Kinetics of Lymphocytes in Chronic Lymphocytic Leukemia. I. Equilibrium Between Blood and a "Readily Accessible Pool"

By J. Manaster, J. Frühling, and P. Stryckmans

Seven patients with chronic lymphocytic leukemia were investigated by isotopic and electron microscopic techniques to study dynamic and morphologic aspects of the kinetics of their lymphocytes. Autotransfusion studies of labeled leukemic lymphocytes showed rapid equilibration of blood lymphocytes between the vascular space and a "readily accessible pool." With increasing absolute blood lymphocytosis (900/cu mm-670,000/cu mm), the relative importance of the readily accessible pool to the vascular space gradually decreases from eleven to one. Electron microscopic studies of the postcapillary venules in lymph nodes suggest a passage of lymphocytes from the vessel lumen, through the endothelial cells and into extravascular sites. About 20% of lymphocytes counted within the perivascular sheet were found in the wall, irrespective of the blood lymphocytosis and akin to the situation found in hematologically normal persons. These studies indicate that the readily accessible pool is complex and consists of at least two spaces, one of which is the classical recirculation space. The vascular pool of leukemic lymphocytes, as do the blood lymphocytes in hematologically normal persons, participates in this recirculation. There is a second space that has yet to be defined anatomically.

STUDIES ON THE KINETICS of lymphocytes play an important role in the elucidation of the function and the fate of cells which a decade ago were still believed to be end elements.

In 1959, Gowans established that normal lymphocytes in the rat recirculate. Electron microscopy showed blood lymphocytes passing through the cytoplasm of the endothelial cells lining the postcapillary venules in lymph nodes; this is presently considered as the efferent arm of the recirculation process. Drainage and extracorporeal irradiation of the thoracic duct (TD) lymph produced lymphopenia in the blood, while transfusion of autologous lymphocytes from the vessel lumen, through the endothelial cells and into extravascular sites. About 20% of lymphocytes counted within the perivascular sheet were found in the wall, irrespective of the blood lymphocytosis and akin to the situation found in hematologically normal persons. These studies indicate that the readily accessible pool is complex and consists of at least two spaces, one of which is the classical recirculation space. The vascular pool of leukemic lymphocytes, as do the blood lymphocytes in hematologically normal persons, participates in this recirculation. There is a second space that has yet to be defined anatomically.
or homologous labeled lymphocytes resulted in the rapid appearance of labeled cells in the TD. These observations led to the conclusion that the TD flow is the afferent arm of lymphocyte recirculation. Finally, studies with homologous blood lymphocytes transfused from normal human female donors to normal male recipients indicated a delay of approximately 10 hr in the appearance of XX-lymphocytes in the thoracic duct.

Although data on cell kinetics in chronic lymphocytic leukemia (CLL) patients are relatively sparse, three interesting points emerge:

1. The daily lymphocytic output from the TD into the blood of CLL patients generally does not exceed that in hematologically normal persons.

2. A radioautographic study done by Binet et al. in one TD-cannulated case of CLL seems to indicate a recirculation time of at least 94 hr. This has, however, been recently challenged by Bremer et al.

3. A rapid equilibrium of part of the blood lymphocytes with part of the extravascular lymphocytes has been suggested.

Point 1, and possibly 2, indicates a hampering of recirculation of lymphocytes in CLL. Various causes for this might be suggested: an inherent membrane defect of lymphocytes, which might be correlated with unresponsiveness to phytohemagglutinin and with hindering passage at the level of the postcapillary venules; an abnormality at the venules themselves; a crowding of the lymphoid extravascular system, etc. Point 3 relates to the fact that, following reinjection of labeled lymphocytes, part of the cells migrates out of the circulating bloodstream into a "readily accessible pool." Theoretically, this could be a marginated vascular pool, the extravascular recirculating space, or a pool totally independent of this recirculation process, etc.

