Peripheral Leukocyte Infusion into Lethally Irradiated Guinea Pigs

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AUTOLOGOUS and isologous peripheral blood leukocytes infused into lethally irradiated mice, guinea pigs and dogs can protect the recipient animals from death. Homologous leukocytes likewise appear to be effective in the guinea pigs.

Despite considerable variation between tissue types and species of animals in their sensitivity to radiation, the outcome of exposure to sufficiently high doses of ionizing radiation is inevitably a fatal one. Since hemopoietic tissues are among the most sensitive ones to injury by radiation, the animals exposed to lethal doses of ionizing radiation usually die of severe aplastic anemia and its complications.

It was shown by Perry et al. that autologous blood leukocytes offer some protection against the effects of whole body irradiation in guinea pigs, but that maximum benefit was derived only from a larger number of cells than could be obtained from a single donor. In the previous experiments, data were accumulated which showed that intracardiac injection of an adequate number of homologous peripheral blood leukocytes protected a large portion of animals from lethal radiation. The purpose of this paper is to present observations made on animals used in these experiments, and to compare these to observations made on animals protected from lethal doses of radiation by injection of autologous peripheral blood leukocytes.

MATERIALS AND METHODS

The Princeton “strain” guinea pigs were used throughout the study. The animals were maintained in an open colony and weighed from 600 to 1000 Gm. Skin grafts taken at random between animals in the colony were consistently rejected in 7 to 12 days.

All animals were exposed to 550 r. of total body radiation. The source of irradiation was a Budd 4 pi cobalt 60 unit. The radiation was administered at a rate of 100 r. per minute as measured in air. The LD100 for animals used in the experiments was determined to be 550 r.

Peripheral blood leukocytes were separated by using a modification of the fibrinogen flotation method of Skoog and Beck. Both fresh and frozen cells were used, but never simultaneously. Blood leukocytes were injected into experimental animals by the intracardiac route. Several postirradiation animals died from hemoparicardium resulting from this procedure. These animals were excluded from consideration in the survival statistics.

The animals were kept in cages and given food and water ad lib. No antibiotics were
given at any time. Blood of experimental animals was collected and examined at predetermined intervals. The examination included WBC count, differential, platelet count, hematocrit, RBC count, and hemoglobin.

When an animal died unexpectedly, it was placed in a refrigerator and then subjected to an autopsy. Animals sacrificed for histologic examination were given injections of Nembutal and autopsied immediately afterwards. In all cases bone marrow was taken from the sternum, ribs, vertebrae and femur. Both smears and sections were prepared.

Sixty-seven guinea pigs were irradiated but received no treatment. These served as a control group for the study. Fourteen animals were sacrificed during the observation period for histologic and hematologic examinations. The remaining 53 animals in the group died between 9 and 14 days after being irradiated, and were also examined histologically.

Thirty-seven animals received 1 x 10⁶ fresh homologous cells within 2 hours of exposure. Fifteen animals were sacrificed at 2-day intervals during the first 23 days after irradiation. Two animals were sacrificed at 57, 2 at 74, 2 at 99, 2 at 128 days, and 1 animal 9 months after exposure. Six animals died unexpectedly, 4 of these within 2 weeks after irradiation, and 2 animals died 9 months after irradiation with severe pulmonary edema following general anesthesia and a cardiac puncture performed to obtain a blood sample. Seven animals are alive and well 1 year after irradiation and injection of leukocytes.

Three animals protected from lethal radiation with 7.8 x 10⁷ frozen autologous leukocytes were sacrificed at 35, 56 and 63 days postirradiation. These animals were used for comparison with guinea pigs receiving homologous peripheral leukocytes. It was assumed that these animals would not show untoward reaction to the injection of their own cells.

Ten animals were given either 1 x 10⁶ or 5 x 10⁶ fresh homologous cells 24 hours after exposure. All of these animals died within 2 weeks after irradiation.

OBSERVATIONS

Control Series

Degenerating cells with pyknotic nuclei were present throughout the bone marrow by the second day following irradiation. By the fourth day bone marrow was hypoplastic and composed of scattered degenerated hemopoietic cells and dilated blood sinusoids engorged with erythrocytes. By the twelfth day, if the animal was still living, the sinusoids disappeared and the marrow was composed of fibrous connective tissue, reticuloendothelial cells, small dark staining cells, and rare plasma cells. In many cases, however, by the twelfth postirradiation day, bone marrow spaces contained clumps of regenerating cells (fig. 1).

