Radioautographic Study of Cellular Migration Using Parabiotic Rats

By Ruth W. Tyler and N. B. Everett

Leukocyte exchange between the hemopoietic tissues of parabiotic rats was studied subsequent to giving multiple injections of 3H-thymidine to one member of each pair while arresting the cross-circulation. Cell types that migrated from one parabiont to the other were segmented granulocytes, small, medium and large lymphocytes, immunoblasts, monocytoïd cells, macrophages or their immediate precursors, and plasma cells. Evidence for the transformation of circulating cells to other cell types was rarely seen. The long-lived small lymphocytes were equilibrated between parabionts, suggesting that this is a single pool of cells with respect to kinetic behavior and recirculation. There was no evidence for a trephocytic function of lymphocytes. A small number of bone marrow lymphocytes coursed directly to lymph nodes and spleen. Evidence is given for a limited recirculation of short-lived lymphocytes of thoracic duct lymph (TDL), as well as for long-lived cells. Only a few immunoblasts of TDL recirculated. The majority of cells that entered the white pulp of the spleen were long-lived small lymphocytes, while the majority of immigrant cells to the red pulp were monocytoïd cells and granulocytes. Many small lymphocytes originated in splenic red pulp and entered the blood. No immigrant cells to the thymic cortex were noted, although some small lymphocytes and monocytoïd cells entered the medullary areas. Immigrant cells to the bone marrow (less than 2% of the cells in marrow) included monocytoïd cells, small lymphocytes, and plasma cells. Evidence for the direct transformation of a circulating cell into a committed blast, based on reduction in grain count, was noted only in bone marrow.

A TRAFFIC OF LEUKOCYTES is maintained within tissues of the body via the blood and lymph. Some of the leukocytes apparently spend their entire life within the circulation, while others may use the blood and/or lymph to migrate from one hemopoietic tissue to another. Experiments of the past decade have emphasized the importance of cellular migration in the development and maintenance of a normal immunologic system. It also has been shown that cells capable of reseeding the hemopoietic tissues of a lethally irradiated animal are present in the blood stream and migrate from one organ to another.

Outstanding among cellular traffic experiments are those of Ford and coworkers who have used the T6 chromosomal marker technique to measure...
the movement of cells capable of proliferation, and they or their progeny are detected when they subsequently enter mitosis. For the most part, the chromosomal marker experiments measure what happens after a prolonged period of time to rapidly proliferating cellular components and represent stem cell movement and exchange. In contrast, the radioautographic experiments reported here were designed to measure the exchange of both "stem" and "end" cells. Radioautography offers the advantage of a morphologic identification of migrating cells.

MATERIALS AND METHODS

A total of 12 pairs of Lewis parabionts were used in this study. An additional seven rats served as nonparabiotic controls. The parabionts were joined unilaterally as previously described, and the 3H-thymidine injections were begun at 2 wk or more following the surgery. The A member of each pair received repeated injections of 3H-thymidine (TTH). Immediately prior to each TTH injection, which was given intraperitoneally to A, the cross-circulation to the B member was arrested by compression. At 15 min post-TTH, the B rat was given a large dose (5 mg) of nonradioactive thymidine intraperitoneally, and the cross-circulation was restored.

For ease of discussion, the animals are divided into three groups. In the first group (1), the A rats received frequent injections over a short period of time (32-56 hr) in order to label a large percentage of rapidly proliferating cells. These parabionts were then sacrificed shortly after the last injection so that the labeled cells seen in the B members represented migration of short-lived cells. In the second group (2), the A rats received one injection of TTH daily for 5-12 days in order to label a significant percentage of both rapidly proliferating, as well as slowly proliferating, cells. These were sacrificed within 24 hr of the last injection, and the labeled cells seen in B rats represented migration of both long- and short-lived cells. In the third group (3), the A rats also received multiple injections of TTH over a long period of time (12 days), but members of this group were all sacrificed at 2 wk or more after the last injection. Thus labeled cells in the A rats represented long-lived cells, and those in the B members were long-lived immigrant cells.

Nonparabiotic controls (C rats) were given TTH injections at the same time as the A parabionts and sacrificed accordingly. They were used in a comparison to the A rats, and the difference in the percentage of labeled cells seen in A and C represented migration of nonlabeled cells from B to A. These cells were the same types as those labeled cells migrating into the B rats from the A members.

