INTERPLAY OF THYMUS AND BONE MARROW REGENERATION IN X - IRRADIATED MICE

by

Klaus-Dieter Hiesche

Department of Radiobiology
Wallenberg Laboratory
University of Stockholm
S-104 05 Stockholm 50, Sweden

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Klaus-Dieter Hiesche
Institutionen för Strålningsbiologi
Wallenberg laboratoriet
Stockholms Universitet
104 05 Stockholm 50

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Klaus-Dieter Hiesche

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University of Stockholm
S-104 05 Stockholm 50, Sweden

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I HIESCHE, K.D. and REVESZ, L.: Cellular Repopulation in Irradiated Mouse Thymus and Bone Marrow. Beitr. Path. 151, 304-316(1974)

II HIESCHE, K.D. and REVESZ, L.: The Role of Bone Marrow in Different Phases of the Cellular Repopulation of Irradiated Mouse Thymus. Beitr. Path. 155, 181-192(1975)

III HIESCHE, K.D., REVESZ, L. and HAOT, J.: Cellular Repopulation and Recovery of the Phytohemagglutinine(PHA) Responding Cell Pool in the Thymus of Sublethally Irradiated Mice. Beitr. Path. 156, 46-55(1975)

IV HAOT, J., HIESCHE, K.D. and REVESZ, L.: Sensitivity of Irradiated and Normal Bone Marrow to H-2 Antiserum. Beitr. Path. 147, 213-220(1972)

V HAOT, J., MÖLLER, E., HIESCHE, K.D. and REVESZ, L.: Cytotoxic Effect of a Xenogenic Antiserum on Bone Marrow Cells from Normal or Sublethally Irradiated Mice. Experientia 28, 956-957(1972)

These communications will be referred to by the Roman numerals I-V in the text.

CONTENTS

1. INTRODUCTION

The thymus is a lymphoid organ located in the upper anterior part of the chest in most species. The general histologic picture is that of a central area, the medulla, with a sparce lymphocyte distribution and a surrounding cortex composed predominantly of densely-packed small lymphoid cells. In young mice the cortex shares about 86 % and the medulla about 14 % of the thymic volume(23).

Lymphocyte proliferation is extensive in the young, adult thymus, as evidenced by frequent mitotic figures(4, 80, 89)and a high rate of DNA turnover(3, 39, 86, 97). The proliferating cells are large, blast-like and they give rise to a progeny of non-dividing small thymocytes which constitute approximately 90 % of the cells in the thymus(23, 86). Cell production is concentrated to the outer cortical rim(22, 28, 40, 89) and to the cortico-medullary junction(85). Whether the cortical and medullary production of small thymocytes are separate events, or whether cells feed from one compartment into the other, is not clear.

An age-involution weight loss of the thymus in adult mice coincides with a fall in thymic mitotic index(80). There is strong experimental evidence which suggests that, from the point of view of proliferative activity of its lymphoid cells, the thymus behaves to a large degree as an autonomous organ(47, 70, 71). The stimulus for cell division may originate in certain secretory cells of the thymic epithelial-reticulum framework(27, 28, 72).

The dividing cell population in the thymus has been shown to be continuously substituted by lymphoid immigrants(24, 35, 56, 73,

74, 77, 78) in a variety of experimental systems. In the adult mouse, the bone marrow is the source of the immigrated cells in the thymus (43, 44, 75, 76, 92, 113). The observations have led to a general assumption that the stem cell compartment in the thymus is not self-sustaining. The validity of this assumption and its application to a normal physiologic condition, remains, however, to be proven.

The irradiated, bone-marrow-reconstituted mouse was found particularly attractive as a model to study the function of bone marrow in thymic lymphocytopoiesis. Conflicting data exist in regard to the relative role of seeded cells and local survivors in thymic regeneration after irradiation. In a series of investigations, we approached this problem by creating experimental conditions in which thymic regeneration could be studied as a function of either the number of bone marrow cells in the organism(I) or the cellular composition of the thymus itself at the time of radiation exposure(II).

There is evidence for a small pool of immunocompetent cells in the thymus(16, 68), but knowledge is incomplete in regard to origin and recruitment of these cells. In the present investigation we studied the recovery of the immunocompetent cell population in the irradiated thymus with particular notice on a possible function of bone marrow cells in this process(III).

Finally, in a series of experiments, the regeneration pattern of irradiated bone marrow was studied in relation to that of the thymus(I). An attempt was also made to characterize a particular cell type in regenerating bone marrow(IV, V), which may be involved in thymic regeneration after irradiation(21).

