

Circulation and Emigration Kinetics of Blood Lymphocytes in Lymphoma Patients

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Summary

In 6 groups of patients [controls (N) with localized solid tumors, untreated or irradiated patients with Hodgkin's disease (HD), irradiated patients with seminoma testis (ST) and patients with chronic lymphocytic leukaemia (CLL) or another leukaemic non-Hodgkin's lymphoma (NHL)], representing a total of 31 patients characterized by different percentages of blood B cells, the circulation and emigration kinetics of each patients' blood lymphocyte population have been studied by their autologous retransfusion after *in vitro* labelling with ³H-cytidine and by simultaneous lymph drainage of a peripheral afferent lymph vessel of the lower leg.

The neoplastic B lymphocytes of the patients with leukaemic NHL exhibited similar increased recovery values in the blood because of prolonged blood transit times and an impaired exchange with extravascular lymphocyte pools; furthermore, a reduced fraction of these leukaemic B lymphocytes has been recovered in the lymph. In contrast, the irradiated patients with ST showed diminished and with HD normal recovery values in the blood, although in both groups of irradiated patients increased fractions of 45% respectively 34% blood B lymphocytes have been determined. On the other hand, lower than the expected numbers of autotransfused lymphocytes appeared in the peripheral lymph of the two groups of HD-patients and of the ST-patients.

Because no B lymphocytes have been observed in the peripheral lymph of all patients studied, the discrepancy between the lymphocyte recovery data in the blood and those in the peripheral lymph of HD-patients as well as of the ST-patients is taken to indicate that in these patients not only the T lymphocytes but also a fraction of the B cells emigrated from the blood, but may remain in the interstitium, since B cells do not reach the lymph. Furthermore, for each of the 6 groups of patients it could be demonstrated that in contrast to B lymphocytes only the blood T lymphocytes are able to recirculate

via peripheral lymphatics thereby also indicating a normal *in vivo* function of these blood T lymphocytes even in the patients with leukaemic B cell NHL.

Introduction

Animal experiments revealed a slower recirculation from blood to lymph of B lymphocytes than of T lymphocytes (1–2). This might explain that in haematologically normal patients as well as in patients with chronic lymphocytic leukaemia (CLL) only few B cells are found in prenodal leg lymph despite a marked B lymphocytosis of CLL blood (3). Alternatively, since autotransfused labelled CLL lymphocytes disappear from peripheral blood at a reduced rate (4–5) and show a decreased reappearance in thoracic duct lymph (6), it might be that the neoplastic CLL B lymphocytes have a defective migration ability.

High-energy irradiation for Hodgkin's disease (7–9) or solid tumors (10–11) regularly induced increased percentages of B lymphocytes in peripheral blood and low lymphocyte counts in peripheral lymph (12) due to a long-lasting blood T lymphocytopenia, while B lymphocytes are rapidly restored. To distinguish between the two possible explanations for the reduced recirculation of CLL lymphocytes, the circulation and emigration kinetics of blood lymphocytes were studied not only in patients with other malignant non-Hodgkin's lymphomas (NHL) exhibiting similar B cell leukaemia but also in patients with radiation-induced relative blood B lymphocytosis. In each of these patients presenting different proportions of blood B cells an autologous retransfusion of *in vitro* labelled blood lymphocytes was performed to examine the disappearance from the blood and reappearance in the peripheral lymph of labelled blood lymphocytes. The cell numbers in

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blood and lymph and the blood B/T lymphocyte ratio of these patients were reported previously (3, 7, 11–12).

Patients and Methods

Patients: 6 groups with a total of 31 patients were investigated:

- 7 patients with newly diagnosed, localized solid tumors, considered as haematologically normal patients (N), showing a mean of 12% blood B lymphocytes;
- 6 patients with untreated Hodgkin's disease (HD), demonstrating reduced percentage of blood B lymphocytes (mean 7%, range 1–10%);
- 8 treated patients with stage II or III extent of HD, 6 of whom having been splenectomized. All had received a total nodal irradiation 1 months to 3 years previously (7);
- 4 patients with seminoma testis (ST) treated by unilateral orchidectomy and either extended or total nodal irradiation; both of these two groups of irradiated patients were characterized by increased proportions of 34 and 45% blood B lymphocytes respectively;
- 4 patients with untreated CLL exhibiting

94% B cells in their blood and

- 2 patients with a leukaemic variant of another type of NHL, a centrocytic lymphoma (13), presenting 95% blood B cells.

Methods: In each patient 500–600 ml of venous blood were incubated with 1 mCi ^3H -cytidine (Radiochemical Centre Amersham, England) for 2 hours at 37 °C (5–6). After washing off the excess of extracellular free radioactivity and the addition of a 1000-fold amount of non-radioactive cytidine, the blood with the labelled autologous lymphocytes was retransfused intravenously within 10 minutes. ^3H -cytidine selectively labels mononuclear cells and is mainly incorporated into lymphocyte RNA (4–5).