This paper deals with the recirculation of leukemic lymphocytes and with the location of the readily accessible pool. Two methods were used: quantification of the equilibrium process occurring between different pools after autotransfusion of 3H-cytidine-labeled lymphocytes, and electron microscopy centered on postcapillary venules in CLL lymph nodes.

MATERIALS AND METHODS

Autotransfusion Study

Seven patients with CLL were studied: they were either untreated or off treatment. (One patient (Rom.) was studied three times.) Their lymphocytosis ranged from 900/cu mm to 700,000/cu mm. Bone marrow examination, blood evaluation, and clinical history (both before and after the study) confirmed the diagnosis in every case. Table 1 shows relevant features with special emphasis on defining volume of lymph nodes, spleen, and liver.

Experiments started in the morning. About 500 ml blood were withdrawn into Fenwal bags containing ACD-A anticoagulant. One-half milliliter (i.e., 500 µCi) tritium-labeled cytidine (Amersham-SA 25,000 mCi/mM) was added, and the blood was incubated for 1 hr at 37°C. A sample was then withdrawn for the trypan blue exclusion test, leukocyte count, differential count, and radioautography (RAG). Reinfusion started within 2 hr after withdrawal and lasted for 10-20 min. Blood sampling started at the end of reinfusion and lasted for approximately 24 hr. Thereafter, blood volume was measured, using the 131I-albumin method. In vivo labeling of circulating lymphocytes by tritiated cytidine present in the reinfused plasma can be dismissed in view of previous work by Fliedner et al.

Radioautography was performed using Kodak Nuclear Track Emulsion NTB-2 for...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex and Age</th>
<th>Date of Experiment</th>
<th>WBC Count (Cells/cu mm)</th>
<th>Lymphocytes (%)</th>
<th>Blood Volume (liter)</th>
<th>Lymph Nodes*</th>
<th>Palpable Liver Size in cm Below Ribs</th>
<th>Palpable Spleen Size in cm Below Ribs</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. Cut.</td>
<td>F 74</td>
<td>Sept. 12, 1969</td>
<td>690,000</td>
<td>97</td>
<td>4.550</td>
<td>2+</td>
<td>+ 4</td>
<td>—</td>
</tr>
<tr>
<td>Rec.</td>
<td>F 88</td>
<td>Aug. 28, 1968</td>
<td>629,000</td>
<td>98</td>
<td>5.330</td>
<td>2+</td>
<td>+ 7</td>
<td>+ 8</td>
</tr>
<tr>
<td>Romb. IV</td>
<td>M 70</td>
<td>Jan. 20, 1970</td>
<td>478,750</td>
<td>97</td>
<td>4.450</td>
<td>+</td>
<td>+ 5</td>
<td>+ 6</td>
</tr>
<tr>
<td>Romb. III</td>
<td>M 69</td>
<td>July 11, 1969</td>
<td>205,000</td>
<td>97</td>
<td>4.450</td>
<td>+</td>
<td>+ 1</td>
<td>—</td>
</tr>
<tr>
<td>Romb. I</td>
<td>M 69</td>
<td>Feb. 28, 1969</td>
<td>188,600</td>
<td>97</td>
<td>4.450</td>
<td>2+</td>
<td>+ 5</td>
<td>+ 7</td>
</tr>
<tr>
<td>Rod.</td>
<td>F 70</td>
<td>Jan. 13, 1969</td>
<td>48,000</td>
<td>95</td>
<td>4.260</td>
<td>2+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sz. II</td>
<td>M 68</td>
<td>Aug. 29, 1968</td>
<td>30,300</td>
<td>91</td>
<td>5.250</td>
<td>+</td>
<td>+ 7</td>
<td>+ 8</td>
</tr>
<tr>
<td>Mat. I</td>
<td>M 61</td>
<td>Jan. 27, 1969</td>
<td>9,066</td>
<td>61</td>
<td>4.750</td>
<td>3+</td>
<td>+ 1</td>
<td>+ 7</td>
</tr>
<tr>
<td>Pet.</td>
<td>F 69</td>
<td>Feb. 27, 1969</td>
<td>1,000</td>
<td>90</td>
<td>4.840</td>
<td>2+</td>
<td>+10</td>
<td>+ 4</td>
</tr>
</tbody>
</table>