2. GENERAL DISCUSSION

2.1. Thymic regeneration after total body irradiation.

Thymic regeneration is cyclic in mice irradiated on the whole body in the nonlethal and sublethal dose range(II, III, 10-12, 14, 15, 31, 33, 62, 63, 105). During the first rapid involution of the organ, pycnotic cells are more frequent in the cortex than in the medulla(40, 107). This observation led to the conclusion that the cortical thymocytes are more radiosensitive than the medullary ones. However, results have recently been reported which seem to contradict such a conclusion(61, 114). They suggest that the differential kinetics of cortical and medullary depletion reflects differences in the rate by which the lethally damaged cells die rather than differences in radiosensitivity. Cortical thymocytes die rapidly in interphase already after moderate radiation exposures(60, 98, 112). Medullary cells, on the other hand, may die only when attempting mitosis. Since the mitotic index is very low in the medullary region (23, 90), irradiation would be expected to have little immediate effect on the size of the medullary cell population.

Thymic regrowth starts within 4-6 days after irradiation and usually continues until the 12th post-irradiation day(II, III, 10-12, 14, 15, 31, 33, 62, 63, 105). Regeneration is preceeded by an increased frequency in the thymus of large, undifferentiated blastoid cells(14, 40, 91), which may function as thymic stem cells. In the present investigation we obtained data which suggest that the proportion of thymic stem cells was indeed increased in the irradiated thymus at the outset of regeneration in comparison to the proportion in the unirradiated organ(II). One explanation is that a fraction of surviving small thymocytes reenter cell cycle in analogy to what has been shown to occur after exposure of lymphocytes to plant mitogens and alloantigens(13, 19, 41, 48, 103). Another explanation is that

blastoid cells in thymus cortex are more radioresistant than the nondividing, small thymocytes which are in radiosensitive interphase.

The thymic seeding from the radiation damaged bone marrow appears to be greatly impaired(II). In addition, the proliferative capacity of the marrow stem cells is depressed(12). Therefore, bone marrow derived cells are probably not involved in the first phase of thymic regeneration after irradiation and regeneration is most likely due to the proliferation of intra-thymic lymphoid survivors.

A second involution of the thymus starts two weeks after irradiation(II, III, 10-12, 14, 15, 31, 33, 62, 63, 105). It is associated with a decreased mitotic index(33) and an exhaustion of the lymphoid stem cells in the thymus(II). As an explanation it has been proposed that the proliferative capacity of the surviving cells is limited and the seeding with new precursors from the radiation-injured bone marrow is impaired(II). During the second thymic atrophy the export of small thymocytes is increased(38). Thus, the second phase of cellular depletion of the thymus can be attributed to both an impaired cell production and a continuous release of thymocytes from the organ.

By the end of the fourth week after irradiation, a final regeneration of the thymus begins. At this time the cellular composition of the thymus is similar to that of the organ in untreated mice of a comparable age(II). The recovery of the thymic stem cell pool can be ascribed to a recovery of the radiation damaged bone marrow with regard to the seeding of the thymus with lymphoid precursors(II).

2.2. Thymic regeneration and bone marrow protection.

In contrast to whole body irradiated animals, no secondary atrophy of the thymus can be observed when a cell suspension prepared from syngeneic bone marrow is administered to the animals immediately after radiation treatment, or when bone marrow tissue

is shielded during exposure(III, 10, 15, 33, 105). There is no evidence that thymus lymphocytopoiesis can be controlled by external factors. Therefore, the inhibition of the second atrophy of the thymus is likely to be due to the activity of the bone marrow cells which were sequestered in the thymus after the radiation treatment. In support of this consideration, bone marrow derived cells were shown to constitute the majority of the dividing cells in the thymus within 12-15 days after infusion into irradiated mice(75, 105, 106). The results were obtained in experiments, in which a chromosome marker was used to identify the dividing cells.

Knowledge is incomplete to what extent marrow-derived cells participate also in the early restoration process of the radiation-depleted thymus. Experiments with partial shielding of the bone marrow from radiation have yielded equivocal results, and findings indicating either no effect(11, 33, 105) or an enhancing effect (15, 55, 66, 84) by the protected marrow on the rate of early regeneration of the irradiated thymus have been reported.

