Influence of a mixture of chemical protectors on the lymphoid regeneration of bone marrow and thymus in irradiated mice

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Summary. In mouse, the administration of chemical protectors before an irradiation induces a more rapid bone marrow regeneration and an increased lymphoid rebound. In the thymus, the late atrophy is reduced. Separately administrated, the protectors decrease greatly the thymocytes number but have no effect on the marrow population.

Whole body irradiations of C57BL mice induce thymic lymphomas. The high incidence is obtained when the total dose is divided into 4 fractions, given a week apart. However, a single exposure is sufficient to increase the lymphoma incidence[1]. During the recovery following a 500 R dose of X-ray, we have observed a transient accumulation of peculiar lymphoid cells in the bone marrow[2]. We have suggested a possible relation between this transient lymphoid accumulation and the development of thymic lymphomas[3]; for instance, the injection of normal bone marrow cells after the irradiation suppresses the lymphoid rebound and reduces the incidence of lymphomas[4]. The lymphoma appearance is preceded by a latent period during which the thymic lymphoid population shows quantitative and qualitative changes[3]. As the administration of a mixture of chemical protectors also decreases the radioleukaemias[5,6], the present experiments were performed to determine whether these radioprotectors have also an effect on the lymphoid peak of the bone marrow, on one hand, and on the thymic cell population on the other hand.

Methods. Female C57BL mice, 3 months old, are separated into 2 groups and X-irradiated under the following conditions: Siemens Stabilivolt apparatus, 190 kV, 18 mA, 0.5 mm^3 filter, 35 cm FD, 210 R/min. The animals of the

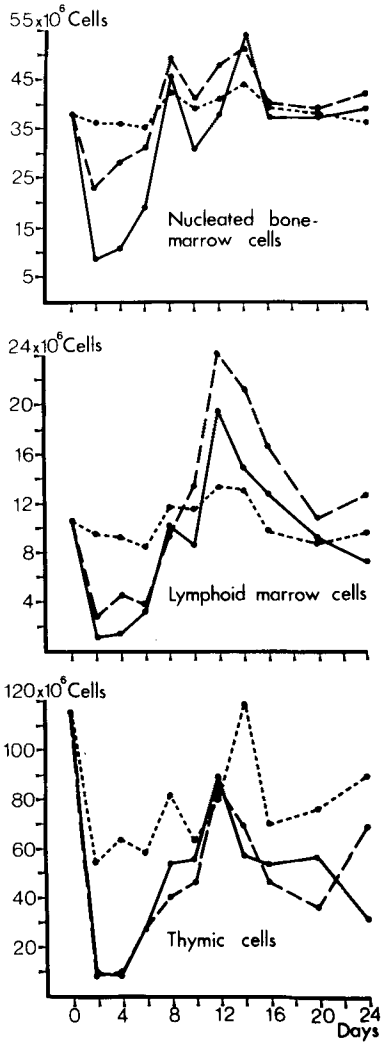

Fig. 1. Absolute numbers of nucleated bone marrow cells, lymphoid marrow cells and thymic cells after 400 R (———), chemical protection and 400 R (----) and after administration of mixture of chemical protectors (.....).

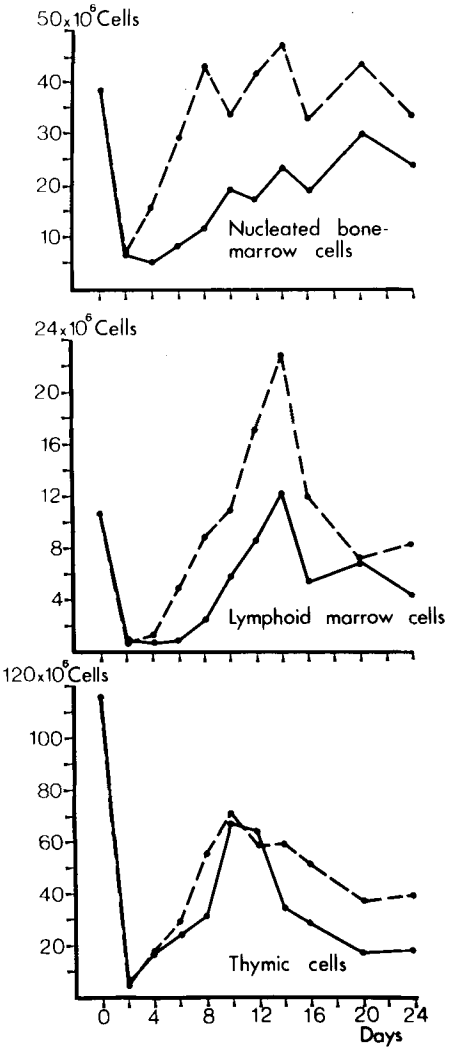

Fig. 2. Absolute numbers of irradiated bone marrow cells, lymphoid marrow cells and thymic cells after 650 R (———) and chemical protection preceding 650 R (---).

