A Radioautographic Study of Hemopoietic Repopulation Using Irradiated Parabiotic Rats

Relation to the Stem Cell Problem

By Ruth W. Caffrey Tyler and N. B. Everett

As early as 1930, Woenckhaus4 showed that if one of two rats joined in parabiosis was exposed to lethal irradiation, both animals survived. Since that time it has been shown that the recovery of lethally irradiated animals may also be attained by shielding bone marrow29 or spleen27 or by transfusing the irradiated animals with large numbers of blood leukocytes.20,33,36,44,49 The use of chromosomal markers has shown that this recovery is due to hemopoietic stem cells which are found in the blood,44 marrow and spleen.4,17,31,32,38,40,52 Stem cells as well as maturing forms of blood cells48 have been shown to be present within newly formed colonies.

The experiments reported in this paper were designed to identify the cells which cross by way of the blood stream in parabiotic rats and promote myeloid and lymphoid recovery following lethal doses of irradiation. The source of the effective cells was restricted to the bone marrow in the hind limbs of one parabiont by lead shielding of the region distal to the mid-thighs while administering 1000 r of total body irradiation to both rats. H3-thymidine (TTH) and radioautography were used to identify the cells originating from the shielded marrow and to follow their course and development in the non-shielded animals.

METHODS

The experimental plan is shown in Figure 1. Pairs of rats (125 Gm. each) from an inbred Lewis colony were joined in parabiosis essentially by the method of Bunster and Meyer5 except for joining the scapulae. At this time the popliteal nodes of the limbs to be shielded were removed. Two weeks later the parabionts were administered 100 r from a Co60 source while the hind limbs of one rat (A) were shielded. At 12 hours postirradiation the shielded rats were given TTH (1 μc./Gm.) in order to label the protected marrow while the cross circulation was arrested by compression. At 15 minutes post-TTH the B rat was given a large dose (5 mg.) of nonradioactive thymidine and the cross circulation was then restored.

Eight pairs of rats served to give the major portion of the information in this paper. (Additional animals are discussed at the end of the Results section.) One pair was sacrificed each day in order to cover the period between 1½ and 8½ days postirradiation. On each successive day the TTH injections were repeated for the pairs remaining in order to insure that a large percentage of the cells crossing from A to B would be heavily labeled.
At the time of sacrifice, differential and total white cell counts were made for each rat. Three milliliters of blood were withdrawn by heart puncture into 0.3 ml. of 2 per cent sodium citrate and after centrifugation, the buffy coat was used for smears. This procedure was necessary to provide adequate numbers of white cells in the smear. Smears and 1 μ tissue sections of spleen, bone marrow, thymus and mesenteric node for radioautographs were made as described previously. All radioautographs were exposed for 8 weeks.

Twelve single rats were given 1000 r of irradiation to serve as irradiated controls. These died between 7 and 11 days postirradiation. Rats from the same inbred colony of an equivalent age served as the nonirradiated controls.

**RESULTS**

**Blood**

Total White Cell Counts and Differentials. The average white cell count of the nonirradiated control rats was approximately 100 × 10^6 cells/ml. of blood. White cell counts of the parabiotic animals ranged from 4 × 10^5 to 22 × 10^6 (Fig. 2). The A rats showed twice the white cell counts of the corresponding B animals between 2½ and 8½ days postirradiation, and both groups approximately doubled their white counts during this period. Irradiated controls receiving 1000 r with no protection showed white counts of less than 1 × 10^5 cells/ml. of blood between 3½ and 8½ days postirradiation. Thus, the majority of the white cells in the blood of the B rats must have resulted from their parabiotic connection with the marrow-shielded A animals.

Three morphologically distinct types of cells were found in the blood of both A and B rats—namely, mature granulocytes, small lymphocytes and large mononuclear cells for which the term “monocytoid” is used (Fig. 3). This latter group includes monocytes as well as other mononuclear cells for which some investigators may prefer the term lymphoid, or consider as medium to large lymphocytes. These cells for which no distinct morphologic boundary could be established to distinguish them from monocytes may be described as follows.

