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THE EFFECT OF IRRADIATION ON OSTEOCLASTS WITH OR WITHOUT TRANSPLANTATION OF HEMATOPOIETIC CELLS

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The sensitivity of the osteoclasts to large doses of irradiation is less than that of the cells in bone marrow and thymus. After irradiation, the number of osteoclasts decreases first after the 7th day. Transplantation of cells from bone marrow and spleen restores the number but cells from thymus, or thymus cells in combination with macrophages, do not have this effect.

Key words: hematopoietic cells; irradiation; osteoclasts; thymus; transplantation

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Sublethal X-ray irradiation results in the disappearance of most of the cells in bone marrow, thymus and spleen. This can be prevented by transplants of bone marrow cells within the first hours after irradiation (Till & McCulloch 1961, Viktora et al. 1978). The osteoclasts are considered to originate from the monocytes of the blood and are thus related to the macrophages (Hancox 1972, Göthlin & Eriksson 1976, Lucht 1980). Milhaud et al. (1978) have found that the osteopetrotic mutation "op" in rats can be cured by injection of thymus or bone marrow cells, the latter, however, not being effective in thymectomized "op" rats. They therefore consider that a normal functioning thymus is necessary for the competence of the osteoclasts. Pelletier et al. (1978) found that an acute inflammatory exudate is able to induce both DNA synthesis and proliferation of macrophages *in vitro*. The ability to stimulate mitoses is reduced should the exudate be obtained from irradiated rats (900 rad). The transfer of thymic syngenic cells not only restored the mitogenical effect of inflammatory exudate from irradiated rats but also increased it. Bone marrow cells, however, lack this competence.

The aim of our study is to investigate the connection between X-ray irradiation and changes in the number of osteoclasts and whether it is possible to restitute the osteoclasts after transplantation of cells from bone marrow, spleen and thymus. So far as we know, no study has been made on the effect of irradiation on the number of osteoclasts. The effect of X-ray irradiation on growing bone has been studied in several papers, see e.g. Hulth & Westerborn (1960).

MATERIAL AND METHODS

In a preliminary experiment, common Wistar rats were used. In the experiments with transplantation of cells, we used inbred syngenic rats. At the start of the experiments the body-weight was about 100 g.

The X-ray dose in the preliminary experiment was 900 rad and in the main experiments 750 rad; 170 rad/

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minute was given. The rats were placed in a plastic box which was of such a height that the rats were confined to one layer. About 25 rats were irradiated at the same time. The width of the box was smaller than the irradiation field. About 25 rats were exposed to irradiation at the same time.

The rats in the preliminary experiment were killed in groups of 4 at 1, 3, 5, 7 and 14 days after irradiation; in the main experiment, in groups of 6 on the 7th or 19th days after irradiation. Before sacrifice, the rats were weighed and blood was collected for hematocrit values.

The relative amount of cells in bone marrow, spleen and thymus was determined in the following way: one femur was cut off with scissors at the lower metaphysis and at the collum. A bone marrow cell suspension was prepared by rinsing the femoral cavity with a known amount of balanced salt solution (BSS). The thymus and the spleen were cleaned of adjacent tissue. Suspensions of these organs were prepared by pressing the organ through a stainless steel mesh into a known amount of BSS. The cells were counted in Büerckl's chamber during microscopy.

From each animal the right fourth and fifth ribs were taken out. The bone was decalcified for approximately 20 hours in a 10 per cent solution of EDTA containing 0.1 M tris buffer. The bone was then washed in cold saline, quickly frozen in liquid nitrogen and then cut in a cryostat into five to six sections 10 μ thick. The sections were stained for succinic dehydrogenase activity by the method of Pearse (Pearse 1960) with nitroblue tetrazolium salt as the H-acceptor (Tatevossian 1973).

The osteoclast count was carried out partly in the primary bone trabeculae of the metaphysis which corresponded to one visual field or less and partly along a predetermined length of the cortex of the metaphysis peri- and endosteally at 160 times magnification. The length was determined by a rule engraved on the eye piece of the microscope.