1st group are exposed to a single dose of 400 R whole body irradiation, those of the 2nd group to a 650 R dose. In each group, half the mice receive prior to irradiation a radioprotector treatment, while the other half is not protected. The radioprotection is performed as follows: 25 min before irradiation, the mice receive 15 mg glutathione orally; 10 min later, they receive i.p. 8.5 mg cysteine followed after 5 min by an i.p. injection of a mixture of 1.4 mg 2-β-aminoethylthiouroneum, 3 mg cysteamine and 0.65 mg 5-hydroxytryptamine[7]. All substances are dissolved in saline. Mice submitted to the mixture of chemical radioprotectors without any irradiation, and mice free of any treatment, represent the control groups. 6 animals of the different experimental modalities are sacrificed every other day from the 2nd to the 16th day and the 20th and 24th days. The variations of the total number of nucleated bone marrow cells and of the lymphoid marrow cells in 2 femurs are scored. The changes in the number of thymic cells are also determined. Our counting techniques have been previously described[8].

Results. After an irradiation of 400 R (figure 1), the marrow nucleated cells are strongly reduced in number between the 2nd and the 4th day; the recovery is fast, with an overshoot at the 14th day; the number of lymphoid marrow cells varies in the same way, but the rebound over the normal level is observed at the 12th day. The administration of radioprotectors prior to the irradiation prevents partially the decrease of the marrow nucleated cells and has little influence on the marrow repopulation. On the contrary, the chemical protectors have only a slight effect on the destruction of the lymphoid marrow cells, but they increase the regeneration with the development of an especially important lymphoid rebound; the mixture of chemical protectors without irradiation does not significantly modify the bone marrow populations.

The modifications of the thymic cell number after a 400 R irradiation, with or without protection and after the administration of chemical protectors only, are also plotted in figure 1. The irradiation induces an important reduction of the thymic cell population; the recovery begins at the 4th day and reaches, on the 12th day, a level clearly lower than the normal values. A secondary atrophy is observed from the 14th day to the end of the observation period. The protectors do not modify the thymic regeneration; they have only a little effect on the secondary atrophy. Indeed, on day 24, the number of thymocytes is significantly higher in protected animals. The administration of the protectors mixture without any irradiation induces a fall of the thymic cell number lasting 6 days (50% of the normal value); the thymic regeneration presents a transient phase of proliferation with a return to the normal level at the 14th day; the regeneration is also followed by a secondary atrophy which is still present at the end of the observation period.

Figure 2 illustrates the modifications of the nucleated bone marrow cells, lymphoid marrow cells and thymic cells after an irradiation of 650 R with or without chemical protection. The evolution of these different populations is rather similar to what is observed after a 400 R dose, but the marrow is not wholly repopulated by the 24th day, the lymphoid peak is less important and the thymic secondary atrophy more pronounced. The protection allows a fast and complete restoration of the nucleated marrow cells and greatly increases the lymphoid rebound. It has no effect on the primary thymic recovery but it reduces the secondary atrophy as early as the 14th day. All the modifications shown here are statistically significant.

Discussion. The sole administration of the mixture of chemical protectors has very little effect on the bone marrow but it induces an important decrease of the thymocytes number. The administration of the same mixture of chemical protectors before the irradiation permits a better bone marrow repopulation and reduces the late thymic atrophy (condition which precedes the apparition of thymic lymphomas). Contrarily to our expectations, the lymphoid peak is considerably increased in radioprotected animals. We are thus confronted with a situation where a lymphoid rebound appears in the bone marrow, whereas, as demonstrated by others[5,6], the development of lymphomas is reduced. However, the composition of this rebound may be quite different from that observed after an irradiation without any chemical protection. Indeed, after irradiation, the bone marrow contains peculiar lymphoid blast cells, called X cells, which have been also observed in the thymus and have been suspected of playing an important role in leukemogenesis. Further ultrastructural investigations are necessary to verify whether X cells are also present in the animals chemically protected.

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Relationship between lymphoid cell population and levels of cholesterol or phospholipids

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Summary. Lymphoid cells obtained from mouse thymus were divided into 3 groups according to the levels of free cholesterol and phospholipids.

The structural lipids of mammalian cell membrane are known to consist primarily of cholesterol and phospholipids, of which the proportions and structure are very important for the properties and functions of the cell membrane[1-4]. Studies on the lymphocyte lipids indicated differences in the cholesterol levels between normal and leukemic cells from man or animals[5-7]. However, relatively little is known about the relation of the lipid levels and various lymphocyte populations. In man and animals, there are 2 major lymphocyte populations (thymus-derived and bone marrow-derived lymphocytes), which are further divided into subpopulations[8]. In this study we have ex-