The size was approximately that of a medium lymphocyte (8–10 μ in nuclear diameter); the nucleus was irregularly shaped with many invaginations and
Folds and was centrally located; the chromatin was not clumped but was in loose strands; the cytoplasm was abundant and pale staining (not basophilic) and azurophilic granulations were often present. The term “monocytoid” is used for these cells in preference to “lymphoid” for several reasons. First, some cells in this category evidenced phagocytosis when exposed to trypan blue or india ink and thus showed the functional as well as the morphologic appearance of typical monocytes. Second, the large and medium cells which have been functionally related* to the small lymphocyte and which are normally present in the thoracic duct lymph, thymus and lymph nodes have a morphology easily distinguishable from that of the monocytoid cell. This morphological type of large and medium lymphocyte was not present in the irradiated parabiotic rats until after 4½ days postirradiation. Third, the cells of the monocytoid group were derived from the bone marrow and showed no evidence of a functional relationship with the lymphocytes of the thoracic duct lymph, lymph nodes, or thymus, nor of the typical small lymphocyte of the bone marrow. This point will be considered further in the Discussion section.

*By “functionally related” we mean those large and medium cells which have been shown to be derived from small lymphocytes following exposure to PHA or more specific antigens (cells which have been shown to divide giving rise to small lymphocytes as well as large and medium).
Fig. 3.—Types of cells in blood of parabionts. A: monocytoid cells. B: mature granulocytes. C: small lymphocyte and monocytoid cell. × 1440.

The data from the differential white cell counts are expressed in numbers of cells/ml. of blood in Figure 4. Approximately 85 per cent of the white cells were small lymphocytes in the nonirradiated controls. At 1½ days postirradiation small lymphocytes were practically nonexistent in the blood of either parabiont. Small numbers of them were encountered between 2½ and 5½ days, and a significant increase occurred in the blood of both rats at 6½ days postirradiation (Fig. 4). This time coincided with the first appearance of small lymphocytes in the mesenteric nodes, thymuses and spleens of the respective rats. No significant increase in the number of small lymphocytes was seen in the bone marrow of either rat at this time.

At 1½ days the blood of both A and B rats showed granulocyte levels approximately 3 times the control values, which is in agreement with the observations of other investigators. Most of these granulocytes had disappeared by 2½ days. The A rats showed normal granulocyte levels after 3½ days, while the B rats showed consistently lower values. An increase in the numbers of granulocytes occurred in the blood of the B rats after 6½ days.

The nonirradiated control rats had a small percentage of monocytoid cells (≈ 8 per cent), the majority of which were phagocytic. After 2½ days 50–90 per cent of the nucleated cells in the blood of the irradiated rats were monocytoid and their actual number/ml. of blood was within normal limits. The white cells of the B animals between 3½ and 5½ days were more then 80 per cent monocytoid. Since the lethally irradiated controls had less than 1 × 10⁵ total white cells/ml. of blood during this same time interval, it is evident that the high monocytoid level was due to the marrow shielding of the A rat and that the monocytoid cell recovery in the B animal was a result of its cross circulation with the A animal.

Labeling Patterns in Blood. The percentages of labeled monocytoid cells and granulocytes were determined in each rat and are shown in Figure 5. Since only a small number of lymphocytes was encountered in the blood of either rat
before 6½ days, it was not possible to make an accurate estimate of the per cent labeled until this time. Between 6½ and 8½ days the percentage of labeled lymphocytes in the A rats was higher than in the corresponding B rats (50 per cent compared to 20 per cent) showing that both animals were producing lymphocytes after 6½ days.

None of the granulocytes was labeled in either rat at 1½ days. Approximately 55 per cent of the granulocytes were labeled in both A and B rats at 3½ and 4½ days, and thus it appears that all the granulocytes of both rats were being produced in the marrow of the A animals at this time. After 5½ days the A rats showed a higher percentage of labeled granulocytes than the B animals, indicating that the latter were producing some granulocytes at the 6½ to 8½ day intervals.

All the A rats showed a greater percentage of labeled monocyteid cells than the corresponding B animals. Thus it appears that the B rats are producing significant numbers of nonlabeled monocyteid cells after 3½ days postirradiation. This production may be attributed to nonlabeled precursors crossing from A to B during the early postirradiation period or to repeated division of labeled precursors finally giving rise to weakly labeled and nonlabeled cells.

