow Transplantation on the Survival of Monkeys Following Sublethal Whole-Body Radiation

By Frances E. Newsome and R. R. Overman

A lethal or supralethal dose of X radiation delays the primary antibody response in animals for a period long enough to permit the initial acceptance of foreign hematopoietic cells. Although late deaths due to "homologous disease" may occur about 30 to 60 days after treatment some animals survive longer and retain the donor cells. Porter found donor neutrophils in rabbits 7 months after homologous bone marrow treatment. Smith found rat platelets in a mouse 82 days after transplantation. Ford et al., Nowell et al., and Makinodan have also reported donor cells in recipients which survive the late death syndrome. Even when donor cells persist for long periods they may be rejected uneventfully. If a sublethal dose of X radiation is given, however, the animal rejects the donor cells much earlier. Congdon et al. found rat granulocytes in the early proliferation areas of the spleen and bone marrow of mice, but not in the peripheral blood after a midlethal dose of 710 r, and Ford et al. found that after a sublethal dose of 575 r homologous donor cells were almost completely replaced by the recipient cells on the twenty-eighth day post-treatment. In some cases the rejection of the donor cells is accompanied by events which tend to increase the mortality rate expected from the radiation alone. Trentin reported that F1 hybrid mouse marrow given to a parental strain 1 to 3 hours after a radiation dose of LD 12 resulted in 100 per cent mortality in 12 days. Gengozian and Makinodin, Congdon et al. and van Bekkum and Vos have also reported increased sublethal radiation mortality after treatment with foreign hematopoietic cells. Santos et al. however, found no increased mortality in mice treated with an LD 20 radiation dose and given rat bone marrow. Van Bekkum and Vos report that at doses lower than 450 r no increased mortality occurs in mice.

The graft rejection has been described by Congdon as a "violent immune response" accompanied by bone marrow failure. In addition, Trentin found lower leucocyte and platelet concentrations in the peripheral blood of mice 2 weeks after treatment with 550 r and F1 hybrid bone marrow than in mice given 710 r and hybrid marrow. The conditions in these experiments may be comparable to those following the injection of isologous lymph cells or spleen or thymus cells as described by Santos and Cole with foreign bone marrow after lethal radiation. In these cases, bone marrow failure occurred.

From the Clinical Physiological Laboratories of the Institute of Clinical Investigation, University of Tennessee College of Medicine, Memphis, Tenn.

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Vos et al. describe the symptoms as similar to those following sublethal radiation and injection of foreign cells.

Because the marrow might recover from these low doses of radiation without the injection of foreign cells, it seems reasonable to suppose that the timing of this "violent reaction" in respect to the process of recovery may influence the ultimate survival of the animals. It was therefore decided to investigate this relationship in the rhesus monkey by giving homologous bone marrow 30 and 48 hours after a sublethal dose of 550 r X radiation.

METHODS AND MATERIALS

The monkeys used were kept in the laboratory at least 3 months before use. A Westinghouse deep therapy unit, operated at 240 kv. and 15 ma. with a filter of 1 mm. Cu and 1 mm. Al, was used for irradiation. The hvl was 2 mm. Cu. and the rate was approximately 13 r/minute. A total of 550 r which is approximately an LD 10 dose for this species was given in two equal periods separated by 15 minutes. The animals were placed in a plastic chair (fig. 1) 100 cm. from the source to mid-body. During the first period they faced the source and during the second period they were placed with their backs to the source. The rate of radiation was measured at least once a month with a Victoreen Model 70 condenser rate meter. This instrument was calibrated with a Co source. The measurements ranged from 13.75 to 12.50 r/minute during the total time used for the experiment.

Three series of monkeys were irradiated. One group made up of 5 animals did not receive bone marrow. A second group of 7 monkeys received bone marrow 30 hours after radiation and the third group of 6 monkeys received bone marrow 48 hours after radiation. The donor monkeys were anesthetized with pentothal to which was added 10 mg. of

Fig. 1.—The position of the rhesus monkey, Macaca mulatta, in the plastic chair.
Table 1.—Effect of the Time of Marrow Transplantation on Survival of Monkeys Radiated with 550r

