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THYMUS HOMING AND RECOSTITUTION IN SPLIT DOSE IRRADIATED PRELEUKEMIC MICE (1)

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The incidence of thymic lymphomas in C57BL/Ka mice treated with four weekly irradiations of 1.75 Gy, reaches more than 90% (1). The tumors appear after a long latent period (4 to 9 months), during which the thymuses contain preneoplastic cells (called preleukemic cells) requiring the thymic microenvironment for their progression into frank neoplasia (2).

During this latency, alterations of the marrow-thymus interactions are observed: the pool of bone marrow prothymocytes is impaired (3, 4), the function and the phenotype of thymic environment are altered (5) and the pattern of thymus repopulation is modified.

Bone marrow grafting immediately after the last irradiation is followed by the restoration of the pool of prothymocytes (3, 4) and the thymic microenvironment (6), facilitates thymus regeneration and prevents the development of lymphomas (7). Under these conditions, the appearance of preleukemic cells is not inhibited but they disappear one month later (6). The mechanisms inducing this disappearance and thus the protection against lymphomas have not been demonstrated. It has been proposed that marrow cells actively repopulating the irradiated thymuses are responsible for the protection against tumors, either by "competition" with preleukemic cells, either by restoring some components of the thymic microenvironment.

Such bone marrow precursors grafted later on after the last irradiation, for example one month later are unable to repopulate the irradiated thymuses and do not inhibit the lymphoma development (8). The present work was undertaken to test whether the injury to thymic stromal cells is the major factor involved in the lack of reconstitution in preleukemic mice. For this purpose, the homing of normal marrow precursors to thymus and their capacity to repopulate the thymus of sublethally irradiated mice were compared in normal and preleukemic thymuses.

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MATERIALS AND METHODS

Mice: One to two-months old C57BL/Ka mice of both sexes were used. Four week old congenic Thy-1.1 C57BL/Ka mice (called BL/1.1) were used as donors of bone marrow cells.

Irradiations: For the induction of lymphomas, mice were given four doses of 1.75 Gy applied at weekly intervals. For the thymus homing and for the repopulation assay, mice were whole-body irradiated with a single dose of 4 Gy. The irradiation was delivered by an X-ray apparatus (Stabilivolt Siemens, 190 Kv, 18 mA, HVL: 0,5 mm Cu) at a dose rate of 1,60 Gy/min.

In vivo thymus homing assay was done as described (9). Briefly, 10.10^6 FITC labelled bone marrow cells (10) were injected i.v. into 4 Gy irradiated animals within 2 hours following X-ray exposure. Twenty-four to 72 hours later, recipients were sacrificed and the number of FITC labelled cells was determined in cell suspensions treated with propidium iodure to exclude death cells.

The thymus repopulation assay was done as described previously (11). Aliquots of 5.10^6 bone marrow cells collected in BL/1.1 mice (Thy-1.1) were injected i.v. into C57BL/Ka mice (Thy-1.2) within 2 hours following a single 4 Gy exposure. Fifty, 20 and 30 days later, recipients were sacrificed and thymus cell suspensions were treated with monoclonal anti-Thy-1.1 and anti-Thy-1.2 antibodies followed by a FITC labelled second stage antibody.

Fluorescence analysis: Cell suspensions were analyzed on a fluorescence activated cell sorter (FACS IV, Becton-Dickinson, Sunnyvale, California). Only the fluorescence of viable cells (discriminated on the basis of labelling with propidium iodure) was considered.

RESULTS

The first experiment was undertaken to test whether radiation-induced injury to thymus inhibits the homing of normal bone marrow precursors. For this purpose, FITC labelled bone marrow suspensions were injected in the four Gy treated C57BL/Ka mice, either normal, either 30 days after the end of the leukemogenic treatment. The percentage of viable fluorescent cells was estimated in the whole thymus 24 and 72 hours after the graft. As seen in Table 1, the proportion of fluorescent cells at each time interval is similar in both conditions. The data indicate that homing of normal precursors is similar in normal and i preleukemic thymuses.