Transplantation of cells

These experiments were performed on syngenic rats. Four to five non-irradiated rats were used as donors of cells to each group. Cells from the bone marrow, thymus and spleen were taken by the same method as in the counting procedure (see above) but the cells were suspended in Hank's solution. The cells were injected into a tail vein in the animals within 3 hours after irradiation. The amount of cells injected was 0.5 ml of a suspension containing 4×10^7 cells per ml.

Macrophages from the peritoneal cavity were produced by injecting 0.25 ml/100 g body-weight of Freund's adjuvant into the peritoneum. Seven days after the injection the peritoneal cavity was rinsed by injection of Hank's solution. The viability of the cells was checked by staining with trypan blue. The number of macrophages injected was 0.5 ml of a suspension of 4×10^7 macrophages per ml. All cell injections were made in a tail vein.

Owing to the time-consuming nature of the experiments, they were performed in three different sets, each set with its own control animals. Therefore, the control values of the number of cells and body-weights vary somewhat in the three sets of experiments. In the first set, irradiation only was compared with irradiation and injection of cells of bone marrow and spleen and controls. In the second set, irradiation only was compared with irradiation and injection of thymus cells and controls. In the third set, irradiation only was compared with irradiation and injection of thymus cells + macrophages and non-irradiation controls.

In the first and second sets, groups of 6 animals were killed on the 7th and 19th days. In the third set the animals were killed on the 19th day only.

RESULTS

In the preliminary experiment after 900 rad irradiation, the number of osteoclasts was unchanged on the 3rd and 5th days. The amount of osteoclasts decreased 30 per cent from the 7th day and remained low even on the 14th day (Figure 1). The effect of the irradiation on thymus, bone marrow and spleen consisted of a very rapid drop in the number of cells so that as early as 3

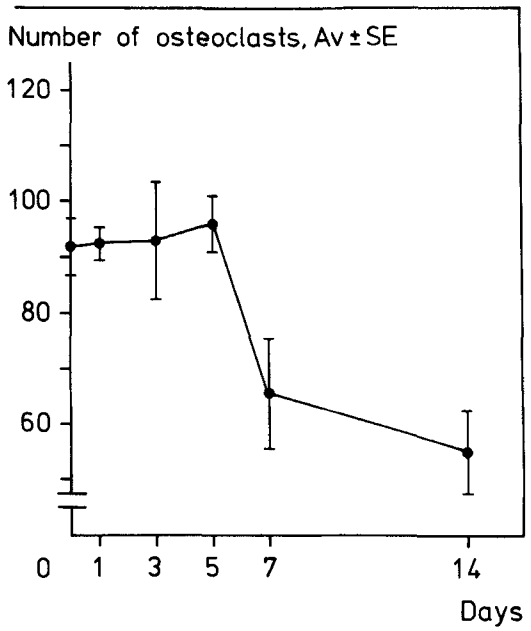

Figure 1. The number of osteoclasts in the rib metaphyses after 900 rad irradiation. The decrease is significant on the 7th and 14th days ($0.05 < P < 0.01$).

