REIMMIGRATION OF BONE MARROW STEM CELLS IN RATS

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One limb (crus) or all four limbs in rats were irradiated with x-rays in a dose of 1500 R, sufficient to inactivate bone marrow, and 3, 24 h, or 6 days later the rest of the body was irradiated in doses of 800 or 900 R, the previously irradiated region being screened. The percentage of rats which survived was the same as when the unirradiated limbs were screened. It was suggested that stem cells migrate from the unirradiated bone marrow into its irradiated part (the crus), in which they survive and are able to migrate again (reimmigrate) in the case of subsequent irradiation of the rats in a lethal dose with screening of the limb. Rats differ from mice in the greater severity of their intestinal syndrome, which depends only to a small extent on migration of stem cells. It is therefore best to judge the effectiveness of stem cell migration chiefly on the basis of survival of the animals during the period of bone marrow death. It can be concluded from analysis of the mortality of the rats in this period that their survival, based on reimmigration after screening of one limb, with a three-hour interval between irradiations, is only half that of mice. Reimmigration of stem cells thus takes place in the rats just as in mice, but it plays a significant role in rats only if the volume of previously irradiated and subsequently screened bone marrow is greater or if the time interval between irradiations is longer.

KEY WORDS: bone marrow; reimmigration; irradiation.

It has been shown in mice that bone marrow stem cells, if they migrate into an irradiated region of marrow, can migrate a second time (reimmigrate) and repopulate the hematopoietic organs in the rest of the body, if the animal is subsequently irradiated with the part originally irradiated now screened [1]. By varying the volume of the irradiated and screened parts and the time interval between irradiations, the survival rate of the mice and other indices can be used to assess the intensity of migration of stem cells and its importance in the development of repair processes.

Data on reimmigration of bone marrow stem cells in rats are given in this paper. Comparative analysis of reimmigration of stem cells in animals of different species is interesting in connection with the fact that the principles of migration of stem cells in the case of nonuniform irradiation vary considerably [3]. In particular, there is evidence that in mice migration of stem cells in response to the local irradiation is more marked than in rats [5].

EXPERIMENTAL METHOD

The scheme of the experiments was the same as in the work on mice cited above. To begin with, one or all four limbs of rats were irradiated in a dose of 1500 R, inactivating the marrow. At various time intervals (3 and 24 h, 6 days) the rest of the body was irradiated in a dose of 800 or 900 R (the first dose incompletely, the second dose completely lethal), the previously irradiated regions being screened with lead. It was assumed that in the interval between the first and second irradiations stem cells would migrate into the irradiated limb, survive there, and after the second irradiation they would reimmigrate into the irradiated part of the body, where they would repopulate the damaged bone marrow and other hematopoietic organs. Irradiation was carried out on the RUM-17 apparatus, with a voltage of 20 kV, current 15 mA, filters 0.5 mm Cu + 1 mm Al, skin-focus distance 60 cm, and dose rate 72 R/min. The animals were tied to a frame for irradiation.

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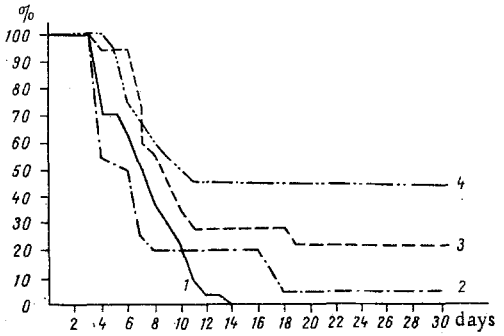

Fig. 1. Survival rate of mice irradiated in a dose of 900 R after screening of one limb irradiated at various times previously in a dose of 1500 R. 1) Whole-body irradiation (47 rats); 2) screening of one previously irradiated limb, interval between irradiations 3 h (20 rats); 3) the same, interval between irradiations 24 h (18 rats); 4) the same, interval between irradiations 6 days (20 rats). Abscissa, time after irradiation (in days); ordinate, survival rate (in percent).

