Leukocyte Labeling With $^{51}$Chromium. III. The Kinetics of Normal Lymphocytes

By James L. Scott, J. Gary Davidson, Joseph V. Marino, and Robert McMillan

The kinetics of radiochromate-labeled autologous blood lymphocytes were studied in normal subjects. The labeled cells equilibrated within hours with a recirculating lymphocyte pool (RLP) distributed between the blood, the spleen, and the bone marrow. The RLP was found to be about twice the size of the blood lymphocyte pool contained within it and turned over through the blood about 12 times daily. The labeled cells had an average half life in the RLP of 18 days.

Radiochromate ($^{51}$Cr) is a particularly useful blood cell label because it allows assessment of in vivo cell distribution. Studies of lymphocyte kinetics with $^{51}$Cr show a complex pattern. There is an initial rapid exponential clearance of the cells from the blood and a coincident rise in body surface counting rates over the spleen and the sacral marrow. There follows a gradual decline in the levels of blood and organ radioactivity. This pattern is compatible with the recirculation of the lymphocytes between the blood and tissues and their subsequent loss from the recirculating lymphocyte pool (RLP) by death or redistribution. In the present investigation, the size, distribution, and intravascular turnover rate of the RLP and the life span of cells in the pool have been studied in normal human volunteers.

MATERIALS AND METHODS

The subjects were paid volunteers who were informed about the procedure and gave written consent. All were healthy males between 21 and 40 yr of age with normal physical examinations and blood counts. The studies were begun between 8:00 and 9:00 a.m. after a normal breakfast.

The leukocytes of two successive 500-ml phlebotomies were separated and labeled. During the separation procedure the subject's blood volume was maintained by an i. v. infusion of normal saline, and the RBC were recovered and reinfused. In the first study, unfractionated leukocytes were labeled, and the blood lymphocyte specific activity (BLSA) curve was determined by isolating the lymphocytes from postinfusion blood samples. This method required serial blood samples of 100 ml and did not allow determination of the organ distribution of the lymphocytes. In studies 2–8, the lymphocytes were isolated prior to labeling to allow body surface monitoring of lymphocyte radioactivity.
To conserve blood, the postinfusion leukocyte samples were not fractionated; 50-ml blood samples were adequate. Daily total and differential leukocyte counts showed no significant variations.

In five other studies, only the final slope of the BLSA curve was determined. The leukocytes were not fractionated because the granulocytes are cleared from the circulation during the first 24 hr after the infusion of labeled, unfractionated leukocytes.5

The leukocytes were labeled, after dextran sedimentation of the RBC and differential centrifugation of the supernatant plasma to reduce platelet contamination, by suspending the cells in Hanks' solution containing 500 μCi of radioactive sodium chromate for 30 min at 37°C. The residual chromate was reduced with 50 mg of ascorbic acid, and the cells were washed twice by suspension in autologous plasma-dextran. After resuspension in 150 ml of plasma-dextran, a sample was taken for determination of the specific activity of the leukocytes and total and differential leukocyte counts, and a weighed volume of the remainder was infused i. v. over a 15-min period. Serial venous blood samples were taken at 15 min, 1, 3, 5, and 8 hr, and daily thereafter, as long as practical. After mixture with Plasmagel (Lab. Roger Billon, Neuilly, France) for RBC sedimentation, the remaining RBC in the supernatant were lysed by suspension in 0.02% ethylhexadecyldimethyl ammonium bromide (Eastman) in formalinized sodium citrate-chloride solution.6 This RBC lysis technique did not cause the disproportionate loss of lymphocytes produced by the gramicidin-lysolecithin method.5

Lymphocytes were isolated and labeled with the same technique after removal of the granulocytes and monocytes from the blood on a nylon column (Fenwal Leukofilter). Stained smears showed that more than 90% of the remaining leukocytes were small lymphocytes. Immediately after isolation and labeling, less than 1% of the lymphocytes stained with trypan blue. In vitro incubation of labeled lymphocyte preparations for a period of 2½ hr was not associated with elution of 51Cr; supernatant radioactivity increased due to cell lysis, but intact cell specific activity remained constant.