Changes in the peripheral blood are summarized in figure 2. The leukocytes which persisted the longest in tissues were eosinophils. These were still found up to 8 days following irradiation. Once these disappeared, foci of infection were surrounded by epithelioid cells and large macrophages.

Changes in lymphatic tissues consisted of pyknosis and fragmentation of nuclei apparent one day postirradiation. These changes were progressive and by the tenth to twelfth day the lymph nodes were composed of connective tissue framework, reticuloendothelial cells, few plasma cells, and macrophages. Still present, however, were scattered lymphocytes around some germinal follicles. The architecture of the spleen was altered and it was composed of fibrous tissue, reticuloendothelial cells, numerous pigment containing macrophages, and occasional lymphocytes.
Fig. 1.—*Twelve-day postirradiation bone marrow*. Note a nest of large hematopoietic cells. H & E x 430.

Germinal epithelium of the testis was destroyed following exposure but it began to regenerate by the tenth day.

With the onset of leukopenia, experimental animals developed overwhelming bacterial and fungal infections to which they succumbed.

*Experimental Series*

In the surviving animals which received $1 \times 10^8$ fresh homologous leukocytes, WBC count returned to normal limits at about 20 days following irradiation. The peripheral blood changes in these animals are summarized in figure 3.

Three days after irradiation bone marrow contained dilated blood sinusoids and many large scattered, proliferating cells (fig. 4). These foci of regenerating bone marrow were composed of large immature cells of myelocytic series, megakaryocytes, large reticuloendothelial cells and plasma cells. Cellular proliferation continued from 3 days on until bone marrow became quite hyperplastic, usually from 10 to 14 days after irradiation and injection of leukocytes. In between the foci of proliferating cells there were scattered numerous mature granulocytes, some of which had hypersegmented nuclei and mitotic figures. Foci composed almost entirely of cells of granulocytic series and mature granulocytes were found in the bone marrow of animals beginning 7 days after irradiation. By 14 days, the bone marrow was quite hyperplastic and abundant with megakaryocytes. It remained hypercellular till about 2 to 3 months following exposure when its hypercellularity began to decrease and by 9 months it was normocellular (fig. 5).
Fig. 2.—Summarizes the changes in the peripheral blood of untreated control animals. Maximum depression of all blood elements was observed between the tenth and the eleventh postirradiation days. This was followed by a rapid rise in all elements. However, this regeneration occurred too late in the postirradiation period to be of benefit to the animals.

The lymphatic system in protected animals regenerated with rapidity. Three days after exposure the germinal follicles of many lymph nodes were surrounded by mature lymphocytes. The sinusoids of the lymph nodes contained large mononuclear cells and scattered lymphocytes. By 7 to 12 days hyperplastic follicles were surrounded by numerous, densely packed small mature
Fig. 3.—Demonstrates the recovery of peripheral blood elements in animals protected with fresh homologous leukocytes. Note that the depression of the platelet and the WBCs was not as severe as in the control animals.

lymphocytes (fig. 6). There was little further change in the appearance of lymphatic system, as is shown in a section taken from a lymph node of an animal 9 months after irradiation (fig. 7). The lymphoid nodules in the lungs and the intestinal tract followed the same pattern as did lymph nodes elsewhere.

Changes observed in the spleens of animals in this group were similar to those in lymph nodes. Initial depletion of lymphocytes was followed by proliferation of the follicles, which became surrounded by mature lymphocytes by 5 to 7 days after exposure. The spleen of 1 animal sacrificed 21 days after injection of leukocyte suspension contained giant cells. The pulp of the spleens of several long-term survivors were moderately fibrotic but in all cases the germinal follicles were well developed.

Protected animals responded to infection in the usual manner as shown in a section of a lung with bronchopneumonia from an animal 2½ months post-irradiation (fig. 8). All animals which survived the exposure to lethal whole body radiation following administration of $1 \times 10^8$ fresh homologous peripheral blood leukocytes failed to demonstrate gross evidence of disease during a 12-
Fig. 4.—Bone marrow 3 days after irradiation and injection of $1 \times 10^8$ fresh homologous leukocytes. Note proliferation of the bone marrow cells. H & E x 200.

month period of observation. The animals gained weight, their fur was glistening and smooth, they took their food readily, and, in general, appeared healthy.