EXPERIMENTAL RESULTS

The fact that reimmigration takes place in rats and that it is important for their survival after lethal doses of radiation was confirmed by comparing the survival rate of rats after total irradiation with their survival rate when the limb was first irradiated and then screened, while the rest of the body was later irradiated.

When one limb (the crus) was irradiated in an inactivating dose of 1500 R and then screened later while the rest of the body was irradiated in a minimal completely lethal dose (900 R) approximately the same percentage of animals was protected against death as when the unirradiated limb was screened during subtotal irradiation in the same dose [2]. Six days proved to be long enough for the restoration of hematopoiesis in the previously irradiated limb. During this period the irradiated limb, when screened, later acquired the ability to exert about the same protective action as the screened unirradiated limb.

If the time between irradiations was shortened the protective effect of the previously irradiated and screened limb was reduced, and when the interval was 3 h the effect was almost absent (Fig. 1).

An increase in the volume of previously irradiated bone marrow had a great effect on the survival rate of the animals irradiated with this region screened. In the corresponding experiments (Fig. 2), with preliminary irradiation of all four limbs, i.e., with an approximately threefold increase in the quantity of marrow subjected to radiation, when the rest of the body was irradiated 3 h after irradiation of the limb, the survival rate of the rats was maximal (about 50%). A higher percentage of survival of the rats could not be obtained through screening of part of the marrow, even that not subjected to preliminary irradiation, if a completely lethal dose of radiation acted on the rest of the rat's body [2]. Under these conditions survival of the rats was limited to their death on the 4th-7th day due to the development of an intestinal syndrome.

The results of experiments to study survival of rats, when the interval between irradiation of all four limbs and of the rest of the body was 3 h, are shown in Fig. 2. Data showing the survival of rats with preliminary irradiation of one limb and the times of death of the rats after whole-body irradiation are given for comparison. Such a comparison shows that preliminary irradiation of one limb, by contrast with preliminary irradiation of all four limbs, led to hardly any increase in the survival rate if the interval between irradiations was 3 h. Meanwhile, an increase in the time interval between irradiations, following preliminary irradiation of one limb (Fig. 1), resulted in maximal survival of the rats, just as in the case of preliminary irradiation of all four limbs with an interval of 3 h between irradiations.

The experiments showed that after preliminary irradiation of one limb, in order to obtain a maximal effect a much longer period was required between inactivation of the bone marrow in the limb and irradiation

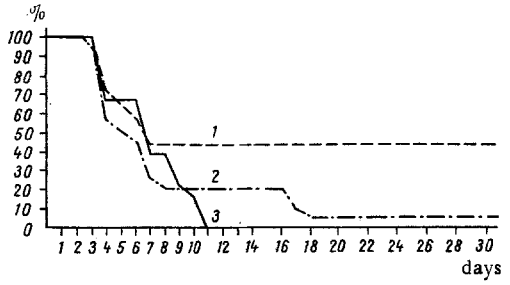

Fig. 2. Survival rate of rats irradiated in dose of 900 R after screening of one or all four limbs, irradiated 3 h previously in a dose of 1500 R. 1) Screening of four limbs irradiated previously (14 rats); 2) screening of one limb irradiated previously (20 rats); 3) whole-body irradiation (18 rats). Abscissa, time after irradiation (in days); ordinate, survival rate (in percent).

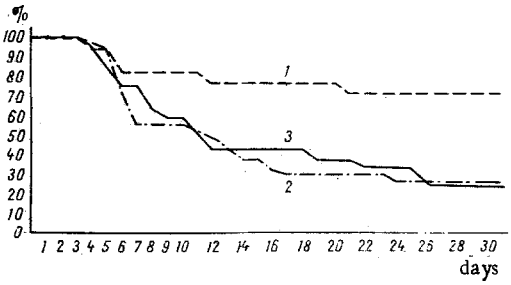

Fig. 3. Survival rate of rats irradiated in a dose of 800 R after screening of one or all four limbs subjected 3 h previously to local irradiation in a dose of 1500 R. 1) Screening of four previously irradiated limbs (18 rats); 2) screening of one previously irradiated limb (30 rats); 3) whole-body irradiation (24 rats). Abscissa, time after irradiation (in days); ordinate, survival rate (in percent).

of the rest of the body than after preliminary irradiation of all four limbs. Within the short time of 3 h, in the latter case, a sufficient number of stem cells could evidently migrate into the screened bone marrow in order to ensure effective reimmigration.