In view of the equivocal findings, we reinvestigated the role of bone marrow cells in early thymic regeneration in heavily irradiated mice by creating experimental conditions, in which the radiation damage of the thymus was kept at a constant magnitude and the number of bone marrow cells in the organism varied(I). In such a system, the role of bone marrow cells in thymic regeneration could be revealed by the presence or absence of a correlation between the number of these cells and the rate of thymus regeneration.

During an initial period of 12 days, thymic regeneration occurred at a rate which was independent of the number of the available bone marrow cells(I). The results were interpreted to indicate that thymic regrowth after irradiation is due to residual survivors within the thymus. This interpretation was supported in complementary experiments by the correlation noted between the radiation dose received by the thymus and the delay in thymic regeneration in mice in which the bone marrow in one femur was totally protected(I).

In a subsequent experimental series, mice were exposed to the

same total radiation dose in two fractions and transplanted with syngeneic bone marrow cells in a defined number immediately after the second irradiation(II). The initial cellular regeneration of the thymus was found to be dependent on the time interval between the two radiation exposures, although the bone marrow population was identical in the different animals. It was concluded that the initial phase of thymic regeneration is greatly dependent on the cellular composition of the thymus at the time of radiation exposure and independent of the bone marrow cells made available after irradiation(II).

Supporting evidence for our conclusion was obtained in the following recent investigations:

(a) In irradiated CBA mice grafted with chromosome-marked bone marrow cells from syngeneic CBA-T6T6 donors(42), all the dividing cells in the regenerating thymus were shown to lack the graft marker during the first 12 days after irradiation(105). It was concluded that early thymic regeneration is due to local survivors and that bone marrow cells take no active part in it.

(b) Mice whose bone marrow was shielded from irradiation or who received injection of large numbers of syngeneic bone marrow cells immediately after irradiation, showed an identical amount of ^{3}H-TdR uptake by the regenerating thymus as those receiving total body irradiation solely(64). It was concluded that the precursor cells responsible for the early post-irradiation phase of thymic regeneration are most likely part of an intra-thymic population.

2.3. Thymic seeding with bone marrow cells.

The frequent failure to detect a function of bone marrow cells early during regeneration of irradiated thymus, however, does not close out the possibility that bone marrow cells seed the thymus and even proliferate there during an early phase. Several observations support such a possibility. In one series of investigations, bone marrow derived cells were identified in the thymus of irradiated rats shortly after intravenous infusion, using a serologic marker for

identification of the seeded cells(81, 82). The newly arrived cells rapidly lost their marrow homeing capacity, indicating the differentiation of the cells into thymocytes. Unfortunately, it was not shown whether the seeded cells did also proliferate within the thymus.

In other investigations, intravenous administration of bone marrow cells to irradiated recipients was also reported to result in the rapid appearence in the thymus of some of the injected cells. In these experiments, marrow cells containing a marker chromosome (33, 69) or cells labeled with ^{3}H-TdR *in vitro*(40, 45) were used. Again, however, a function of the seeded cells in thymic regeneration was not conclusively demonstrated.

In the present investigations we used an indirect approach to study the possible early seeding of the irradiated thymus with bone marrow cells(II). Mice were irradiated with a nonlethal dose either on the whole body or with one hind limb shielded. The shielding was shown to protect against approximately 90 per cent of the radiation dose received by the nonshielded part of the body(20). After two weeks the animals received a large, second radiation dose followed by syngeneic bone marrow graft of identical size. After the second irradiation, the regeneration of the thymus occurred at a markedly faster rate in the mice which had part of the bone marrow shielded during the first irradiation compared to the non-shielded animals. This observation suggests a difference in the proportion of stem cells in the thymus at the time of the second irradiation in the two groups of mice. It was concluded that the difference is due to the thymic seeding with a large number of cells from the shielded marrow during the interval between the two radiation exposures.

From the foregoing evidence it is conceivable that the thymus sequesters circulating, bone marrow derived cells during an early period after irradiation when no active participation in thymic regeneration of the seeded cells can be detected. One explanation may be that the seeded cells do not multiply immediately after arrival in the thymus. Another explanation is that the number of marrow derived cells which initially seed the thymus is below a level that

can be detected by techniques used to detect proliferating cells. In support of this explanation, it has been deduced from kinetic studies on thymic regeneration in radiation chimaeras that the dividing donor cell population within the thymus can derive from very few precursor cells, possibly as few as one or two(109).