Radioautographs of smear preparations of thoracic duct lymph, blood, spleen, thymus, mesenteric lymph node, and bone marrow were made as previously described. One set of slides was exposed for 4 wk and the other for 8 wk. One micron sections were made from Zenker-fixed tissues and processed for radioautography as previously described by a method recently developed in this laboratory. The tissue sections were exposed 8 wk for assessing the types and locations of immigrant cells and 6 mo to detect their more weakly labeled division products.

RESULTS AND DISCUSSIONS

Reutilization

In experiments of this type it is of primary importance to ascertain whether effective occlusion was maintained between the parabionts at the time of the TTH injection to A members and to consider the possibility that labeling in the B members could be due to reutilization of 3H-thymidine. The fact that reutilization of 3H-thymidine does occur is well documented. It apparently can occur both on a widespread tissue basis and locally within confined tissue compartments. In widespread reutilization, the DNA is degraded into nucleosides and...
nucleotides that may enter the body fluids and effect a low degree of labeling in all rapidly proliferating cells. A check on widespread reutilization was easily made by examining a large variety of tissues and cell types. As an example, the intestinal epithelium of all A rats that were sacrificed within 24 hr of the last TTH injection was heavily labeled, while that of the B members showed no evidence of radioactivity even in sections that were exposed for 6 mo (Fig. 1). Other tissues that served as controls were liver, kidney, lung, and testis.

In the case of local reutilization, the radioactivity of $^3$H-DNA is apparently only made available within the tissue in which it is degraded. Thus, it is conceivable that a labeled cell from an A rat could "home" to a tissue of the B member, and after degeneration its labeled nucleotides would be available to proliferating cells at the site of degradation. However, the following observations indicated that such local reutilization did not occur to a detectable degree in the present experiments: the average grain counts of more than 99% of all labeled cells seen within the B rats were the same as those in the corresponding A rats, and the cells were heavily labeled (more than 25 grains per cell in smear preparations); the heavily labeled cells were types that do not normally incorporate $^3$H-thymidine and are known to enter circulation, i.e., small lymphocytes, mature granulocytes, and monocytes; and the small percentage (<1%) of labeled cells with one-half to one-eighth the average grain count of circulating cells that were found with the tissues of the B rats were all primitive in their morphologic appearance. In contrast, no proliferating cells toward the end of a morphologic sequence, such as the metamyelocyte or basophilic erythroblast, were labeled in the tissues of the B rats. These observations leave little doubt that all labeled cells encountered in the B rats were either immigrant cells or division products of immigrant cells. The grain counts over immigrant cells were adequate in most cases to allow for detecting labeled descendants for approximately four generations, provided the radioautographs of smear preparations were exposed for 8 wk or those from 1-m tissue sections for 6 mo.

Fig. 1. Radioautographs of intestinal epithelium from rats joined in parabiosis. (A) received 12 daily injections of TTH; (B) is from control member.
Morphology and Kinetics of Migrating Cells

The only cell types that were found to be heavily labeled in the tissues of the B parabionts were the same as those known to circulate within the bloodstream. They were classified in smears and tissue sections as belonging to one of seven cell types: segmented granulocytes, small lymphocytes, medium to large lymphocytes, immunoblasts, monocytoid cells, macrophages, and mature plasma cells. The morphology and kinetic behavior of these cell types is summarized briefly below.

**Segmented Granulocytes:** In the rat the majority of the granulocytes are neutrophils; a small percentage are eosinophils. The first labeled neutrophils were seen in the blood of control rats sacrificed at 36 hr post-3H-thymidine. There was a significant variation between animals in the rate of increase of labeled cells with time, which was probably dependent upon the granulocyte reserves. In control rats that had received multiple injections of 3H-thymidine over a 56-hr period, approximately 97% of the circulating granulocytes were labeled.

**Small Lymphocytes:** Cells with a nuclear diameter of 7 μ or less were classified as small. They did not incorporate 3H-thymidine, and the first labeled cells appeared in blood and lymph at approximately 4 hr post-TTH. Circulating small lymphocytes have been divided into two classes with respect to life span. In the rat the short-lived population has been shown to comprise approximately one-third of those in blood and has a circulating life span of 4–5 days. The long-lived small lymphocytes that comprise the remaining two-thirds of the cells in blood recirculate from blood to lymph, and some remain labeled as long as 1 yr post-TTH.

**Medium Lymphocytes:** Medium lymphocytes were defined as cells with a nuclear diameter of more than 7 μ but less than 10 μ. A small percentage of these cells incorporated 3H-thymidine. However, all of them were not labeled even after 11 days of multiple TH injections, and a small percentage may be labeled at 2 wk or more post-TTH. They may arise from either the hypertrophy of small lymphocytes or by the division of larger cells, and therefore they represent a mixed category with respect to their kinetics.