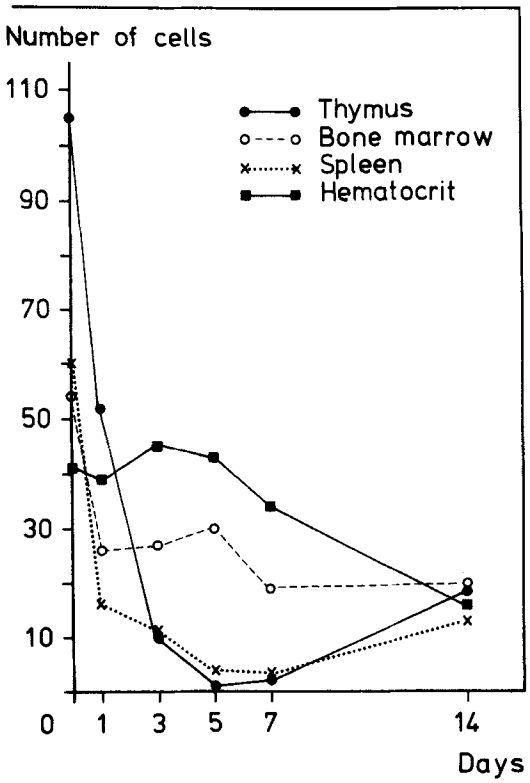

Figure 2. The number of cells in thymus, bone marrow and spleen after 900 rad irradiation. The percentage changes in hematocrit are also shown. The decrease in the number of cells is significant: for thymus $0.01 < P < 0.001$, for bone marrow and spleen $P < 0.001$.

days after irradiation, the amount of cells in thymus, spleen and bone marrow was 10, 20 and 50 per cent, respectively, of the original values. The amount of cells in these organs remained at these low levels until the 14th day after the irradiation (Figure 2). The hematocrit value was continually decreasing from the 5th to the 14th day post-irradiation.

In the main experiment (700 rad) the amount of cells in bone marrow, spleen and thymus was very low on the 7th day after irradiation, independent of whether transplantation of cells was performed or not. The number of cells in the three organs decreased to a third or lower than that of the control animals ($P < 0.001$). Without cell transplantation, the amount of cells remained low even on the 19th day ($P < 0.001$), except in the spleen which spontaneously repopulated.

On the 19th post-irradiation day (700 rad) there had occurred a repopulation of cells in the three organs after transplantation of bone marrow or spleen cells (Figure 3 I) but not after transplantation of thymus cells (Figure 3 II). The spleen seems to be capable of repopulating its cells even without transplantation (see histogram on Figure 3). The combined transplantation of cells from thymus and macrophages gave the same result as transplantation of thymus cells only.

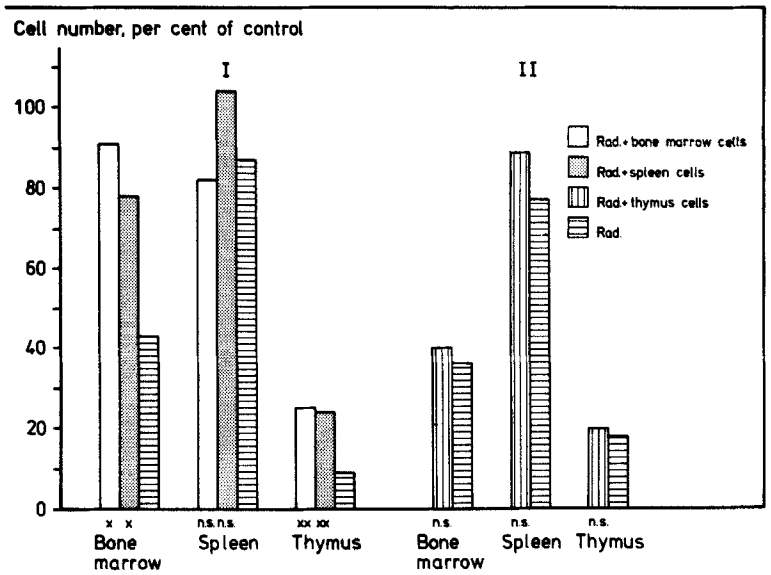

Figure 3. Histogram showing the number of cells as a percentage of the controls on the 19th irradiation day (700 rad) in bone marrow, spleen and thymus after transplantation of bone marrow or spleen cells (I) or thymus cells (II). Each column represents 6 animals. Differences from irradiated controls: n.s. = non-significant, $x = 0.01 < P < 0.05$, $xx = 0.001 < P < 0.01$.