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fraction Surviving 30 days</th>
<th>Times of death in days after Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>550r only</td>
<td>5/5</td>
<td>—</td>
</tr>
<tr>
<td>550r + marrow 30 hours</td>
<td>1/7</td>
<td>10, 11, 12, 13, 14, 15</td>
</tr>
<tr>
<td>after radiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>550r + marrow 48 hours</td>
<td>5/6</td>
<td>23</td>
</tr>
<tr>
<td>after radiation</td>
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h-Kronin.* After 15–20 cc. of cardiac blood were taken they were killed; the femora, tibiae and humeri were taken and the diaphyses removed. The marrow was forced out of each bone with a syringe containing a small amount of peripheral blood. It was then mixed with the blood, strained through a coarse nylon screen and injected immediately into the saphenous vein of the recipient. The number of nucleated cells injected was estimated by counting a sample of the mixture in a solution of 1 per cent HCl. The counts of only 2 of the 7 injections given 30 hours after radiation were determined. These counts were 4.1 x 10⁹ and 3.6 x 10⁹ cells. The cellular content of the injections given 48 hours after radiation ranged from 9.2 x 10⁸ to 6.5 x 10⁹ with a mean of 3.4 x 10⁹ cells. The monkeys were slightly lethargic for a short time immediately after the injection.

Peripheral blood samples were taken periodically. The monkeys given bone marrow 30 hours after radiation were sampled weekly until death while the 48-hour-transplanted monkeys and the control animals were sampled three times a week for three weeks, twice a week for two weeks and then once a week. The animals were weighed each time blood was drawn. The hemoglobin concentration was determined by Drabkins method.17 Reticulocytes were counted after vital staining with New Methylene Blue.18 Leucocytes were diluted with 1 per cent HCl and counted in a hemocytometer. Smears for differential reading were made for the control group and for the 48-hour-transplant group.

RESULTS

Of the 7 monkeys given bone marrow 30 hours after radiation, 6 died between 10 and 15 days (table 1) with gross symptoms resembling those produced by a lethal dose of radiation. Facial edema, skin peteciae and generalized hemorrhage in the lungs and intestine were common. Diarrhea and hyporexia were evident just prior to death. Histopathological studies1 of 2 of these animals indicated marked lymphoid atrophy in the lymph nodes and spleen, associated with reticuloendothelial cell hyperplasia, hemosiderosis and erythrophagocytosis. The bone marrow of one of these animals was examined and showed marked atrophy. The other monkey had acute passive congestion, edema and hemorrhage in the lungs. One of the 6 monkeys receiving bone marrow 48 hours after radiation died 23 days later apparently of jaundice and general infection. On the eleventh day post radiation, the nucleated red cell count in the peripheral blood of this animal was 30/100 WBC and this increased to 378/100 WBC by the eighteen day, suggesting hemolytic jaundice. The plasma appeared noticeably yellow from the fourteenth day to time of death. Just prior to death, hyporexia and diarrhea were evident. The gross

*The sodium pentothal and heparin used in this work was generously supplied by Abbott Laboratories, North Chicago, Ill.

†Dr. A. C. Upton of the Oak Ridge National Laboratory very kindly examined the tissues taken at the time of autopsy of nonsurvivors.
autopsy indicated pulmonary hemorrhage and congestion. The gall bladder was filled with clotted blood. Tissue studies showed lymphoid atrophy, pleuritis, perisplenitis and bronchopneumonia. Inflammatory infiltrations in the periportal connective tissue of the liver suggest that the jaundice may have also been caused by an obstruction. The mucosa of the intestine was disorganized. None of the control monkeys died.

The maximum average weight loss in the control monkeys was 7.5 per cent. The monkeys given bone marrow 30 hours after radiation lost 16.9 per cent and those given bone marrow 48 hours after radiation lost 23.3 per cent. Although the average weight losses in the experimental monkeys was greater than that in the controls, these differences are not significant statistically, and are due to especially large weight losses in 5 animals. The original weights of all monkeys ranged from 2.71 to 12.43 Kg. All except one regained the lost weight. The monkey which originally weighed 12.43 Kg, lost 51 per cent of his weight and one year after treatment weighed but 7.10 Kg.

The peripheral blood data are shown in figures 2, 3 and 4 indicating averages and standard errors. The hemoglobin values (fig. 2) for the monkeys receiving bone marrow 30 hours after radiation provide a suggestion that the graft may have functioned briefly. The hemoglobin concentrations in 5 of these monkeys increased by about 1 Gm. per cent by the seventh day, while in 2 animals, including the single survivor, the hemoglobin concentrations remained approximately the same as the control values. Unfortunately, reticulo-
Reticulocytes were not counted during the first week. Reticulocytes (fig. 3), however, were not found in this group on the seventh or fourteenth days post radiation. After the seventh day the hemoglobin concentrations fell rapidly. Samples taken within 24 hours of the death of two animals had hemoglobin concentrations of 9.0 and 6.7 Gm. per cent.