**Immunoblasts:** This category is composed of cells with a nuclear diameter of more than 10 μ and a distinctly basophilic cytoplasm and includes precursors to both the lymphocytic and plasmacytic series. Although one cannot distinguish between these precursors in smear preparations, ultrastructural studies have shown that some of them contain a well-developed rough endoplasmic reticulum and are usually referred to as plasmablasts. Others have many free ribosomes found in clusters with little or no rough endoplasmic reticulum and are known as “large pyroninophilic cells.” Both cell types enter the blood via the lymph. Essentially 100% of this cell type was labeled in all tissues except thymus of control rats given TTH for 36 hr. The average generation time for the large pyroninophilic cells has been calculated to be 12 hr, while that of the plasmablast is approximately 9 hr.

**Monocytoid cells:** This category includes all cells with the morphology of the monocyte. Evidence has been presented in previous studies that a stem
cell that is capable of repopulating lethally irradiated rats belongs to this morphologic class. However, it should be realized that in the present study the great majority (more than 99%) of these cells that were observed were probably true monocytes and were not capable of differentiating into any other cell line except the macrophage. A small percentage of the monocytoid cells of normal human blood incorporate TTH \(^{24}\) and a larger percentage of those in blood of irradiated marrow-shielded rats are in DNA synthesis.\(^{11}\) Whitelaw\(^ {25}\) studied the turnover time of rat monocytes that he defined as peroxidase-positive mononuclear cells. He reported that 25% of those in blood were labeled after 1 day of frequent TTH injections, and that 82% were labeled by 8 days of injections. Both labeled and unlabeled monocytes disappeared from the circulation as an exponential function with a half time of 3 days. Our studies are in agreement with those of Whitelaw in that they show that the monocytoid cells have a slower turnover time than the short-lived lymphocytes, blast cells, or granulocytes of blood, although none of them was labeled in the blood of rats sacrificed at periods exceeding 14 days post-TTH. More than 99% were labeled in the blood of animals receiving TTH for 11 days, thus they were not long-lived as compared to recirculating small lymphocytes.

**Macrophages:** Although macrophages or histiocytes are rarely seen circulating within the blood of normal animals, in pathologic conditions they are often encountered. It has been shown that the majority of macrophages in subcutaneous exudates are derived from blood monocytes that originate in the bone marrow.\(^ {26}\) However, the source of the majority of free macrophages seen in other tissues has not been established (Roser\(^ {27}\)). Occasionally in B parabionts heavily labeled macrophages were encountered in the spleen, mesenteric node, or bone marrow. No labeled macrophages were seen in the B rats sacrificed at 2 wk or more post-TTH, although they were present in the A rats. It is to be noted, however, that these animals were not stimulated for macrophage production.

**Mature Plasma Cells:** As in the case of macrophages, mature plasma cells are not normally encountered in blood. In certain diseased states, however, they may constitute a large percentage of the cells present. The plasma cells of the mesenteric lymph nodes and spleen have a rapid turnover rate, and 100% of them can be labeled in rats by 5 days of \(^{3}H\)-thymidine injections.\(^ {20}\) Miller,\(^ {28}\) however, has reported some long-lived plasma cells within the popliteal lymph nodes of rats. It has been routinely observed in our laboratory that rats sacrificed at periods ranging from 2 wk to 6 mo post-TTH have labeled plasma cells in the bone marrow and occasionally in the thymus, although the same animals showed no labeled plasma cells in the mesenteric lymph nodes or spleen. The evidence from the parabiotic rats indicates that the mature plasma cells in bone marrow and thymus are not only long-lived but that they are also immigrant cells to these organs.