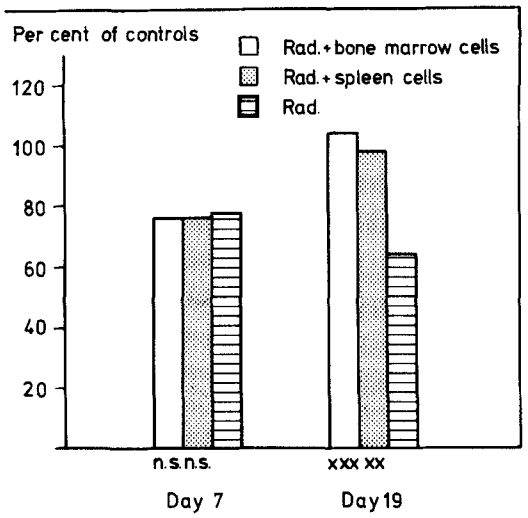

Figure 4. Histogram showing the number of osteoclasts as a percentage of the controls on the 7th and 19th post-irradiation days (700 rad). Transplantation of bone marrow and spleen cells restored the original number. Differences from irradiated controls: n.s. = non-significant, xx = 0.001 < P < 0.01, xxx = P < 0.001.

The osteoclasts were fairly resistant to the amount of irradiation given (700 rad) (Figure 4). On the 7th day the number of osteoclasts was about 75 per cent of the control values, whether transplantation of bone marrow or spleen cells was performed or not. After the 19th day, the repopulation of osteoclasts was complete, when BM cells or spleen cells had been given. The number of cells was the same as in the controls. The animals which were irradiated without cell transplantion had a cell deficit of about 40 per cent ($0.01 < P < 0.001$). Transplantation of thymus cells did not have any effect, neither did transplantation of thymus cells in combination with macrophages.

The body-weights differed considerably after the various forms of treatment. On the 19th day, the irradiated animals (700 rad) weighed only 140 g. The animals transplanted with bone marrow or spleen cells weighed approximately 170 g (significantly more than the irradiated controls, $0.01 < P < 0.001$). The control animals weighed 210 g. Transplantation of thymus cells resulted in a small increase in the body-weight (different to irradiated controls, $0.01 < P < 0.001$). The hematocrit values were almost normalized in the animals transplanted with bone marrow or spleen cells; the animals which were given thymus cells did not differ from the animals which had been only irradiated.

DISCUSSION

The series of experiments performed in the present investigation show that osteoclasts can resist irradiation rather well, reacting more slowly than, for example, bone marrow, thymus and spleen cells, to sublethal and lethal irradiation. After the 7th day, the amount of osteoclasts was significantly lower than in the control animals. The fall in the amount of osteoclasts was, however, not so great as that of the cells in bone marrow, spleen and thymus. Transplantion of bone marrow cells and spleen cells brought about a normalization of the number of osteoclasts, where there was no change in the osteoclasts in the non-transplanted animals. This shows that the osteoclasts in themselves are more resistant than the progenitor cells of the osteoclasts. It also shows that the life-span of an osteoclast might be 1 week or more.

During the last decade it has been postulated that osteoclasts originate from the monocytes of the blood (Göthlin & Eriksson 1976, Lucht 1980). The present experiments show at least that osteoclasts originate from cells existing in bone marrow and spleen. Thymus cells, on the contrary, do not seem to have anything to do with the formation of osteoclasts, also when they are combined with macrophages. This negative finding does not exclude the possibility that the thymus can have a surveillance capacity over the function of the osteoclasts as has been previously supposed by Milhaud et al. (1978). On the other hand, Marks & Schneider (1978) showed that osteopetrotic i.a. rats could be cured by irradiation and immediate injections of either spleen cells or thymus cells, which we cannot explain. We found that the thymus cells had no effect in spite of a 99 per cent viability of the injected cells.

When considering the transplantation experiments it is evident that spleen is able to repopulate itself after 700 rad irradiation, but the cells of bone marrow do not have this capacity during the

period of time (19 days) studied in these experiments.

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