**Bone Marrow**

**Cell Counts and Differentials.** The total number of nucleated cells in the marrow of one tibia from each rat was estimated by DNA determinations.
according to the method of Ceriotti. The A rats had a normal number of marrow cells (80–100 × 10⁶); the B rats had between 5 and 10 per cent of the control numbers. Differential cell counts were made using tissue section and smear preparations. These data are expressed in terms of marrow cells per tibia for the B rats in Figure 6. The number of cells of the fixed reticular network in the tibia showed no significant reduction or increase as compared to that of nonirradiated controls. A large percentage of the cells at 1½ days were mature granulocytes (65 per cent); however, in terms of cell number, this represented one half that found in controls and could easily account for the granulocytosis in the blood. Thereafter, the number of mature granulocytes decreased and then increased again at 6½ days following the same pattern of recovery as observed in the blood of the B rats. The number of granulocytic precursors showed a rise at 5½ days. Thus the bone marrow analysis as well as that of the blood showed that the B rats were producing granulocytes after 5½ days postirradiation. Erythropoiesis was first evident at 6½ days and erythroid precursors increased in number thereafter. Both erythrocytic and granulocytic clones were seen in tissue sections at intervals greater than 6½ days (Fig. 7). The number of lymphocytes remained small throughout the 8½ day period and there was no significant lymphoid recovery in the marrow. A small percentage of hemocytoblasts was seen and there was a significant increase in this
cell type at 5½ days just prior to erythroid recovery. The monocytoid cells, which comprise 2–3 per cent of the cells in the normal rat marrow, represented 30 per cent of the cells in the irradiated marrow between 3½ and 5½ days. These cells were not seen in the marrow of lethally irradiated rats given no protection.

**Labeling Patterns in Marrow.** The experiments were not designed to label every new cell formed in the A marrows. Since the A rats did not receive TTH until 12 hours postirradiation and thereafter only once every 24 hours, it is probable that many unlabeled stem cells crossed to the B rats during the early postirradiation period giving rise to unlabeled progeny. However, the radioautographs of the A marrows did show that by 4½ days 100 per cent of the nucleated erythroid, granulocytic, lymphocytic, monocytoid and blast cells were labeled with more than 50 grains/cell (average grain count > 100 grains). This high intensity of label over all cells formed in the A marrow after 4½ days made it possible to distinguish 2 groups of cells in the marrow of B
Fig. 7.—Radioautographs of one-micron sections of bone marrow from nonshielded parabiont 7½ days postirradiation. A: small erythrocytic clone and a labeled immigrant cell. B: granulocytic clone. × 1440.

Fig. 8.—Radioautographs of cells in bone marrow of nonshielded parabiont 6½ days postirradiation. A: labeled and nonlabeled monocytoid cells above and weakly labeled erythroblast below. B: heavily labeled monocytoid cell in division. × 1440.

by virtue of relative labeling intensities. One group had more than 50 grains/cell and was considered as direct immigrants from the marrow of A. The other group having 12–25 grains/cell was considered as a division product of the labeled immigrant cells.

The heavily labeled group of cells in the marrow of the B animal contained 3 types of cells only: mature granulocytes, small lymphocytes and monocytoid cells. Of these, the monocytoid cells were by far the most prevalent cell type
Fig. 9.—Radioautographs of blast cells in the marrow of the nonshielded parabiont at 6½ days postirradiation. A and B: labeled myeloblasts. C: early erythroblast. D: erythroblastic islet. × 1440.

which migrated into the B marrow. This was especially evident in the early postirradiation intervals when more than 90 per cent of the labeled cells were monocytoid. It was the only one of the 3 cell types showing both weak and heavy label during the later postirradiation intervals (5½–8½ days) indicating the capacity to divide and to produce cells of the same morphology through several generations (Fig. 8).

Labeled hemocytoblasts, pronormoblasts and myeloblasts were seen in the B marrow at intervals after 5½ days (Fig. 9). These showed 9 to 15 grains/cell and are believed to be products of the second or third division of labeled stem cells which migrated into the marrow from the A animals. No heavily labeled (>50 grains) blast cells were observed in the B marrow.

Weakly labeled phagocytic reticular cells were also seen in the marrow of B rats. Shortly after irradiation (24–48 hours) the marrow cavity was filled with
erythrocytes and the tissue sections showed hemorrhage into the marrow par-
enchyma. At 3¼ to 4½ days labeled phagocytic cells (monocytes) were ob-
served with phagocytized red cells in their cytoplasm. At 6½ to 8½ days some
of the phagocytic reticular cells in the center of the erythroblastic islets were
labeled with 15–20 grains. This sequence of events suggested that the blood
monocyte may be the precursor of the phagocytic reticular cells within the
erthroblastic islets and it is in accord with the work of Bessis, suggesting that
these reticular cells play an important role in iron metabolism by reutilizing
red cells.