More than 99% of all labeled cells (both weakly and heavily labeled) observed within the tissues of the B parabionts belonged to one of these seven morphologic cell types. In view of the fact that the B rats had a large percentage (approximately 25%) of all types of circulating cells labeled in some members
Table 1. Percentages of Labeled Cells in Blood and Lymph (Smears) of Parabionts and Controls

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Injection Schedule</th>
<th>Time Post-TTH (hr)</th>
<th>Blood</th>
<th>Thoracic Duct Lymph</th>
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</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1012A</td>
<td>6 inj/2 days</td>
<td>2</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>0.8</td>
<td>39</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>14</td>
<td>0.5</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>912A</td>
<td>3 inj/3 days</td>
<td>24</td>
<td>4.5</td>
<td>3.1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>3.0</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>5.6</td>
<td>41</td>
</tr>
<tr>
<td>914A</td>
<td>5 inj/5 days</td>
<td>24</td>
<td>9.5</td>
<td>5.5</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>4.8</td>
<td>26</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>7.8</td>
<td>97</td>
</tr>
<tr>
<td>916A</td>
<td>8 inj/8 days</td>
<td>24</td>
<td>16.2</td>
<td>6.9</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
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<td>65</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>28.0</td>
<td>100</td>
</tr>
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</table>

for periods of 2 wk or more, it seems highly unlikely that a large number of circulating cells can give rise to any other cell type in the hemopoietic tissues by direct transformation not accompanied by many subsequent divisions. This observation would also seem to repudiate the theory advocating that the primary function of a circulating cell (i.e., lymphocyte) is trephocytic in the sense that it donates its DNA to be used on a large scale by other proliferating cells.29-33

More weakly labeled cell types that had one-half to one-eighth the grain count of immigrant cells in the B parabionts and therefore represented division products included the following: immunoblasts in the spleen, lymph nodes, and Peyer’s patches; monocytoid cells in the nodes, spleen and bone marrow; macrophages in the nodes, spleen, and bone marrow; and large primitive cells in bone marrow that appeared to be primitive blasts of the erythroid, granulocytic, or megakaryocytic series.

Extent of Cross-Circulation in Parabionts

The percentages of labeled cells in the blood and thoracic duct lymph of parabionts sacrificed within 24 hr of the last TTH injection (groups 1 and 2) are recorded in Table 1. From the data for blood, it is obvious that leukocytes of the respective parabiotic members were not equilibrated through the cross-circulation. In all cases the percentage of labeled small lymphocytes, granulocytes, or monocytoid cells was significantly higher in the blood of the A rat than in the corresponding B member. Experiments in which $^{59}$Fe-labeled erythrocytes were injected into the blood stream of one parabiotic rat showed that the radioactivity was completely equilibrated between the two rats within a few hours. Thus the reason for this nonequilibration of leukocytes probably relates to their rates of entry and exit from the blood stream and tissues and to their short circulating life for same. However, by comparing the percentages

\[
\text{Blood} \quad \text{Thoracic Duct Lymph}
\]

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Injection Schedule</th>
<th>Time Post-TTH (hr)</th>
<th>Small Lymphocytes</th>
<th>Granulocytes</th>
<th>Monocytoid Cells</th>
<th>Small Lymphocytes</th>
<th>Immunoblasts</th>
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<tbody>
<tr>
<td>1012A</td>
<td>6 inj/2 days</td>
<td>2</td>
<td>2.5</td>
<td>70</td>
<td>38</td>
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<td>95</td>
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<td>39</td>
<td>14</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>912A</td>
<td>3 inj/3 days</td>
<td>24</td>
<td>4.5</td>
<td>54</td>
<td>72</td>
<td>3.1</td>
<td>96</td>
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<tr>
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<td>23</td>
<td>2.0</td>
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<tr>
<td>C</td>
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<td>5.6</td>
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<td>78</td>
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<td>914A</td>
<td>5 inj/5 days</td>
<td>24</td>
<td>9.5</td>
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<td>76</td>
<td>3.0</td>
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<td>4.8</td>
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<td>19</td>
<td>2.3</td>
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<tr>
<td>C</td>
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<td>7.8</td>
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<td>100</td>
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<td>100</td>
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<tr>
<td>916A</td>
<td>8 inj/8 days</td>
<td>24</td>
<td>16.2</td>
<td>63</td>
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<td>13.0</td>
<td>39</td>
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<td>C</td>
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</table>
of labeled granulocytes and monocytes in the A and B rats with those of the corresponding controls (C rats), it may be estimated that these cell types had approximately a 25%–30% chance of crossing from A to B or B to A. The average grain counts of all circulating cells found in B rats were the same as those of the corresponding cell type seen in the blood of A rats at all time intervals, and since no immediate precursors of the granulocytes were labeled in these B rats, it must be assumed that all labeled neutrophils were formed in the A member.