2.4. Phytohemagglutinin-reactivity of irradiated thymus.

Cell suspensions prepared from the thymus of adult mice exhibit a low immunological reactivity compared to that of cells from other lymphoid organs(8, 9, 25, 108). It was, therefore, assumed that the reactivity of individual thymocytes is low. However, results of investigations on cell suspensions from thymi of mice 48 hours after cortisone-injection, contradict this assumption. Cortisone-treatment of mice eliminates most of the cortical small cells(59) but seems also to eliminate larger cells including those from the medulla(34). In contrast, the small thymocytes in the medulla seem to resist the steroid treatment. These medullary small thymocytes were shown to carry a number of immunological functions such as reactivity *in vitro* to phytohemagglutinin(PHA)(19, 103, 104) and alloantigens(17, 19), graft-versus-host reactivity(13, 16, 29, 68) and helper activity for thymus dependent antibody responses(2, 18, 30). It may be argued that cortisone-treatment enhances the reactivity of thymocytes *per se*. The observation that a highly reactive cell population can be obtained also by density-gradient centrifugation(67, 88, 102) of cell suspensions prepared from whole thymi, however, strongly contradicts such an arguement.

The thymus provides the inductive stimulus for stem cells to mature into immunocompetent cells(T cells). The differentiation pathway of the T cells in the thymus is only recently in the process to be understood. For many years the cortisone-sensitive thymocytes in the cortex could not be correlated with any function and were, therefore, considered immature precursors of the functionally competent cortisone-resistant cells in the medulla. This concept receives some support from experiments using the *in vivo* labeling of outer cortical DNA-synthetic cells by ^{3}H-TdR. Within 1-4 days after thymidine administration, a gradual movement of labeled cells in the

direction of the medullary region is observed(22, 40). Parenteral administration of hyrdocortisone at the time of labeling prevents the appearance of labeled medullary descendants, indicating that hydrocortisone-sensitive cells may give rise to medullary thymocytes(110). Fractionation by density-gradient centrifugation of the thymus cell population at various intervals after labeling, and the characterization of the fractionated cells with regard to antigenic markers, provides some evidence for a maturation pathway of cortical to medullary thymocytes(111).

Turning to a quantitative aspect, no information is available to what extent the cortex-derived cells transform into immunocompetent medullary thymocytes. Using the in vitro-response of thymocytes to PHA as a criterion of immunoreactivity(19, 103, 104), we have studied the recovery of the immunocompetent cells in the irradiated thymus under experimental conditions in which the number of bone marrow cells in the organism - and, consequently, the number of cortical cells in the thymus(109) - varied. For this purpose, mice were irradiated with a sublethal dose either on the total body or with one hind limb shielded(III).

The results show that thymic PHA-reactivity remained low for about 12 days after irradiation. Thereafter, it increased at a rate which was largely independent of the bone marrow, suggesting that the PHA-reactive cells were recruited from intra-thymic survivors. The increase in PHA-reactivity is preceeded by complete cellular and morphological restoration of the thymus(III, 62, 105). Therefore, we are in ignorance about the intra-thymic origin of the cells responsible for the early phase in PHA-recovery. Studies on intra-thymic cellular migration patterns may give information in regard to this question.

By the 20th post-irradiation day, thymic PHA-reactivity was restored to about 50 % of that in unirradiated mice, in agreement with the findings of other investigators(62). During the remainder of the observation period - between 20 and 40 days after irradiation - two observations need particular notice:

(1) A new and temporary decrease in PHA-reactivity occurred which was, however, less evident in the leg-shielded mice than in the total body irradiated animals.

(2) Reactivity in neither case exceeded a value approximately 50 % of that in unirradiated mice. In the leg-shielded animals, the deficiency in thymic function was associated with a complete restoration of the nonfunctional cell population in the thymus.

In pointing out these findings, the author is very much aware of the considerable error which is connected to the determination of PHA-reactivity in each individual case. However, he also feels that the consistency of the observations justifies a close inspection of the data.

The first observation may indicate a certain late effect of bone marrow on thymic PHA-reactivity, in analogy of the previously discussed late effect of bone marrow on the number of nonfunctional cells in the thymus. The bone marrow may carry either one type of precursor cell in common for both the functional and nonfunctional cell populations, or separate precursors for each of the two cell compartments in the thymus. As an entirely different interpretation, the difference in PHA-reactivity between the total body irradiated and leg-shielded animals may reflect differences in intrathymic migration of cells from the medullary into the cortical region. It has been proposed that PHA-reactive cells can be utilized in cortical regeneration(62). They may be induced for such a function under particular circumstances, e.g. after total body irradiation, when the cortex is severely depopulated and new precursors from the radiation damaged bone marrow are not available. In leg-shielded mice, on the other hand, in which the cortex is completely repopulated and the thymus is continuously seeded by precursors from the shielded bone marrow, medullary thymocytes would not be expected to be needed for cortical regeneration.