An alternative explanation for the labeled reticular cells as well as the blast
cells is the reutilization of labeled DNA. Previous experiments have shown that
reutilization of DNA does occur within the marrow. However, the labeling
of cells due to reutilization is much the same as that seen in marrows exposed
to continuous low levels of TTH; i.e., the intensity of grain count over the cells
has no correlation with the level of cell maturation and 100 per cent of the
rapidly proliferating cells are weakly labeled. In contrast, the early blast cells
of the parabiotic rats were either labeled with 9–15 grains or not at all. Also,
the more primitive forms in both the erythrocytic and granulocytic series had
the highest grain count and this grain count decreased with the level of
maturation. If reutilization were the explanation for the labeled reticular and
blast cells (15–20 grains/cell), it would need to be a highly specific process in
which the reticular cell retained at least 1/4 of the label from a phagocytized
cell. Such a highly specific process for reutilization seems extremely unlikely.

**Thymus**

*At 1½ days postirradiation* extensive cell destruction was evident in the
thymus of both A and B rats. Cell debris was seen in the phagocytic reticular
cells and the macrophages. Approximately 99 per cent of the cells in smear and
tissue sections were cells of the fixed reticular network and free macrophages
at 2½, 3½ and 4½ days postirradiation as observed in both the A and B rats. A
few small lymphocytes were seen and these were not labeled in either
animal. The only labeled cells in the thymus of B were of the monocytoid type.
Monocytoid cells and a small percentage (<1 per cent) of reticular cells were
labeled in the thymus of A animals. Large lymphocytes first appeared in the
thymus of both rats at 5½ days; they were all labeled in the A rat but none
was labeled in the B rat, indicating that they were formed within the respec-
tive thymus of each animal. Since none of these large cells was labeled in B, it
is evident that their immediate precursors were not labeled. Good production
of small lymphocytes was evident in the thymus of both rats by 8½ days.
More than 99 per cent of the lymphoid cells in the thymuses of A rats were
labeled and in sharp contrast the thymus of B showed no labeled lymphoid
cells. Because of this, the thymus served as a good control showing that there
was no leakage of TTH into the B rat nor widespread reutilization of label.
The only labeled cells found in the thymus of the B rats at any time were small
percentages of monocytoid and phagocytic reticular cells. These phagocytic
reticular cells had about ½ the grain count of the monocytoid cells. The grains
were always directly over the nucleus and no phagocytized labeled material
was ever seen in the cytoplasm. Thus it is suggestive that the labeled reticular cells were derived from the phagocytic cells (monocytes) of the monocytoid group.

**Mesenteric Lymph Nodes**

At 1½, 2½ and 3½ days postirradiation, more than 99 per cent of the cells in the mesenteric node of both rats were those of the fixed reticular network or mature plasma cells. None of these was labeled in the B rats and only a small percentage (<1 per cent) of reticular cells incorporated TTH in the A animals. The only labeled cells which were found in the B nodes at these intervals were monocytoid cells. Some nonlabeled (<1 per cent) small lymphocytes were seen in both rats throughout this time. At 4½ days an increase in the number of lymphoid cells was seen in both rats. The nodes of both animals showed essentially equal rates of recovery between 4½ and 8½ days. At 5½, 6½, 7½ and 8½ days, 100 per cent of the blast cells in the A nodes were labeled with more than 50 grains, while essentially 100 per cent of the blast cells of the B nodes were nonlabeled at these same intervals. Labeled small lymphocytes were observed in the nodes of both rats at 6½ days with approximately 50 per cent labeled in A and 20 per cent labeled in B. It is, therefore, believed that all these labeled small cells were produced in A and that there was a continuous exchange of small lymphocytes by means of the cross circulation. The plasma cells were highly labeled in the A rats between 5½ and 8½ days (95 per cent with more than 50 grains). A small percentage of the plasma cells in the B nodes had 5 to 8 grains. No labeled plasma cells with 50 grains were seen in the B rats. Thus it appears that some labeled plasma cell precursors had migrated from the A to the B animals to give rise to the weakly labeled cells.