The percentage of labeled small lymphocytes was also higher in the blood of the A members than in the corresponding B rats. In contrast, the percentage of labeled small lymphocytes in the thoracic duct lymph was approximately the same in all corresponding parabionts. This observation can only mean that the recirculating lymphocyte pools of the parabionts were completely equilibrated within 24 hr of their entry into the circulation. In group 1 in which the A rats received frequent injections of TTH over a short period of time, almost all of the labeled small lymphocytes appearing within the lymph would be of the short-lived variety. However, no significant differences were seen in the percentage of labeled small lymphocytes in TDL of A and B members. This observation shows that the short-lived small lymphocytes found in thoracic duct lymph also pass from blood to lymph and thus recirculate at least in this limited sense. The short circulating life span of these cells would limit their recirculation (lymph to blood to lymph). It remains to be determined if this limited recirculation has physiologic significance.

Approximately 95% of the immunoblasts in the thoracic duct lymph of the A rats in groups 1 and 2 were labeled, whereas 5% were labeled in the B members (Fig. 2). Controls showed 100% of this cell type labeled. This observation confirms previous studies that have indicated that the majority of immunoblasts of lymph do not recirculate but are formed in mesenteric nodes or Peyer’s patches and enter the blood via the lymph.

The Long-lived Lymphocyte Pool

The major traffic of cells between these parabiotic rats was by far that of the long-lived small lymphocytes. The long-lived lymphocyte pool is defined here as that group of small lymphocytes that have a circulating life span of more than 2 wk. We have presented evidence elsewhere that the long-lived small lymphocytes are a single group of cells with respect to their kinetics, circulation, and life cycle; however, the most direct evidence comes from these experiments with parabionts.

As stated above, the percentages of labeled small lymphocytes in the thoracic duct lymph of parabionts sacrificed within 24 hr of the last TTH injection were approximately the same. This observation was also the case for the third group of parabionts sacrificed at 2 wk or more post-TTH. Furthermore, in group 3, both the percentages and locations of labeled small lymphocytes were comparable within all tissue compartments of the respective members as well. These observations can only mean that the great majority of the long-lived small lymphocytes of lymph node, spleen, and Peyer’s patches had entered
### Table 2. Comparison of Percentages of Labeled Cells in Hemopoietic Tissues of Parabiotic Rats

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Injection Schedule</th>
<th>Time Post-TH</th>
<th>Smears</th>
<th>1 µ Tissue Sections</th>
<th>1 µ Tissue Sections</th>
<th>1 µ Tissue Sections</th>
<th>1 µ Tissue Sections</th>
<th>1 µ Tissue Sections</th>
<th>Bone Marrow</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blood</td>
<td>TDL</td>
<td>MLN Cortex</td>
<td>Red Pulp</td>
<td>White Pulp</td>
<td>Medulla</td>
<td>Cortex</td>
</tr>
<tr>
<td>Group 1</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>6.2</td>
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<td>4.2</td>
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<td>0.76</td>
<td>1.44</td>
<td>0.45</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1002A</td>
<td>12 inj/56 hr</td>
<td>4 hr</td>
<td>55.0</td>
<td>7.0</td>
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<td>919A</td>
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<td>% migration of labeled cells*</td>
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*Per cent migration of labeled cells equals per cent labeled in A/per cent in B × 100.
Fig. 2. Radioautographs of thoracic duct lymph from parabionts. (A) received three daily injections of TTH; lymph samples were collected from both members 24 hr after last injection. Note labeled blast and small lymphocyte. (B) Note nonlabeled blasts and labeled small lymphocyte.

circulation during the 2-wk post-TTH period and were a part of a single pool that was equilibrated by recirculation.

Extent and Type of Cellular Traffic Into Hemopoietic Organs

Information relating to the extent of cellular traffic into the various hemopoietic tissues is recorded in Table 2. The ratio of the percentage of labeled cells in the A rat divided by the percentage of labeled cells in the corresponding B member represents the fraction of labeled cells that are immigrant to that tissue, provided there is complete equilibration with the corresponding member. A ratio of one would mean that 100% of the labeled cells were migrating, as was the case for blood and lymph of rats sacrificed at 2 wk or more post-TTH. A low ratio shows that the labeled cells in the tissue are primarily of in situ origin. Examples of the latter are thymus cortex where less than 1 in 10,000 cells was labeled and bone marrow where less than 0.5% was labeled. However, in some cases it is probable that these ratios are smaller than they should be, since it was shown that the monocytes and granulocytes of the blood had only a 25–30% chance of crossing from one animal to another.