To allow subsequent simultaneous analysis of blood and lymph, in 25 out of the 31 patients a peripheral lymph vessel of the lower leg had been cannulated for continuous lymph drainage during 3 to 10 days (14). From serially taken blood and lymph samples leukocyte rich blood smears (4–5) or cytocentrifuged lymph cell preparations were performed followed by autoradiography and, finally, microscopic evaluation (4–5).

To determine the percentage of B or T cells, in contrast to lymph cells the blood lympho-

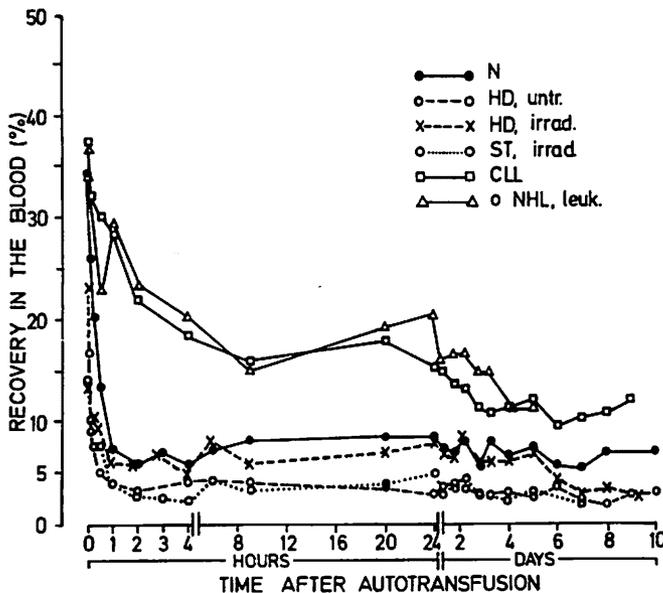


Fig. 1 Patterns of recovery values of autotransfused ^3H -cytidine labeled blood lymphocytes in blood volume of haematologically normal controls (N) and of patients with untreated or irradiated Hodgkin's disease (HD), with irradiated seminoma testis (ST), with chronic lymphocytic leukaemia (CLL) or with another leukaemic non-Hodgkin's lymphoma (NHL).

cytes had to be separated by Isopaque-Ficoll gradient centrifugation. After washing one part of the cells were stained in suspension with a fluorescein isothiocyanate-labelled antiserum directed against the F(ab')₂ fragment of human IgG as described earlier (15). Lymphocytes bearing detectable amounts of surface immunoglobulins were considered as B cells. To the second part of blood or lymph lymphocytes a 100-fold number of sheep erythrocytes was added; the formation of spontaneous rosettes with 3 or more erythrocytes was taken to indicate T cells (16).

Results

Lymphocyte recovery in the blood: Immediately after completion of autotransfusion (time "zero") only about half of the autotransfused, labelled blood lymphocytes or less were circulating in each patient's blood volume; recovery values on the average of 34 to 38% have been found in the N-, CLL- and other NHL-patients as compared to mean recovery values of 14 to 23% in the 2 groups of HD-patients and the ST-patients (Fig. 1). Within the following hour these recovery values of labelled blood lymphocytes decreased rapidly in all patients but to different extents. From 1 to 4 hours after autotransfusion onwards rather constant recovery values have been observed (Fig. 1). The level of this plateau at 1-4 hours after autotransfusion was significantly lower in the irradiated ST- and the untreated HD-patients (2.9 and 4.0% recovery respectively) as compared to the normal controls and the treated HD-patients (6.4 and 6.0% recovery respectively). The highest recovery values of 22.9 and 24.2% respectively were met with the CLL- and the other leukaemic NHL-patients.

By means of the half-times of the initial disappearance rates of labelled lymphocytes from the blood the lymphocyte blood transit times can be calculated yielding similar transit times of 32 to 43 minutes for the blood lymphocytes of the 4 groups of aleukemic patients, but prolonged blood transit times of about 100 to 500 minutes respectively for the neoplastic lymphocytes of the 2 groups of leukaemic NHL-patients. Despite these different blood transit times the total number of blood lymphocytes

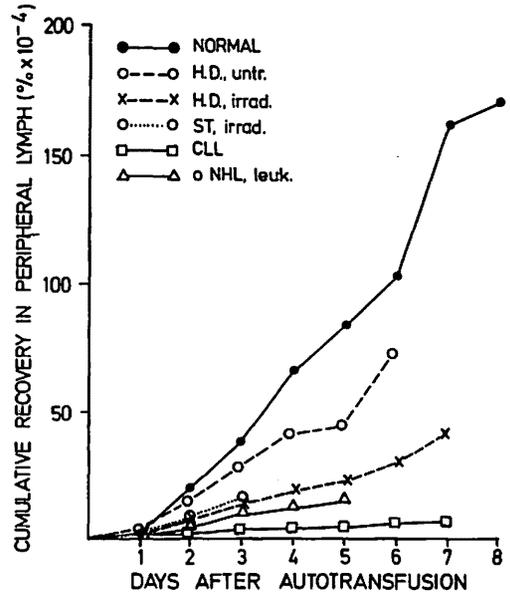


Fig. 2 Cumulative recovery values of autotransfused ³H-cytidine labelled blood lymphocytes in the peripheral lymph of normal controls and of patients with different lymphatic disorders. For abbreviations see text of fig. 1.

phocytes leaving the circulation was considerably higher in the leukaemic NHL-patients than in the 4 groups of aleukaemic patients.