*Lymph nodes: +, less than 1 cm (diameter); 2+, between 1 and 3 cm; 3+, greater than 3 cm.
Table 2. Localization of Removed Lymph Nodes and Blood Lymphocytosis at the Same Moment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Localizations of Lymph Node</th>
<th>Blood Lymphocytosis (cells/cu mm)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rec.</td>
<td>Auxillary</td>
<td>622,000</td>
<td>CLL</td>
</tr>
<tr>
<td>Rod.</td>
<td>Cervical</td>
<td>47,000</td>
<td>CLL</td>
</tr>
<tr>
<td>Rh.</td>
<td>Cervical</td>
<td>About 1,000</td>
<td>Hematologically normal</td>
</tr>
<tr>
<td>Ks.</td>
<td>Abdominal</td>
<td>About 1,000</td>
<td>Hematologically normal</td>
</tr>
</tbody>
</table>

dipping. Slides were exposed at 4°C for periods ranging from 29 to 115 days. When at least 50% of the lymphocytes reinjected into a patient showed labeling, all slides pertaining to this patient were developed and stained with May-Grünwald-Giemsa. At least 5000 lymphocytes were then counted per slide (except in case No. 1 where only 3000 lymphocytes could be counted per slide); only lymphocytes with more than three grains were considered as labeled.

Symbols are as follows: N⁺, total number of labeled lymphocytes reinjected; N, total number of lymphocytes present in circulation; L⁺, ⁰ / N of labeled lymphocytes in blood; N⁺/N, theoretical L⁺, assuming that no labeled lymphocyte leaves circulation after reinfusion and no unlabeled lymphocyte enters it; R, recovery in blood of labeled reinfused lymphocytes, expressed as % of N⁺/N; MR, mean of all the Rs calculated on the samples drawn from 1 hr post-transfusion to approximately 24 hr thereafter.

**Electron Microscopy Study**

Lymph nodes were obtained from two hematologically normal persons and from two CLL patients. Table 2 lists the localization of the removed lymph nodes and the blood lymphocytosis of these subjects.

Each lymph node was transferred to physiologic saline, was dissected free from the adipose and connective tissue, and was divided along its longitudinal surface. A 1-mm-thick section was undercut and immediately covered with fixative. Medullary and cortical parts were identified and separated. Each part was divided into small cubes (0.5 mm side). Fixation started within 10 min after surgical removal.

Fixation, dehydration, and embedding procedures were performed classically.¹⁰⁻¹² One micron thick slices were first examined, in order to localize postcapillary venules. After preparation of ultrathin sections,¹³ a systematic scanning for these venules was performed, both in the medullary and in the cortical fragments, until a reasonable total number of vessels and lymphocytes had been scored. Each lymphocyte was identified to avoid duplicate counting.

**RESULTS**

**Autotransfusion Study**

Figure 1 shows a representative experiment for the group of patients with a blood lymphocytosis over 30,000/cu mm. Like patient Rod. (45,600 lymphocytes/cu mm), they presented with an initial ⁰ / N blood-labeling index close to N⁺/N (the theoretical labeling if blood were a closed compartment); L⁺ fell rapidly, leveled off with in 60 min, and remained stable for the ensuing 24 hr (continuous line). The dotted line represents the recovery (R) in the blood of labeled reinfused lymphocytes.

In Fig. 2, patient Mat. I illustrates CLL cases with a lymphocytosis under 30,000/cu mm. Initial ⁰ / N of blood labeling was always much lower than N⁺/N; “plateauing” of L⁺ occurred at a lower level, and finally during the hours following an early nadir, the ⁰ / N of blood labeling showed a distinct
Fig. 1. Patient Rod. (45,600 lymphocytes/cu mm). Per thousand labeling indices and per cent recoveries in blood during the 24 hr following autotransfusion of labeled lymphocytes.