Lymph Nodes

Gowans and Knight showed that the major route of recirculation for long-lived lymphocytes is via the postcapillary venules of the mesenteric lymph nodes and Peyer’s patches. More specifically, they showed that labeled thoracic duct lymphocytes that were transfused to recipients penetrated the deep cortex of the mesenteric nodes and entered the medullary lymph sinuses and efferent lymphatics. Results of the present experiments indicate, however, that the long-lived recirculating small lymphocytes are by no means confined to this
Fig. 3. Radioautograph of mesenteric lymph node cortex from a B parabiont showing labeled small lymphocytes in cuff of cells surrounding germinal center (GC). (A) member of this pair had received six injections of TTH over a 12-day period.

route of recirculation. The data from the B rats, which were sacrificed at 2 wk or more post-TTH, are conclusive in showing that the majority of cells in the corona surrounding the germinal centers were a part of the long-lived recirculating pool and are immigrant to these areas (Fig. 3).38

Preliminary experiments in which Freund's complete adjuvant was injected into the footpads of parabionts showed that large numbers of long-lived small lymphocytes home to the popliteal node within the first 48 hr after stimulation and that they occupy the outer cortex. Later, they comprise the majority of small lymphocytes in the coronas surrounding germinal centers. This observation is of particular interest, since it suggests that this is the region where long-lived small lymphocytes most likely make contact with the antigen. It has been shown by electron microscopic studies that following an injection of labeled antigen, the antigen is retained on the surface of long branching processes of specialized dendritic reticular cells located in the cortical lymphoid follicles where it makes free contact with surrounding lymphocytes.39,40 Thus, it appears that recirculation serves to bring a large percentage of cells in the long-lived pool in contact with a local deposition of antigen.

The germinal center cells of A rats of groups 1 and 2 were essentially 100% labeled, whereas less than 1% of these cells were labeled in the corresponding B members. The labeled cells within the germinal centers of the B animal were often seen in clusters of two or three cells, were heavily labeled, and were either small or medium lymphocytes. Similar results have been previously reported by Austin.41 Results from local marrow labeling experiments have shown that these cells are immigrants from the bone marrow.37,42 A previous study15 showed that the migration of marrow lymphocytes to lymph nodes was more pronounced in irradiated parabiotic animals in which the A member had the marrow shielded. In single rats following 1000 R of total body irradiation.
tion, there was no evidence of lymphoid regeneration within spleen or lymph nodes during the first 8 days postirradiation. However, all rats of the irradiated parabiotic series had mitotically active lymphoid cells between 4 and 8 days postirradiation. The labeled cells of marrow origin observed in lymph nodes of the B member were confined to two regions: areas normally occupied by germinal centers, and areas in the deep cortex bordering the medullary cords where plasma cell proliferation was first evident in the border of the red and white pulp in clusters of large blast cells. These regions might be termed the marrow-dependent areas of spleen and nodes as opposed to the thymus-dependent areas described by Parrott et al.

The large pyroninophilic cells or immunoblasts distributed throughout the lymph node cortex were essentially 100% labeled in A rats of groups 1 and 2, whereas less than 1% of these cells was labeled in the corresponding B rats (Fig. 4). This observation confirms previous studies that showed the majority of these blasts are formed in situ and that they are derived primarily from proliferation of their own kind. Although it is well established that small lymphocytes can transform to large pyroninophilic cells, it should be emphasized that relatively few of the total population seem to respond to any one antigen and that the chances of observing transformation in vivo are small.

The plasma cells and their precursors found within the medullary cords of mesenteric nodes were essentially 100% labeled in all A rats receiving TTH for 5 days or more, whereas essentially none of this cell type was labeled in the corresponding nodes of the B rats (Fig. 5). It may be concluded that the majority of plasma cells and their precursors found in nodes are formed in situ and that any cellular transformation into this category by an immigrant cell is obscured by the extensive cellular proliferation that followed. Occasion-

Fig. 4. Radioautographs of mesenteric lymph nodes (corticomedullary region) of parabionts. (A) member received seven injections of TTH over a 32-hr period. Note labeled blast cells. (B) Note nonlabeled blast cells and two labeled small cells.
Fig. 5. Radioautographs of mesenteric lymph nodes (medullary cords) from parabionts. (A) member received one injection of TTH daily for 5 days prior to sacrifice. Note labeled plasma cells. (B, right) No plasma cells were labeled.

ally, weakly labeled immunoblasts in nodes (less than 5%) were present, and these most probably represented division products of immigrant cells.