The second observation, on the one hand, is surprising in view of the postulated feeding of nonreactive, cortical thymocytes into medullary, immunoreactive cells. As an explanation, only a minor

fraction of the cortical cells may function as precursors of the medullary thymocytes. It is also conceivable that maturation of the cortex-derived cells occurs at a low rate. On the other hand, the second observation may be interpreted to indicate the existence of separate developmental pathways of thymocytes in thymus cortex and medulla. Evidence in support of such an interpretation was obtained in the following experiments:

(a) When thymi from CBA-T6T6 mice were grafted to normal CBA recipients, the cortex of the grafted thymi was rapidly repopulated by cells of the host. The PHA-reactive cell population in the medulla, in contrast, remained of donor origin even 300 days after grafting (36, 37). At this time most of the PHA-reactive cells in peripheral blood are known to be of host origin(32, 79). Nonreactive thymocytes may thus not necessarily function as precursors of the mature pool of thymus cells, but may feed directly into the peripheral pool of mature T cells. On the other hand, functionally competent thymocytes of the medulla may never leave the thymus.

(b) After repeat injection of tritiated thymidine into young, adult CBA mice, both low θ and high θ small lymphocytes in the thymus(representing roughly the medullary and cortical cell populations, respectively) showed continuous and close to linear accumulation of labeled cells with no evidence for a marked lag in the labeling of low θ, i.e. medullary cells(99). The data suggest independent developmental pathways from large to small thymocytes both in the cortical and medullary compartments.

(c) During an early period in the age-involuting thymus, a decrease in the number of nonreactive cells was associated with an increase in thymic PHA-reactivity(III), indicating a function of the PHA-reactive medullary cells independent from the nonreactive, cortical cells.

The concept of thymocyte generation within the thymus may turn out to be even more complex than outlined in the foregoing. The identification of subpopulations of lymphocytes in cortex and medulla of the thymus(34) justifies such a supposition. Future studies combining cell

separation techniques with other approaches, especially with studies on cell proliferation kinetics, cell surface antigens and immunological responsiveness, may greatly increase our understanding of the mechanism by which the thymus-derived cell population in the organism is built up during ontogeny or after X-ray exposure.

2.5. Bone marrow regeneration after X-irradiation.

a. Comparison to thymic regeneration.

As already discussed in detail in an earlier paragraph, thymic regeneration after irradiation is initially independent on available bone marrow cells. In contrast, bone marrow regeneration in heavily irradiated mice was found to be also initially dependent on available undamaged marrow cells(I). As an explanation, it can be assumed that thymic stem cells are more radioresistant than bone marrow stem cells. Accordingly, after irradiation a sufficient number of precursors may remain in the thymus to regenerate the organ even in the absence of immigrating cells. A particular radioresistant lymphoid cell type has been indeed suggested to occur in the thymus(58, 65, 107), but contradictory results have recently been presented(61, 87, 114). It is also possible that the absolute number of the stem cells is larger in the thymus than in the femoral bone marrow in untreated mice. In such a case a larger number of stem cells would be expected to survive in the thymus after irradiation than in the bone marrow. Unfortunately, no experimental system has so far been developed, by which the number of stem cells in the thymus can be measured quantitatively. Finally, it is conceivable that precursors seed the bone marrow in a larger number than the thymus. In support of this consideration, local labeling of guinea pig bone marrow with tritiated thymidine resulted in the appearance in the thymus of only few labeled cells(83).

b. Induction and characterization of a particular cell type.

Irradiation of mice on the whole body in the nonlethal and sublethal dose range, induces in the regenerating bone marrow the trans-

sient production of a small, mononucleated cell type, denoted X cell (51, 52, 100). The cells appear within 8 days after irradiation; they accumulate in greatest absolute and relative number 4 days later and they cannot be detected in the bone marrow after the second post-irradiation week(49). It seems likely that the X cells disappear due to emigration from the marrow tissue and not as a result of transformation into other marrow cell types(49). Their destination is not known for certain, though the thymus has been suggested as a possible target (21).