**Spleen**

More than 99 per cent of the cells present in the spleen of both A and B rats were a part of the fixed reticular network at 1½, 2½, 3½ and 4½ days postirradiation. A small percentage of the reticular cells were labeled in the A rats (1–3 per cent). None was labeled at these intervals in the B group. The only labeled cells found in the spleen of the B animals at 1½, 2½, and 3½ days were monocytoid cells. Labeled small lymphocytes were seen at intervals after 4½ days. Labeled reticular cells were also found in B rats after 4½ days and the grain count was the same as that of the early monocytoid cells. In contrast to the mesenteric node and thymus, labeled blast cells with basophilic cytoplasm were seen in the spleen of the B rats after 5½ days. They were either direct immigrants from A or products of perhaps one division, since they were heavily labeled. No erythroid or granulocytic development was evident in the spleen of either rat at 8½ days.

**Further Studies Using Parabiotic Rats**

A second group of parabiotic rats was used in order to facilitate the above studies for cell estimation and to provide additional information on the mono-
cytoid cells. This group was also given 1000 r while shielding the tibial regions of the A rats. One parabiotic pair was sacrificed each day between 1\(\frac{1}{2}\) and 8\(\frac{1}{2}\) days postirradiation as in the previous series, but none of this group received TTH. At the time of sacrifice the blood and bone marrow from each rat were exposed to TTH in vitro at a concentration of 1 \(\mu\)c./ml. of serum for 15 minutes in order to label the cells in DNA synthesis. These studies showed that the only cell type in the blood of the parabiotic rats during the early postirradiation interval (1\(\frac{1}{2}\)-4\(\frac{1}{2}\) days) which incorporated TTH was the monocytoid cell. Other cell types normally found in blood\(^7\) and capable of TTH incorporation, such as the medium and large lymphocytes, large basophilic blast cells, and "basket cells," were not found in the blood until after 4\(\frac{1}{2}\) days. Approximately 1–3 per cent of the monocytoid cells in the blood of the parabionts were in DNA synthesis. From 40–60 per cent of the monocytoid cells in the bone marrow from the B rats of this group were in DNA synthesis.

**DISCUSSION**

The term "stem cell" is applied to a cell having the capacity for extensive proliferation resulting in renewal of its own kind as well as giving rise to fully differentiated cells.\(^5,48\) In accord with this definition irradiation studies have shown that hematopoietic stem cells are found in bone marrow,\(^4,16\) spleen\(^27,38\) and blood.\(^44\) Cells from these sources can recolonize the myeloid as well as the lymphoid organs. The experiments of Berman and Kaplan\(^5\) suggest that the marrow may contain separate stem cells for the lymphoid and myeloid elements. These investigators found that marrow pretreated with Thio-Tepa or urethan was ineffective in promoting thymic recovery, while such pretreatment did not diminish its ability to protect against irradiation death usually caused by anemia and hemorrhage. It would appear, however, that the erythroid, granulocytic and megakaryocytic series may be derived from one common stem cell.\(^4,30\)

In the present experiments, the bone marrow shielding did promote thymic and node regeneration as reported by others.\(^17,19,23,27,26\) Although labeled monocytoid cells did appear in the thymus and node of the nonshielded animal, there was no label observed in the large lymphocytes of either organ. However, the inability to find label in the large lymphocytes of thymus and node may mean that the shielded marrow contributed stem cells during the early postirradiation period when a large percentage of the marrow cells were not labeled, or that labeled stem cells underwent so many divisions before giving rise to a detectable number of large lymphocytes that the radioactivity was diluted below the level of radioautographic detection.

Since labeled large lymphocyte-like cells did appear in the spleen of the nonshielded animal at the late postirradiation intervals (after 5\(\frac{1}{2}\) days), it seems reasonable to assume that the majority of those seen in the blood at these same times were destined for the spleen.

As lymphocyte recovery became evident in the blood, spleen and nodes of the parabionts, there was an exchange of small lymphocytes between the 2 animals. This observation is in accord with an extensive recirculation of small
lymphocytes\textsuperscript{15,19} and with the experiments of Harris et al.,\textsuperscript{24} who found that the exchange of cells in parabionts was greatest in the spleen and lymph nodes, less in thymus and least in bone marrow.

With respect to myeloid recovery, it is significant that labeled pronormoblasts, basophilic erythroblasts and myeloblasts were seen in the marrow of the nonshielded animals. This observation shows that it is possible to use TTH and radioautography in order to follow the products of stem cell division through cell differentiation. The pronormoblasts and myeloblasts encountered had grain counts which indicate that they were the second and third division products of labeled immigrants and were not derived by direct transformation of stem cells. In view of the time lapse before the first committed blast cells appeared at 5\textsuperscript{1/2} days postirradiation and the need for renewal in the stem cell compartment, it is not surprising that several divisions would occur between stem and blast—especially under the conditions of irradiation recovery. Direct transformation from stem to blast may be more common in the nonstimulated marrow where there is no need to expand the size of the stem cell compartment.