The percentages of labeled small lymphocytes in the mesenteric lymph node smear preparations were approximately the same (differences of 1%-2%) for all corresponding A and B members irrespective of the post-TTH interval, and their average grain count was the same in the two animals. This observation indicates that the short-lived lymphocytes, as well as the long-lived cells of lymph nodes, are migrating cells. If they are the progeny of the large pyroninophilic cells distributed throughout the cortex, then they are rapidly equilibrated by recirculation. This observation raises the question of what, then, is the function and fate of the many immunoblasts observed in the node cortex if they are not producing significant numbers of small lymphocytes. Three possibilities exist. First, they divide by giving rise to several generations of cells of their own kind. This appears to be true and would account for one-half of their cell number every 12 hr (G.T.). Second, they leave the nodes by way of the lymph, migrating to other nodes and other tissues. The data from the percentage of labeled blasts in TDL of the respective members support this concept. Third, many of them die within the nodes where they are phagocytized by macrophages. The extent of labeled phagocytized debris in A rats suggests that cell death may be significant.

Small and medium lymphocytes accounted for more than 95% of all labeled cells found within the lymph nodes of the B rats. The remaining 5% were made up primarily of monocytoid cells found within the medullary or cortical sinuses.

Spleen

The radioautographs of the spleen tissue sections were analyzed with respect to the red and white pulp. The white pulp is composed primarily of
small lymphocytes with a small percentage of immunoblasts. A comparison of the percentages of these cells labeled between corresponding A and B rats confirmed that the small lymphocytes of the white pulp are a part of the long-lived recirculating pool.\textsuperscript{44} In contrast, the blast cells of the white pulp were essentially 100\% labeled in A, and approximately 10\% were labeled in the corresponding B member. This incidence was significantly more frequent than in mesenteric lymph nodes and would indicate that the spleen is the destination of many of the immunoblasts entering the blood from lymph nodes. This concept finds further support from transfusion experiments in which labeled immunoblasts obtained from MLN were found to home to the spleen of the recipients in greater numbers than to any other hemopoietic organs (unpublished results).

The majority (75\%-90\%) of the labeled cells found in the red pulp of spleens from B rats of groups 1 and 2 were monocytoid cells and segmented neutrophils. The remainder were made up of medium lymphocytes and the occasional immunoblast. Only rarely were typical small lymphocytes seen. This observation may relate to the route of circulation of small lymphocytes through the spleen\textsuperscript{45} in that the lymphocytes are removed from the blood and concentrated in the white pulp in such a way as to leave the granulocyte and monocytoid cells concentrated within the red pulp.

Although the splenic red pulp of B rats was greatly deficient in labeled small lymphocytes, that of A rats and of controls from Group 1 showed a significant number of labeled small lymphocytes. (Group 1 animals received multiple injections of TTH over a short interval of time.) The lymphocytes were found in clusters around venules, and some heavily labeled small lymphocytes were within these vessels (Fig. 6A). This observation is interpreted as evidence that the splenic red pulp contributes short-lived small lymphocytes directly to blood and is in accord with similar observations reported by others.\textsuperscript{46,47}
Further evidence that the spleen is producing significant numbers of short-lived lymphocytes came from an analysis of the percentage of labeled small lymphocytes in smear preparations. The differences in the percentages of labeled small lymphocytes in spleens of respective A and B members were large in comparison to the differences in the corresponding lymph nodes or lymph.

**Thymus**

The radioautographs of thymus sections were analyzed with respect to the medullary and cortical areas, since the lymphocytes in each of these compartments have a different proliferative behavior. In thymic sections of control rats, 50% of the thymocytes were labeled in the cortex by 36 hr of TTH injections, while only 6.5% of those in the medullary areas were labeled.

The thymic cortex of A members of groups 1 and 2 showed a high percentage of labeled cells, whereas essentially no labeled cells of any type were seen in the thymic cortex of the corresponding B members (Table 2). This observation in no way conflicts with the evidence that supports a stem cell migration from bone marrow into thymus,23,34 but it must be concluded that such a migration within the normal adult animal involves only a small number of cells that would be hidden within the extensive cellular proliferation that subsequently occurs. This proposal is in accord with chromosomal marker studies that show that it takes approximately 20 days for one-half of the mitotic cells in a transplanted thymus to be replaced by cells of recipient origin.48