The fact that the development of an intestinal syndrome limited the possibility of survival after screening of the previously irradiated parts of the bone marrow was confirmed in a series of experiments in which a smaller dose was used (800 R) than in the previous series. Under these conditions, among the control irradiated rats 25% survived, whereas about 30% died from gastrointestinal involvement. When all four previously irradiated limbs of the rats were screened and the rest of the body irradiated 3 h later about 70% survived, 20% more than in the experiments with a minimal lethal dose of radiation (Fig. 3).

The results obtained in experiments on rats can be compared with the results of similar previous observations on the reimmigration of bone marrow stem cells in mice. However, there are considerable differences between mice and rats as regards the development of an intestinal syndrome. In mice, after lethal doses of irradiation death takes place predominantly because of damage to hematopoiesis. In rats, death under such conditions is largely due to an intestinal syndrome, which depends only to a small extent on migration of stem cells from the screened region of bone marrow or after bone marrow transplantation, leading to restoration of hematopoiesis [2, 4]. The effectiveness of stem cell migration can therefore best be judged

from survival of the animals until the time of their death from bone marrow damage (from the seventh day after irradiation). It can be concluded from analysis of mortality of the rats in this period that their survival, as a result of reimmigration following screening of one limb and an interval of 3 h between irradiations, is only half of that found in mice: The percentage of surviving rats did not exceed 20, whereas the percentage of surviving mice under similar conditions was considerably greater, namely 46%. Approximately the same survival rate (36%) as a result of screening of the previously irradiated limb was achieved in rats when this was done, not 3 h beforehand, as in mice, but 24 h beforehand. In rats, just as in mice, reimmigration of stem cells thus takes place, but it plays a significant role only if a larger volume of marrow is previously irradiated and later screened, or if the time interval between previous irradiations of the region of bone marrow and subsequent irradiations of the rest of the body is considerably longer. The limited importance of migration of bone marrow stem cells in rats compared with mice is in agreement with observations obtained earlier by other methods [3, 5]. The comparison of reimmigration of bone marrow stem cells in rats and mice made in this paper confirms previous observations and characterizes migration of bone marrow stem cells from a new aspect.

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CHANGES IN THE CYCLIC NUCLEOTIDE CONTENT IN WOUND TISSUES DURING HEALING

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Changes in the content of cyclic nucleotides (cyclic AMP and cyclic GMP) in wound tissues (muscle and granulation tissue) were investigated in rats. The experimental model was a wound with a skin defect in the dorsal region with pulping of the underlying muscle. The cyclic AMP level in muscle tissue was shown to rise to two peaks: on the first day and more especially on the 7th day. The cyclic GMP content rose a little on the 1st-4th days, fell on the 7th day, and rose again until the 14th day. The cyclic AMP concentration in granulation tissue followed a similar course to that in the muscle tissue: a rise on the 7th day and a fall on the 14th day. On the other hand, the curve of the cyclic GMP content in the granulation was more uniform. Only a small increase toward the 7th day was observed.

KEY WORDS: cyclic nucleotides; regeneration; cell; wound.

In the modern view, cyclic nucleotides play the leading role in the regulation of many different processes in the cell, including those processes of cell division and differentiation that are directly related to wound healing [1, 2, 3, 6, 7, 9-13]. Meanwhile, investigations of cyclic nucleotides during regeneration have been undertaken chiefly on tissue cultures [8]. It was therefore decided to study the dynamics of the cyclic nucleotide content in wound tissue at different stages of healing, for the information so obtained, it was considered, could shed some light on the basic principles of healing, and so could lead to the formulation of recommendations aimed at promoting or accelerating healing.

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