Leukocyte specific activity, as cpm/mg of cell nitrogen, and body surface counting rates were measured by previously described methods.5 Leukocyte sample counting rates of less than twice background were considered to be insignificant. Sufficient counts were accumulated to reduce the error of counting to ± 3%.

The percentage of infused cells recovered in the circulation, the size of the RLP, and the lymphocyte turnover rate (LTR) were calculated from formulas derived from those used by Athens et al. in studies of granulocyte kinetics.7,8 In studies 2–8, cell recovery was calculated from the formula:

\[
\text{Recovery} = \frac{\text{Blood WBC specific activity} \times \text{total WBC count} \times \text{blood volume}}{\text{Infused lymphocyte specific activity} \times \text{No. infused}} \times 100
\]

Blood volume was estimated as 7% of the body weight. The total blood leukocyte count was used in the calculation because the BLSA determinations were made on unfractionated leukocytes.

RLP size was estimated by multiplying the blood lymphocyte pool, derived from the blood lymphocyte count and the blood volume, by the ratio between the BLSA level immediately after infusion of the labeled cells (To) and the BLSA level after equilibration (Te) of the injected cells with the RLP. LTR per 24 hr was calculated from the formula:

\[
\text{LTR} = 24 \times \frac{0.693}{T/Te} \text{hr}.
\]

RESULTS

Blood Lymphocyte Kinetics

The individual and the mean BLSA curves of studies 1–8 are shown in Fig. 1. The BLSA curve of isolated blood lymphocytes after the infusion of unfractionated labeled leukocytes (study 1) resembles the curves found with lymphocytes isolated before labeling. The labeled lymphocytes initially left the blood in a rapid exponential with an average T½ (±1 SD) of 1.57 ± 0.54 hr. The
BLSA curves reached a nadir 3–5 hr after infusion and rose by 8 hr to levels about one-half the immediate postinfusion values. There followed a slow exponential fall in BLSA. In studies 3 and 6, determinations of BLSA level were continued long enough to allow extension of the exponential to derive $T_{1/2}$ values of 20 days. In five other studies only this last portion of the BLSA curve was determined (Fig. 2). Daily determinations of BLSA following clearance of the labeled granulocytes yielded exponential curves with $T_{1/2}$ values of 16, 16, 17, 18, and 20 days. The mean $T_{1/2}$ of this exponential in the seven studies is $18 \pm 1.9$ days.

Body Surface Counting

The mean body-surface counting rate curves of the seven subjects infused with isolated labeled lymphocytes, expressed as per cent of the initial 15-min counting rate, are shown in Fig. 3. The spleen and sacral counting rates rose
Fig. 3. Mean body-surface counting rates, expressed as per cent of 15-min value, in seven subjects infused with isolated, labeled lymphocytes. Vertical brackets enclose ± 1 SD.

during the first 4 hr and fell gradually thereafter. The average spleen counting rate was three times that of the sacrum. The average counting rate curves of the lung, heart, and liver fell gradually throughout the course of the studies. Individual variations were observed in the liver and lung counting rates that may have resulted from labeled cell sequestration. These changes were most pronounced in the studies with the lowest cell recovery values (Table 1).

Calculations

Data derived from studies 1–8 are summarized in Table 1. The percentage of infused cells recovered in the circulation in the seven studies in which this could be calculated averaged 59%, with a range of 20–83%; an unsatisfactory nitrogen determination on the infused leukocyte suspension prevented calculation of this value in the first study. The ratio between the initial BLSA level (T₀) and that following equilibration (Tₕ) averaged 2.2, with a range of 1.6–3.4. The average size of the RLP calculated from this ratio and the size of the blood lymphocyte pool was 23 ± 6.5 × 10⁸ cells, or 31.9 ± 7.6 × 10⁷