The sequence in which labeled cells appeared in the blood and bone marrow of the parabionts as well as the recovery of granulopoiesis and erythropoiesis in the marrow of the nonshielded rats established that the cells responsible for this recovery had their origin in the shielded marrow. The only morphologically distinct white cell types which left the shielded marrow and entered the blood during the early postirradiation period were mature granulocytes, small lymphocytes and monocytoid cells. It may be assumed, then, that the only possible cells types to be considered as stem cell precursors for the erythrocytic and granulocytic series in these experiments were small lymphocytes and monocytoid cells. The following observations suggest that it was cells of the monocytoid category which served as stem cells for both the erythrocytic and granulocytic cell lines.

1. They were the first and by far the major cell type crossing from the marrow of the shielded animal to the marrow of the nonshielded rat.

2. They evidenced the ability to divide and to reproduce themselves through several generations, as judged by the observation that cells of the same morphology but with reduced grain counts were encountered in the recovering B marrow at late postirradiation intervals. This ability for continuous self renewal is a requirement of a stem cell compartment.\textsuperscript{48}

3. In the parabiotic rats sacrificed at early postirradiation intervals, the monocytoid cells were the only cells found in the blood which incorporated TTH. Approximately 1 to 3 per cent of those in blood were in DNA synthesis. It has previously been reported that cells approaching the monocytic series in appearance and found circulating in the human peripheral blood incorporate TTH.\textsuperscript{7}

4. Cells with morphologic characteristics intermediate between the monocytoid cells and the committed blasts were common in the recovering marrow. It would be presumptive to place too much emphasis on these “intermediate forms,” since the boundaries between cell types are rarely clearly defined,
especially in the immature cells. However, when taken into consideration with the other evidence, such as the labeling patterns of the cells involved, the presence of intermediate forms becomes more significant. In this regard, it may be said that the per cent label, grain count, and time sequence with which these “intermediate forms” appear fit well with that which would be expected if these cells were intermediate between monocytoid cells and differentiated blast cells.

5. Tissue sections and imprints showed that the monocytoid cells were sometimes present within the erythrocytic and granulocytic clones which developed in the recovering marrow of the nonshielded parabiont. No small lymphocytes were seen within these clones. This observation is in agreement with the reports of Mekori and Feldman, who found no lymphoid elements in the intrasplenic myeloid colonies developing in irradiated marrow of transfused mice. These myeloid colonies have been shown to contain new colony-forming units.

6. The monocytoid cells were radiosensitive and were not present in the blood or marrow of lethally irradiated controls. Hemopoietic stem cells are known to be highly radiosensitive; 150 r destroys 90 per cent of them. These results would appear to be in conflict with those of Cudkowicz et al. and those of Thomas et al. who have presented evidence implicating small lymphocytes of the marrow as myeloid stem cells. Both of these laboratories reported that the “lymphoid” cells which they believed to be stem cells incorporated TH. In the present experiments, no cells which were morphologically distinctive as small lymphocytes incorporated TH. In the rat and guinea pig marrow the small lymphocyte is approximately 5 to 7 μ in nuclear diameter, and it has a small amount of basophilic cytoplasm and dense nuclear chromatin. Slightly larger lymphoid cell 8–10 μ in nuclear diameter with less dense chromatin and a narrow rim of deep blue cytoplasm are also seen in rat and guinea pig marrow, and these do incorporate TH. These are the “transitional cells” of Yoffey. Recent work shows that in both the rat and guinea pig small lymphocytes are formed in the marrow and that these larger lymphoid (“transitional”) cells are probably their precursors. The present study provided no evidence that the small marrow lymphocytes (as defined above) or “transitional cells” of Yoffey gave rise to the myeloid elements, although a relatively small number of small lymphocytes (as compared to the monocytoid cells) did migrate from the shielded to the nonshielded marrow.