Neither the mechanism by which the X cells are induced nor their function is known. The production of X cells is not a prerequisite for bone marrow regeneration. Thus, when when damage was caused to the bone marrow by other agents than X-rays such as cyclophosphamid and cortisone (57), regeneration occurred without the appearance of X cells in the marrow cell population. The growth of undamaged marrow cells after transplantation to irradiated recipients also proceeds without the production of X cells(I, 49). It has been suggested, therefore, that X-irradiation per se induces the production of the X cells by some unknown mechanism. This suggestion, however, is contradicted by the finding that the production of X cells in the marrow of an irradiated femur can be inhibited when the rest of the body was exposed to heavy irradiation(I).

The X cells have a characteristic ultrastructure(100) and a volume which is slightly larger than that of normal bone marrow lymphocytes(49, 52, 100). Attempts made to associate the cells with either the erythroid(54, 94), granulocytic(53) or bone marrow stem cell compartment(IV, 50, 93) have failed. In preliminary experiments, X cells could neither be correlated to a function in humoral antibody production(5-7).

In the present investigation we have focused on a surface antigenic characterization of the X cells. Results of analysis of volume distribution, cytologic study of myelograms and determination of cell viability were consistent in indicating that about 1/3 and 2/3 of the cell population from unirradiated and irradiated bone marrow, respectively, were killed by treatment in vitro with an antiserum directed against the H-2 antigens of the marrow donor strain(IV). The cells killed in the unirradiated marrow cell population had a volume in the range corresponding to normal marrow lymphocytes, in confirmation of earlier results(54). In irradiated marrow, in addition, cells with slightly larger volume, conceivably X cells, were also destroyed. Cytologic examination of the surviving cell population confirmed that cells with lymphoid morphology had been killed. The results can be interpreted to indicate that X cells are as sensitive to in vitro treatment with H-2 antiserum as normal bone marrow lymphocytes. The results and the finding that the sensitivity of Xcells to cortisone treatment in vivo is similar to the sensitivity of the lymphoid cells in untreated bone marrow(95, 96), makes it conceivable that Xcells represent a particular lymphoid cell type.

Notwithstanding their lymphoid character, X cells can be clearly distinguished from the normal lymphocytes in the marrow of adult, unirradiated mice(100). The X cells are, however, similar to some elements in the marrow and thymus of new-born mice (26, 101). This finding and observations on resurgence of cells with embryonic antigens in the adult organism under certain conditions(1, 46), suggested a preliminary investigation of the possible embryonic character of the X cells. For this purpose an antiserum was used which had been raised in rabbits against mouse embryo tissues. After proper absorption, X-cell-rich marrow was found to be more sensitive to in vitro treatment with such a serum than marrow obtained from unirradiated mice(V). Since the investigation included neither cytologic examination nor volume distribution analysis of the treated marrow, it cannot be excluded that a cell type other than the X cells is sensitive to the antiserum. Complementary experiments using a more specific antiserum in combination with immunofluorescence and cell separation techniques can be expected to give a more definite answer on the possible embryonic nature of the X cells.

3. SUMMARY

The aim of the present investigation was to study the modifying effects of bone marrow cells on regeneration, after X-irradiation, of thymus and bone marrow cell populations.

Data are presented which indicate that the cellular composition of the thymus and, in particular, the frequency of the stem cells in the organ at the time of radiation exposure determines thymic regeneration for about two weeks after irradiation. After this period, regeneration depends on new precursors from the bone marrow which have previously seeded the thymus. In contrast to the thymus, cellular restoration of the bone marrow is already initially dependent on the number of protected or transplanted marrow cells.

Two phases in the recovery of thymic PHA-reactivity after irradiation were observed: one initial phase which is independent on the number of the available bone marrow cells, and a subsequent phase during which PHA-reactivity is slightly increased in mice irradiated with partly protected bone marrow in comparison to in total body irradiated animals. During the entire observation period, PHA-reactivity remains at a low level not exeeding 50 % of that in untreated mice. In contrast, the thymus is fully repopulated with regard to the number of nonreactive cells. Alternative pathways of thymocyte development within the thymus are discussed.

Bone marrow X cells were shown to be as sensitive to in vitro treatment with a specific H-2 antiserum as were lymphocytes from normal bone marrow. This finding was taken to indicate that the X cells represent a particular lymphoid cell type.

A xenogeneic rabbit-anti-mouse embryo antiserum was more toxic to pre-irradiated bone marrow, with high proportion of X cells, than to bone marrow from untreated mice, using in vitro cytotoxicity test. A possible embryonic character of the X cells is discussed.

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