Fig. 2. Patient Mat 1. (5500 lymphocytes/cu mm). Same as in Fig. 1.

tendency to ascend. This increase was observed only in cases with a very low blood lymphocytosis.

Figure 3 pictures the recovery curves during approximately 24 hr of sampling. The recovery appears to correlate with absolute blood lymphocytosis, the highest cell counts showing the highest recoveries.

Table 3 lists MR (mean recovery) vs. blood lymphocytosis. Between 900/cu mm and 150,000/cu mm the mean recovery increased from 8% to 45%; above 200,000/cu mm, this ascent levels off at about 45%–50%.

Electron Microscopy Study

On electron micrographs, the postcapillary venules were identified according to the criteria set forth by Marchesi and Gowans.2 The endothelium consists of sharply delimited, plump, cuboidal, or flattened cells, often forming a monolayer when the vessel is filled up with blood elements (Fig. 4). No pores or gaps in between the cells can be seen. A large nucleus with well-defined nucleoli and a cytoplasm with normal amounts of endoplasmic reticulum,
mitochondria, Golgi apparatus, and lysosomal bodies characterize these cells. Desmosomes and terminal bars between the endothelial cells (Fig. 5, d and arrow) are easily identified. A basal membrane that is often multilaminated sustains the endothelium and separates it from concentrically arranged pericytes (Figs. 4–7, bm).

All lymphocytes seen exhibited the known features of normal and leukemic lymphocytes. The striking fact of the electron microscopy study was the presence of lymphocytes in all the structures of the venule wall, i.e., in the endothelial cells and in the peripheral layers both in normal and leukemic lymph nodes. Table 4 shows the incidence of this finding as a function of lymphocytosis. Thus, all stages of suggested passage can be described morphologically: margination in the lumen (Fig. 4, Ly 1), engulfment within the endothelial

Table 3. Mean Recovery in Blood of Labeled Reinfused Lymphocytes vs. Blood Lymphocytoses

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood Lymphocytosis (cells/cu mm)</th>
<th>N*/N%</th>
<th>MR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. Cuts.</td>
<td>870,000</td>
<td>73.00</td>
<td>46.2</td>
</tr>
<tr>
<td>Rec.</td>
<td>618,000</td>
<td>67.3</td>
<td>46.9</td>
</tr>
<tr>
<td>Romb. IV</td>
<td>464,500</td>
<td>90.0</td>
<td>45.5</td>
</tr>
<tr>
<td>Romb. III</td>
<td>199,000</td>
<td>88.5</td>
<td>36.7</td>
</tr>
<tr>
<td>Romb. I</td>
<td>163,500</td>
<td>108.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Rod.</td>
<td>45,600</td>
<td>83.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Sz. II</td>
<td>27,600</td>
<td>53.0</td>
<td>14.1</td>
</tr>
<tr>
<td>Mat. I</td>
<td>5,530</td>
<td>69.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Pet.</td>
<td>900</td>
<td>44.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

N*/N, theoretical labeling index expected if blood were a closed compartment. MR, mean of all the recoveries calculated on the successive samples drawn from 1 hr postautotransfusion of labeled lymphocytes to approximately 24 hr thereafter.
LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

Fig. 4. Patient Rec. (622,000 lymphocytes/cu mm). General view of postcapillary venule in transversal section. El, endothelial cells; bm, basal membrane, often multilaminated; Pc, pericyte; E, erythrocyte in the lumen; Ly, lymphocyte in the lumen; Ly 1 to Ly 5, lymphocytes in different stages of passing through the vessel wall; *, lymphocyte (Ly 5) seemingly thrusting the cytoplasm of the endothelial cell and the basal membrane outwards; Ly 6, lymphocyte between the elements of the perivascular sheet. Technical conditions (relating to Figs. 4 to 7): Fixative, glutaraldehyde 2.5% PO₄ 0.1 M; postfixative, OsO₄ 1%, PO₄ 0.13 M; embedding, epon; coloration, uranyl acetate 1% in water, followed by lead citrate.¹³ X 4600.

cells (Fig. 4, Ly 2 and Fig. 6, Ly 1), localization in these cells (Fig. 4, Ly 3 and Fig. 6, Ly 2), coming out of the cells (Fig. 4, Ly 4), contact with the basal membrane (Fig. 4, Ly 5 and Figs. 5–7) and outward thrusting of it, and finally escape into the layers of the peripheral sheet (Fig. 4, Ly 6).