Table 1. Lymphocyte Kinetic Data in Eight Normal Subjects

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Weight (kg)</th>
<th>Recovery %</th>
<th>T 1/2 (hr)</th>
<th>Blood Lymphocyte Turnover Rate (times/24 hr)</th>
<th>Blood Lymphocyte Count* (per cu mm)</th>
<th>Blood Lymphocyte Pool (× 10⁹)</th>
<th>Recirculating Lymphocyte Pool (× 10⁹)</th>
<th>T₀/Tₕ</th>
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<td>1.75</td>
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<td>1.8</td>
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<tr>
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<td>Mean</td>
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<td>±1 SD</td>
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<td>±3.8</td>
<td>±251</td>
<td>±1.84</td>
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* Mean of four to seven total and differential WBC counts.
cells/kg of body weight. Turnover of the RLP through the blood, based on the initial exponential disappearance rate, averaged 11.7 times/day. From the average $T_{1/2}$ of the slow exponential of 18 days, 3.8% of the RLP, or about $0.9 \times 10^9$ cells, were lost from the RLP each day.

**DISCUSSION**

The RLP has been succinctly defined by Ford and Gowans, from the results of animal studies, as a population of nondividing, small lymphocytes with an average life span of several weeks that recirculate from the blood to the lymphoid tissues and back to the blood in a cycle measured in hours. The results of the present studies are consistent with a pool of this description. The sharp initial fall in the BLSA and the rise in the spleen and marrow counting rates indicate that the labeled cells rapidly migrated from the blood to these organs. The following coincident rise in the BLSA and the fall in the spleen and marrow counting rates are compatible with cell recirculation from these organs back to the blood. The subsequent parallel course of the BLSA and the body-surface counting rates indicate that the labeled cells became equilibrated within the RLP. The occurrence of equilibration within hours is compatible with a turnover rate as rapid as that defined by the initial blood clearance rate.

The initial distribution of the labeled cells in the spleen and marrow is similar to that found shortly after the transfusion of autologous labeled lymphocytes in the rat. A fraction of the recirculating lymphocytes then migrate to the mesenteric lymph nodes and presumably return to the systemic circulation by way of the cisterna chyli and thoracic duct. The splenic lymphocytes, which are initially found in the marginal zones surrounding the lymphoid follicles, migrate within a few hours to the periarteriolar lymphoid sheaths of that organ and reenter the blood. Comparable knowledge of the lymphocyte recirculation pathway within the marrow is lacking, but the almost parallel spleen and sacral curves observed in this and a previous study in man indicate that the migratory behavior of the spleen and marrow lymphocytes is similar.

These studies indicate that the RLP is at least twice the size of the blood lymphocyte pool contained within it. The failure to recover a portion of the injected cells in the circulation suggests that the RLP may be somewhat larger; the unrecovered cells may have equilibrated rapidly with a pool of lymphocytes distributed in a marginal position within small vessels, analogous to the marginal granulocyte pool. Calculation of the size of the marginal lymphocyte pool from the present data by the isotope dilution principle would be of doubtful validity, because there was evidence that suggested organ sequestration of damaged cells in some of the studies.

Chronic thoracic duct drainage results in the mobilization of a cell pool that is on the average substantially larger than the RLP of approximately 20 billion cells measured in these studies. Over a period of weeks, the cumulative cell yield has been reported to range from 10 to over $200 \times 10^9$ lymphocytes. The larger yields have been associated with striking lymphopenia.
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and lymphocyte depletion from the lymph nodes, spleen, and intestinal wall. The daily yield of cells stabilized at a level between 0.5 and $1.2 \times 10^8$ cells,\(^1\) which may reflect the maximum daily rate of lymphocyte production.

We calculate that a similar number of lymphocytes is lost from the RLP each day. This balance may indicate that the majority of the cells leaving the RLP die in the tissues, as concluded by previous investigators,\(^3,\) but the evidence that some lymphocytes in the RLP have a life span of several years\(^13,\) cannot be dismissed. It seems reasonable to conclude that $T_\beta$ values in the range of 13\(^12\)–18 days represent the median sojourn in the RLP of a population of cells that is heterogeneous both in life span and function.\(^15\) Because of their very long life span, it is likely that some cells make a slow circuit through the tissues and reenter the RLP. From the general magnitude of daily lymphocyte production, it is also likely that the very long-lived cells are a minor fraction of the heterogeneous population.

REFERENCES


8. —, et al.: Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turn-
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