Average hemoglobin in the monkeys receiving bone marrow 48 hours after radiation increased very slightly between the time of radiation and marrow injection. This average increase was due to gains of about 1 to 2 Gm. per cent in 3 animals. This effect has been noticed in other animals and is suspected to be caused by a decrease in the plasma volume shortly after radiation. The hemoglobin concentrations did not increase during the second week and by the twenty-first day post radiation the hemoglobin values and the reticulocyte counts were not statistically different (P = 0.95) from those found in the group receiving 550r only. The differences in the hemoglobin averages (fig. 2) may suggest, however, that the erythroid elements were somewhat less active during the third week in this group. Leucocyte concentrations in all groups fell to below 1000 cells/cu. mm. in one week. The recovery occurred at about the same time in all survivors and was due to an increased output of both lymphocytes and neutrophils in the 48-hour group and in the control group. Differential smears were not made for the 30-hour group. The increase in the white cell count in the sixth week post radiation was due to a marked eosinophilia in both groups. Eldred27 also reports an increase in the eosinophil count in the peripheral blood of monkeys during the sixth week following radiation with 550r.

Fig. 3.—Average reticulocyte counts in the peripheral blood showing standard errors greater than 0.5 per cent.
Fig. 4.—Average leucocyte concentrations in the peripheral blood showing standard errors greater than 300.

Discussion

Animals receiving bone marrow 30 hours after radiation died with symptoms characteristic of the effects of higher doses of radiation. The injection of homologous marrow appeared to inhibit the recovery from radiation. These symptoms were not seen in the monkeys which received 550 r only nor in those which received bone marrow 48 hours after radiation. As it is generally known that a sublethal dose of radiation will not permit the prolonged acceptance of foreign marrow, it might be assumed that graft rejection did occur in both groups receiving bone marrow after a radiation dose of approximately LD 10. The reticuloendothelial cell hyperplasia in the lymph nodes and spleen of the monkeys which died after the 30-hour injections also suggests that there was some reaction against the foreign cells since Congdon et al. also found these symptoms in mice during the rejection of rat marrow cells. As no tag for the homologous cells was used, it cannot be definitely stated when the marrow cells were rejected. A comparison of the effects of marrow injection given 30 and 48 hours after radiation suggests that rejection in the 30-hour group was rather violent and that it occurred at a critical time during recovery from radiation. As the marrow had apparently little effect when given 48 hours after radiation, it might be supposed that the process of recovery was well under way at the time of the reaction. It is also possible that the host's immune response had recovered sufficiently to react against the foreign cells before they became as well established as did the marrow cells given 30 hours after radiation, thus producing, perhaps, a less disturbing reaction.
SUMMARY

Homologous bone marrow given to the rhesus monkey 30 hours after LD 10 radiation dose of 550r increased the mortality rate expected from the radiation alone. The symptoms produced resembled those obtained at higher doses of radiation. The effects of marrow given 48 hours after radiation differed little from those produced by the radiation alone. It is suggested that the 30-hour injection elicited a more violent reaction or that the reaction occurred during a more decisive time in the process of recovery.

SUMMARIO IN INTERLINGUA

Medulla ossee homologe, administrate a rhesos 30 horas post 550 r (DL_{10}) de radiation, augmentava le mortalitate expectate ab le radiation sol. Le symptomas produceite esseva simile a illos obtenite per plus alte doses de radiation. Le effectos de medulla administrate 48 horas post le radiation differeva pauco ab le effectos produceite per le radiation sol. Es hypothetisate que le injection post 30 horas evocava un plus violente reaction o que le reaction occurreva a un momento plus critic in le processo del restablimento.

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mice treated with rat bone marrow.


The injection of rat bone marrow into mice lethally irradiated allowed the survival of the animals and caused an improvement of the peripheral blood counts, as well as the repopulation of the bone marrow and of the spleen. The granuloblastic cells were shown to belong to the species of the donor on the basis of their histochemical characteristics. Furthermore, a regeneration of the lymphatic cells took place, both in the circulating blood and in the spleen. The lymphatic reaction was interpreted as the consequence of the immunological reaction following the introduction of the heterologous bone marrow.—P. d. N.

Marrow Treatment of Irradiated Dogs. K. A. Porter and N. P. Couch.


Twenty-nine male dogs received 450–700r whole body irradiation followed after 1–3 hours by intravenous infusion of bone marrow from female puppies. Eight dogs received 450r but no bone marrow, and all of these died within 18 days.

In 10 dogs, the leukocyte count rose rapidly 4–7 days after marrow infusion and included female neutrophils. Seven of these dogs, including all those receiving over 500r, died between 11 and 20 days after irradiation from overwhelming infection and intestinal damage; in the remaining three, the leukocyte counts fell again between the 14th and 18th day, and female neutrophils disappeared from the blood. The counts then rose again, but no female neutrophils were found. Only temporarily successful marrow homografts were thus achieved.—R. M. H.
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