Neither phagocytic nor excessive pinocytic activity was demonstrated by any of the affected endothelial cells; there was no excess of lysosomal structures. The surface of the endothelial cells, surrounding the imprisoned lymphocyte, was intact (Figs. 5 and 7), as was the membrane of the lymphocyte. The space separating both elements in each case of passage (Figs. 4–7) was accentuated on the micrographs by the use of a hypertonic aldehyde fixative. The examination of not strictly serial, but nevertheless sequential, sections likewise suggested the formation of an intracellular invagination. We saw no lymphocytes directly penetrate any of the intercellular junction complexes between two adjacent endothelial cells. Figure 5 shows such an intact junction (d and arrow), with a completely intracellular lymphocyte close to it.
Fig. 5. Same patient as Fig. 4. Lymphocyte (Ly) passing through a tunnel formed by one endothelial cell (El 1). Immediately contiguous (d and arrow) desmosomes between the plasma membranes of two distinct endothelial cells (El 1 and El 2). E, erythrocyte in the lumen. In the lymphocyte: N, nucleus; Nc, nucleolus; m, mitochondrion; r, ribosomes and polysomes; bm, basal membrane on the external side of the vessel. Double arrow, direct contact between the lymphocyte and the basal membrane. Same technical conditions as Fig. 4. × 16,200.

Morphologic aspects of lymphocyte passage in normal lymph nodes were identical to those seen in CLL lymph nodes. Quantitative results are reported in Table 4.

DISCUSSION

The Dilutional Pool of Blood Lymphocytes in CLL

In a recent review on lymphocyte kinetics in CLL, Schiffer proposed a model based on results of extracorporeal irradiation of blood and consisting of three overlapping compartments. Compartment 1 includes the blood lymphocytic pool and a "readily accessible pool;" lymphocytes circulate freely between both pools. Compartment 2 still exchanges with compartment 1, but cells have different mobilities depending on where they are located. Compartment 3 is a nearly immobile tissue lymphocytic pool.

The technique of transfusion of 3H-cytidine-labeled lymphocytes, as initiated by Fliedner et al., enables one to study the equilibration process occurring within compartment 1 between blood and readily accessible pool. Within approximately 1 hr, a plateau of blood labeling is reached; it seems very unlikely that, within so short a period, the total volume of distribution
of the labeled lymphocytes could exceed compartment 1 in favor of less mobile compartments, and the plateau, moreover, reflects equilibrium within compartment 1. The experiment is limited to 24 hr following reinfusion, as degradation of RNA thereafter introduces important errors. Recovery is calculated over this time interval; it should, however, be emphasized that no significant modification in results occurs if calculations are made over 12- or 6-hr periods following reinfusion. Finally, radioactive chrome, the other major cell label used in this technique, offers no advantages; it does not allow RAG and labels platelets and erythrocytes extensively. Therefore, tritiated cytidine is preferred.

The mean recovery expresses the fraction of lymphocytes of compartment 1 constituting the circulating blood pool, while the missing part represents, by definition, the readily accessible pool. Thus, this pool is defined in a purely operational way; it has not been located anatomically. One could argue that the missing fraction simply corresponds to the destruction of lymphocytes occurring during in vitro manipulations. However, supravital dye exclusion tests before reinfusion showed less than 1% lethality. Moreover, under certain conditions, e.g., prednisolone therapy, a much higher mean recovery in blood can be obtained.