The return of peripheral WBC count to normal in animals which received frozen autologous leukocytes was similar to that in animals receiving fresh homologous cells. White blood cell count 56 and 63 days after exposure was 7800. The bone marrow was hyperplastic in the 35-day animal, and normocellular in the 56- and 63-day animals.

The lymph nodes contained active germinal follicles and were packed with numerous mature lymphocytes. Moderate fibrosis was observed in the pulp of the spleen. The germinal follicles were composed of large reticuloendothelial cells and were surrounded by small, densely packed lymphocytes.

In the lungs were noted perivascular and peribronchial accumulations of mononuclear cells. Changes in experimental animals dying within control animal survival period were similar to those in control animals, the only difference being that some animals which received leukocytes 24 hours after irradiation contained extensive foci of regeneration in the bone marrow.

**DISCUSSION**

Injection of pooled homologous leukocytes into lethally irradiated guinea pigs precluded death in a majority of experimental animals. Gradual decrease, rather than a sharp rise in the peripheral WBC count following infusion of homologous leukocytes, indicates that the infused cells probably did not remain in circulation for prolonged periods of time. The presence of immature granulocytes with mitotic figures and of numerous mature granulocytes in the bone marrow of experimental animals within 1 week postirradiation suggest
Fig. 5.—Bone marrow from a guinea pig 9 months after lethal irradiation and injection of homologous peripheral blood leukocytes. H & E x 100.

Fig. 6.—Section from a spleen of a 12-day postirradiation animal injected with homologous peripheral blood leukocytes. Note the active germinal follicle. H & E x 250.
that most infused cells probably settled in the bone marrow and that at least a partial, transient graft was established.

Regeneration of lymphatic tissue and particularly persistence of germinal follicles throughout the period of observation are in contrast to findings of Thomas et al. on lethally irradiated dogs which were transfused with homologous bone marrow. Usual inflammatory reaction encountered with bronchopneumonia further points to immunologic competence of experimental animals. Failure to demonstrate gross evidence of disease, the healthy appearance, and the gain in weight of experimental animals rules out homologous disease. Almost identical appearance of tissues in animals which received autologous cells substantiates this contention. It appears that both the establishment of at least a transient homograft and regeneration of host's own hemopoietic tissues are important in facilitating recovery. Regeneration alone does not produce adequate numbers of leukocytes soon enough to prevent lethal infection, as is evident in the control animals.

Time of injection is of importance since injected cells apparently do not remain in circulation. Sufficient number of newly formed leukocytes must be produced by the time the host's original leukocytes are depleted. Bone marrow of animals which received peripheral blood leukocytes 24 hours after irradiation, although partially repopulated, was still hypocellular in comparison with bone marrow of animals which were injected within 2 hours after exposure.

It is not clear as to why the 4 animals which received 1 x 10^8 cells did not respond to injection. One possibility is that cells were not injected into the vascular system, but rather into the pericardial or pleural cavities. This was difficult to ascertain since the animals died from 10 to 12 days after injection.
Fig. 8.—Focus of bronchopneumonia in a lung of a guinea pig 2½ months following irradiation and injection of homologous peripheral blood leukocytes. H & E x 200.

Lung was the only organ where peculiar lesions were found. These consisted of perivascular and peribronchial collection of mononuclear cells. These lesions were found in both experimental and control animals and probably represented either foci of organized unresolved pneumonia or early formation of the lymphoid nodules. If one were to assume, as was suggested by Lorenz et al.9 that some noncellular, humoral factor was responsible for early regeneration of host’s hemopoietic tissue, then the fate of injected cells would be of little importance. The results in this study suggest that injected cells actively contributed to protection, at least until recovery of hemopoietic system was established. However, this still does not preclude release of some humoral factor or detoxification effect by the injected cells.

SUMMARY

Homologous peripheral blood leukocytes were injected into lethally irradiated guinea pigs. Histologic examination of these showed a rapid recovery of the bone marrow and the lymphatic tissue following an injection of a sufficient number of leukocytes. All control animals died with a hypoplastic bone marrow. Protected animals did not develop “secondary” disease during 9 months of observation.

SUMMARIO IN INTERLINGUA

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REFERENCES


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