In contrast to the thymus cortex, the medullary areas of thymus sections from B rats revealed a significant number of immigrant cells (Table 2). This migration was especially evident in rats of group 1 that received multiple injections of TTH over a short period of time (Fig. 6B). The labeled cells were not present in significant numbers at 2 wk or more post-TTH; thus it may be concluded that they are from a rapidly proliferating source and have a short life-span within the thymus. The immigrant cells consist of both monocytoid cells and small lymphocytes, and the immigrant small lymphocytes had a grain count of three to five times that of the majority of thymocytes. Thus, in observing the thymus sections or smears of the A rats or of controls, it was evident from the intensity of label that cells were immigrants. No labeled blast cells were seen, and the significance of this migration into the thymic medulla is not known. A migration of small lymphocytes into the thymic medullary area has also been reported by others.49

**Bone Marrow**

Counts of labeled cells in sections of bone marrow from the B parabionts sacrificed within 24 hr of the last TTH injection (group 1 and 2) showed less than 0.5% of the cells of bone marrow to be labeled and, therefore, immigrants. In contrast, a high percentage (74%–94%) of the bone marrow cells were labeled in the A members. Approximately 54%–75% of the immigrant cells in the B marrows were monocytoid cells, while 15%–30% were segmented neutrophils. Five to ten per cent of the labeled cells were small lymphocytes, but this represented only 2%–3% of the total marrow lympho-
cyte population. Approximately 2% of the labeled cells were mature plasma cells (Fig. 7A), and another 2% were medium lymphocytes.

The bone marrow of the A parabionts of group 3 (sacrificed at 2 wk or more post-TTH) had three cell types labeled (reticular cells, rosette center cells, and mature plasma cells). The corresponding B members showed only one cell type to be labeled (plasma cells); these were present in numbers comparable to those in the A rats, and they were heavily labeled. These experiments can only be interpreted as meaning that plasma cells of rat bone marrow are long lived and are immigrant to the marrow.

Preliminary transfusion experiments have been carried out in order to investigate the sources of the labeled cells migrating into marrow. The donors of the labeled cells were normal adult rats that had received $^{3}$H-thymidine for 7 days (two injections daily, $1\mu$/g). Recipients of labeled mesenteric lymph node cells showed that the only labeled cells in the bone marrow were plasma cells or their precursors. This indicated that lymph nodes served as a site of origin for immigrant plasma cells of bone marrow and is in agreement with the work of Chaperon et al.$^{5}$ who reported that some antibody-producing cells move from spleen to bone marrow and thymus following an antibody response.

Recipients of the labeled bone marrow cells showed the following cell types to home to the marrow: plasma cells, small lymphocytes, monocytoid cells, and segmented neutrophils. Of these, the plasma cells homed more efficiently than any other cell type.

The previous experiments employing bone marrow transfusion suggested that small lymphocytes, monocytoid cells, and segmented neutrophils that entered the marrow reflected a bone marrow exchange of cells (unpublished results). This concept was confirmed by other experiments$^{37}$ in which the tibial marrow of one hind limb was given an injection of TTH while arresting the limb circulation by a rubber compression bandage. Nonradioactive thymidine was administered to the animal intraperitoneally to control further labeling in other organs, and the circulation was restored after 15 min. The tibial marrows from the noninjected limbs of rats sacrificed at 24–48 hr after injection showed labeled cells of the following types: monocytoid cells, segmented neutrophils, and small lymphocytes. Thus it was concluded that these cell types were involved in bone marrow exchange.

The bone marrow was the only tissue of the B parabionts that showed evidence that a circulating cell would give rise to committed blast cells with an incidence frequent enough to be followed by radioautography. However, it is necessary to survey many bone marrow cells in order to detect the evidence. In one smear (Fig. 7) where numerous cells on the slide were viewed (approximately $5 \times 10^{5}$ cells), 91 labeled cells were encountered that could not be classified as one of the immigrant cell types. Most of these had one-fourth to one-half the grain count of immigrant cells (i.e., 7–23), and although they varied greatly in morphology, they were not typical of any committed blast cells abundant within the bone marrow (Fig. 7D–F). The monocytoid cell was the only immigrant cell type within the B marrow that showed a
Fig. 7. Radioautographs of bone marrow smear from B parabiont showing types of labeled cells encountered. (A), plasma cell; (B), small lymphocyte; (C), monocytoid cell; (D and E), blast cells; (F), two blast cells and a neutrophil.
reduction of average grain count as compared to that of the circulating monocytedoid cells in blood of A animals. This observation indicates that it was the only cell type entering the marrow of the B rats that had the ability to reproduce itself without first undergoing a morphologic transformation.

REFERENCES


Radioautographic Study of Cellular Migration Using Parabiotic Rats

Ruth W. Tyler and N. B. Everett