The experimental data suggest that the readily accessible pool always exceeds in size the blood pool but that, with increasing blood lymphocytosis, the relative prevalence of the former decreases from 11 times the blood to 1.1 times the latter. At a blood lymphocyte count of about 200,000/cu mm, both subcompartments are nearly equal, and with higher lymphocytosis both pools increase in parallel.

**Fig. 6.** Same patient as Fig. 4. Detail of postcapillary venule. L, lumen; El 2, endothelial cell of rather cuboidal form; Ly 1, engulfment of lymphocyte by an endothelial cell; m, mitochondria of the lymphocyte; Ly 2, lymphocyte in the tunnel formed by the endothelial cell El 2; bm, basal membrane, multilaminated (arrows); Pc, detail of pericyte. Same technical conditions as Fig. 4. X 6200.
The existence of a specific relationship between blood pool and readily accessible pool raises several questions. Below 200,000 lymphocytes/cu mm blood, the readily accessible pool seemingly behaves as a subcompartment that has to be filled up, with the equilibrium blood pool \( \approx \) readily accessible pool being, therefore, largely in favor of the latter. At higher blood lymphocytosis, however, the readily accessible pool by its load of cells seemingly counterbalances this filling-up process.

Fig. 7. Same patient as Fig. 4. Lymphocyte (Ly 1) passing through an endothelial cell (El) and showing one process (P 1) in the lumen of the vessel and a second process (P 2) already behind the endothelial cell and in contact with the basal membrane (bm); E, erythrocyte in the lumen; Ly 2, lymphocyte in the lumen; N, nucleus; Nc, nucleolus; m, mitochondria; Pc, pericyte. Same technical conditions as Fig. 4. \( \times \) 19,800.
LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

Table 4. Quantitative Results of Electron Microscope Studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood Lymphocytosis (cells/cu mm)</th>
<th>Venule Lymphocytes/ cu mm</th>
<th>Lymphocytes in Venule Wall/ Lymphocytes in Wall + Lumen</th>
<th>Lymphocytes in Wall (%)</th>
<th>95% Confidence Interval (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL</td>
<td>622,000</td>
<td>1458</td>
<td>45/175</td>
<td>25.7</td>
<td>19–33</td>
</tr>
<tr>
<td>CLL</td>
<td>47,000</td>
<td>187</td>
<td>10/45</td>
<td>22.2</td>
<td>11–36</td>
</tr>
<tr>
<td>NL₁ + NL₂*</td>
<td>1,000</td>
<td>61</td>
<td>7/39</td>
<td>18</td>
<td>7–32</td>
</tr>
</tbody>
</table>

*Calculated number of lymphocytes present within perivascular sheet of postcapillary venules per sq mm scanning surface.
†Number of lymphocytes in the wall structures of postcapillary venules relative to total number of lymphocytes counted within perivascular sheet.
‡NL, normal lymph node; results in the two hematologically normal persons are totaled.

The Return of Lymphocytes to the Blood in CLL

A major problem in defining anatomically the readily accessible pool is that the route of return of lymphocytes from it into the blood remains unclear. If one equates this readily accessible pool with the classical recirculation space, the TD could constitute such a return path. As stated earlier, it is however well established that the daily lymphocytic output from the TD into the blood in CLL patients does not exceed the one in hematologically normal persons.

At a low lymphocytosis, this does not give rise to any difficulty. In fact, CLL patients with a number of circulating lymphocytes identical to that of hematologically normal persons show not only a similar TD flow of lymphocytes into the blood, but also an identical compartment 1. Figure 8 is a nomogram deduced from our studies and showing the number of lymphocytes present in

![Fig. 8. Nomogram showing in CLL the number of lymphocytes per kg body weight present in blood (dotted line) and in blood + readily accessible pool (RAC) (continuous line). Points represent results in nine kinetic studies. Both scales are logarithmic.](image-url)
the blood (BLP) and in compartment 1 (blood pool, BLP and readily accessible pool, RAC) in CLL patients. At a blood lymphocytosis of 1000/cu mm, compartment 1 contains about $10^9$ lymphocytes/kg body weight; this is precisely the size of the recirculating pool of lymphocytes calculated by Revillard et al. for hematologically normal persons using lymphocyte depletion by continuous TD drainage. At these low levels of lymphocytosis, the readily accessible pool could, therefore, be viewed as an integral part of the TD recirculation process; the fact that, at these levels, blood labeling tends to rise after an early nadir (Fig. 2) enforces this view.