Thus it seems that part of the disagreement with regard to the identification of stem cells may be the morphologic definition of the cell types, together with the possibility that cell morphology may vary slightly between the different species which have been used (rat, mouse and dog). The cells which were identified in the present study as monocytoid cells were approximately the size of a medium lymphocyte (8–10 μ in nuclear diameter). The majority have an irregular-shaped nucleus with many invaginations and folds, while the nucleus of the lymphocytic series tends to be round. The nucleus is usually located centrally while the nucleus of the lymphocytic series is more eccentric. The chromatin does not form dense masses as in the small lymphocytes. The cyto-
plasm is not as basophilic as in the lymphocytic series and it is much more abundant than that seen in the majority of medium to large lymphocytes. These monocytoid cells do have some morphologic characteristics in common with the activated, long-lived, recirculating, small lymphocytes of thoracic duct lymph and nodes which appear in response to immunologic stimuli, and this is the only lymphoid cell over which confusion might arise. The long-lived small lymphocytes are not found in bone marrow, and transfusion experiments show that they have no capacity for repopulating depleted myeloid elements. Also, numerous irradiation experiments have shown that shielding node, thymus or Peyer’s patches, or in fact transfusion of lymphocytes from thoracic duct lymph, node or thymus does not promote myeloid recovery. Furthermore, since Cudkowicz et al. have emphasized that the small "lymphocyte," which they have proposed as a stem cell, is found only in myeloid tissue and even then is not representative of the majority of the lymphoid population in the marrow, it would seem more appropriate to refer to the myeloid stem cell by a name other than "lymphocyte." To judge a cell type on morphologic characteristics alone without considering its function, origin or relationship to other cells seems inadequate and adds confusion to an already complicated problem. The use of such loosely drawn morphologic definitions does not allow an investigator to express the data accurately without numerous qualifying statements.

In regard to the use of the term "monocytoid cell," it should be emphasized that the term is not synonymous with monocyte. The term "monocyte" is usually restricted to a cell with phagocytic capacity. Approximately one-half of the monocytoid cells observed in the blood of these parabionts displayed no phagocytic activity when exposed to trypan blue or india ink. It seems reasonable to believe that these blood monocytes were the precursors of the labeled macrophages and phagocytic reticulum cells found in the thymus, node, spleen and bone marrow of the nonshielded animal, since tissue culture experiments have shown that blood monocytes can transform into macrophages and fibroblast-like cells. On the other hand, the stem cell for the erythroid and granulocytic series is most probably a nonphagocytic cell and may bear no functional nor developmental relationship to monocytes.

SUMMARY

These radioautographic studies using parabiotic rats and partial marrow shielding showed that cells responsible for recovery of irradiated bone marrow had their origin in the shielded marrow. Three morphologically distinct cell types appeared in the blood of these parabionts, mature granulocytes, small lymphocytes and monocytoid cells. The monocytoid was the major cell type which crossed from the shielded to nonshielded marrow, and the observations suggested that it is this cell which served as a stem cell for both the erythrocytic and granulocytic cell lines.

Labeled erythroblasts and myeloblasts were observed in the recovering marrow, and the labeling intensity of these cells indicated that they were the second or third division products of labeled immigrant cells.
The effect of marrow shielding upon the recovery of lymphopoiesis in spleen, thymus, lymph nodes and bone marrow is also discussed.

**SUMMARIO IN INTERLINGUA**

Iste studios radioautographic utilisante rattos parabiotic con partial protection del medulla ha demonstrate que le cellulas responsabile pro le restablimento de irradiate medulla ossee ha lor origine in le medulla sub protection. Tres morphologicamente distincte typos cellular appareva in le sanguine del parabiontes, i.e., granulocytos matur, micre lymphocytos, e cellulas monocytoide. Iste ultime esseva le major typo cellular transient ab le protegite ad le nonprotegite medulla, e le observationes suggestiona que il es iste cellulas que serviva como cellulas primordial tanto pro le linea erythrocytic como etiam pro le linea granulocytic.

Marcate erythroblastos e myeloblastos eseva observate in le medulla in stato de restablimento, e le intensitate del marçage de iste cellulas indicava que illos eseva le producto del secunde o del terti division de marcate cellulas immigrante.

Le efecto del protection del medulla super le restablimento del lympho-poiese in le splen, le thymo, le nodos lymphatic, e le medulla ossee es etiam commentate.

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889


A Radioautographic Study of Hemopoietic Repopulation Using Irradiated Parabiotic Rats: Relation to the Stem Cell Problem

RUTH W. CAFFREY TYLER and N. B. EVERETT