The following experiment was designed to compare the capacity of normal marrow precursors to repopulate 4 Gy irradiated normal and preleukemic thymuses. Bone marrow from BL/1.1 mice (Thy-1.1) were injected in 4 Gy irradiated C57BL/Ka mice (Thy-1.2) either normal, either preleukemic. Fifty, 20 and 30 days later, the percentage of donor (Thy-1.1) thymocytes was scored. As seen in fig. 1, the percentage of donor cells in normal thymuses increases gradually from the 15th to the 30th day after the irradiation to reach about 90%. This evolution is quite different in preleukemic mice: the percentage of donor cells decreases from day 15 to day 20, before to increase and to reach normal values at day 30. The data indicate that reconstitution of preleukemic thymuses is delayed as compared with that of normal thymuses.

Table 1. Migration of FITC labelled bone marroe cells in 4 Gy treated thymuses

Delay after bone marrow grafting (hours)	Frequency of FITC labelled cells	
	In normal thymuses	In preleukemic thymuses (30 days after the leukemogenic irradiation)
24	8.5/10.000	10/10.000
72	10/10.000	12/10.000

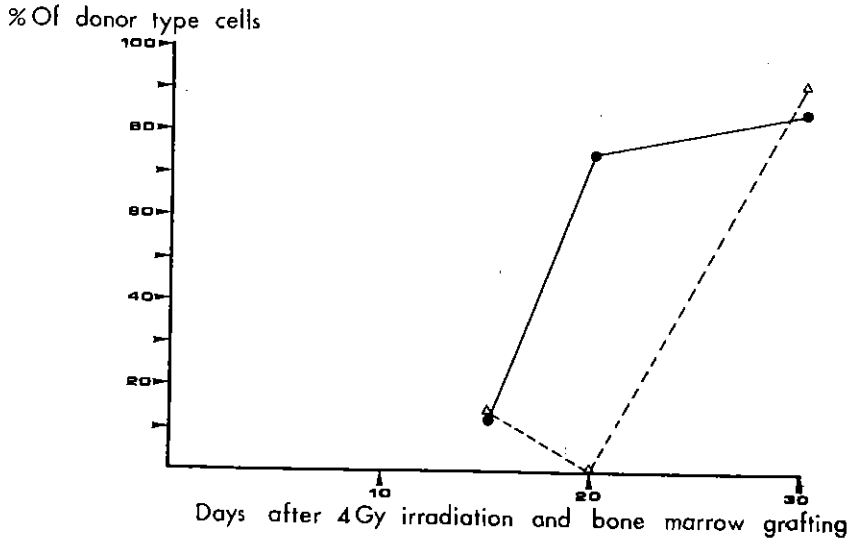

Fig. 1. Evolution of donor type cells after a 4 G y irradiation and a bone marrow graft in normal (●——●) and preleukemic (Δ---- Δ) mice treated 30 days after the leukemogenic irradiation.

DISCUSSION

Previous studies have shown that marrow thymus interactions are altered in preleukemic C57BL/Ka mice (3, 4, 6, 7). Therefore we have designed experiments to test whether the homing or normal marrow precursors to preleukemic thymuses and/or their repopulation capacities were modified.

It has been demonstrated that in vitro fluorochrome labelling of lymphocytes does not alter their migration properties when they are injected into animals (10). With this technique, we studied the migration of normal bone marrow precursors to thymuses of normal and preleukemic mice (taken 30 days after the end of the leukemogenic irradiation), and we demonstrated that it was similar in both conditions.

By contrast, using radiation chimeras, we showed that reconstitution of preleukemic thymuses by normal bone marrow cells is delayed as compared with that of normal thymuses.

There is no evidence that short term thymus homing bone marrow cells in fact are the precursors that allow long term thymic reconstitution. Because fluorescence fades rapidly, it is not possible to follow the evolution of immigrants for a long period of time. However, their phenotype (12) and their localization in the outer region of the thymus (data not shown) which is known to contain precursors of thymocytes suggest that they might be responsible for thymus repopulation. If it is true, one may conclude that the altered thymic microenvironment during the preleukemic period is responsible for the impaired proliferation of precursors within the thymus. In agreement with this hypothesis, we have shown that the phenotype and the function of thymic nurse cells which are responsible for some early steps of T cell proliferation and differentiation (13) are altered (5). During the preleukemic period, they are unable to establish in vitro interactions with immature thymocytes.

It remains to explain why the thymus reconstitution is only delayed and not totally abolished during the preleukemic period and how this phenomenon contributes to the leukemogenic process.

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