At higher blood lymphocytosis, the bottleneck constituted by a low TD flow of lymphocytes into the blood associates with an accumulation of cells rearward. The persistence in this situation of an equilibrium within compartment 1 suggests that the exchanges between its constituting pools occur, at least in part, independently of the recirculation through the TD. The readily accessible pool does not then have to be considered, in its entirety, as an obligatory stage in the TD recirculation. The return of part of the readily accessible pool lymphocytes to the blood in CLL possibly proceeds through channels other than the TD, e.g., the lymphovenous shunts demonstrated in hematologically normal persons.

The kinetics at different levels of blood lymphocytosis indicate that the readily accessible pool is a complex one, consisting partly of the classical recirculating space, as defined by Gowans, and partly of a second as yet anatomically undefined space. The fluctuations in recovery during the 24-hr time interval at high blood lymphocytosis could be related to equilibration processes occurring within this second space.

The Route of Exit of CLL Lymphocytes From the Blood

The electron microscopy studies were primarily aimed at morphologically defining, in normal persons and in CLL patients, the passage of lymphocytes in the lymph nodes. They showed quite conclusively the existence of such a passage in humans, thereby confirming previous studies in rodents. That the basal membrane is always pushed away from the lumen by any of the crossing lymphocytes, as can be nicely demonstrated in Fig. 4 (*), suggests a passage from blood toward the perivascular structures, in accordance with earlier studies. The lymphocytes cross through cells that show none of the characteristics of macrophages; both protagonists display intact membranes; no passage through any desmosome or terminal bar joining two endothelial cells is ever seen. The mechanism of passage is, of course, unknown; one possibility would be the formation of intracellular tunnel-like structures. The electron microscopy studies also showed no qualitative difference between lymph nodes of hematologically normal and CLL patients, as far as passage of lymphocytes is concerned. Quantitatively, however, important information was gained: the fraction of lymphocytes detected in the wall structures amounts to nearly 20% of all cells counted within the lumen and wall structures of the postcapillary venules, although blood lymphocytosis ranged from 1000 to 600,000/cu mm and although two lymph nodes were derived from hematologically normal persons and two from CLL patients with widely
LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

437

different blood counts. Every lymphocyte counted was cut at several levels to ensure that it was either entirely intravascular on the one hand, or partially or completely engaged within the wall structures on the other hand. The percentage of cells in the wall differs from the values of lymphocytic egress as defined by the kinetic studies. However, this is not surprising, as neither the time needed for passage across the wall nor the total surface available for passage is known. Within these limits, the data obtained from the electron microscopy studies correlate quite well with those derived from the autotransfusion studies. In both cases, the absolute quantity of passage increases almost linearly with blood lymphocytosis. Thus, it can be concluded that if there exists at all some hampering of recirculation of lymphocytes in CLL patients, this is certainly not at the level of the postcapillary venule in the lymph node.

ACKNOWLEDGMENT

The authors are grateful to Mr. G. Vamecq for the statistical analysis, to Mrs. M. Socquet and Mr. R. Badjou for the valuable technical assistance, to Mr. J. Rummens for the preparation of the thin sections, and to Mr. J. Verheyden for the photographic work. We would also like to thank Dr. E. P. Cronkite for his interest in this work.

REFERENCES


Kinetics of Lymphocytes in Chronic Lymphocytic Leukemia. I. Equilibrium Between Blood and a "Readily Accessible Pool"

J. Manaster, J. Frühling and P. Stryckmans