Lymphocyte recovery in the lymph. The first autotransfused labelled lymphocytes appeared in the peripheral lymph within 1 to 4 hours after autotransfusion; the mean of this time interval, similar for each of the 6 groups of patients, was 2 hours and is considered to represent the minimal blood-lymph migration time. During the following hours the numbers of labelled lymphocytes increased continuously reaching peak values at day 3 or 4 in the majority of patients, which is taken to indicate the "modal" migration time of blood lymphocytes.

Summarizing the output of labelled lymphocytes in the lymph in a cumulative may the irradiated HD-patients exhibited tentatively decreased recovery values after 3 and 5 days of continuous lymph drainage as compared to the controls, whereas those of the untreated HD- and the ST-patients were not significantly reduced (Fig. 2). The lowest recovery values

have been disclosed in the two groups of leukaemic NHL-patients.

Discussion and Conclusions

Intravascular lymphocyte distribution. Including trypan-blue dye exclusion by more than 90% of in vitro labelled lymphocytes, up to 100% of all autotransfused lymphocytes may be considered as viable cells (4–5). Since in humans trapping or sequestration of living autotransfused lymphocytes in lung and/or liver is negligible (17–19), the low initial recovery values of about 50% or less in the blood of all patients (Fig. 1) indicate that within the 10 minutes of retransfusion-time the autotransfused lymphocytes are distributed in an intravascular lymphocyte pool larger than the “circulating lymphocyte pool (CLP)” of the blood volume (4–5). In analogy to similar rapid distribution patterns of other leukocytes it is concluded that the total blood lymphocyte pool consists of at least 2 intravascular subcompartments, the CLP and the “marginal lymphocyte pool (MLP)”, presumably located in the vascular areas of slow blood flow (4–5). Nevertheless, CLP and MLP must be considered as a functional entity, e.g., with respect to the rapid disappearance of labelled lymphocytes from the blood within the first hour after autotransfusion.

Emigration of blood lymphocytes. The observation that the peripheral lymph of aleukaemic and leukaemic patients contained no or only few B lymphocytes (3) but almost exclusively T lymphocytes (20) agrees with animal experiments indicating a preferential recirculation of T cells (1–2). Due to the increase of B cells in the blood of the irradiated HD- and ST-patients and an excessive B cell augmentation in the blood of the 2 groups of leukaemic NHL-patients, correspondingly diminished (9–11) or only small percentages of T cells (20) were found in the blood of these patients. Therefore, a reduced emigration of autotransfused lymphocytes and, hence, higher than normal recovery values in the blood have been expected in these 4 groups of patients with enlarged blood B cell fractions. The increased blood recovery values in the 2 groups of leukaemic NHL-patients are consistent with these considerations. However,

the lower than expected recovery values in the blood of the treated HD- and ST-patients led to the assumption that in these 2 groups of irradiated patients not only the T lymphocytes but also a fraction of the B cells emigrated from the blood.

Furthermore, reverse to the recovery data in the blood, increased recovery values of autotransfused lymphocytes must be expected to appear in the peripheral lymph of the untreated HD- and the irradiated ST-patients, normal recovery values for the treated HD-patients and the lowest recovery fractions in the leukaemic patients. Whereas the in fact lowest lymph recovery values in the CLL- and other leukaemic NHL-patients are in agreement with these considerations, the actually observed recovery data in the lymph of the 2 groups of patients with HD- and the ST-patients are lower again than the expected ones (Fig. 2). This discrepancy between the blood and lymph recovery values in both groups of HD-patients as well as the ST-patients is taken to indicate that in these patients either the fraction of B cells emigrated from the blood may remain in the interstitium or that B cells do not pass through the endothelial cells of postcapillary venules within nonlymphoid tissue.

The increase of the B cell fractions in the blood of the irradiated and the leukaemic patients correlated with correspondingly reduced lymphocyte recovery values in the peripheral lymph. On the other hand, regarding separately the recirculation of the labelled blood T lymphocytes, normal T lymphocyte recovery values have been determined even in the leukaemic patients. It is concluded that only T lymphocytes are able to recirculate via the peripheral lymph even in the leukaemic NHL-patients.

Defective migration ability of neoplastic B cells. The constant recovery values at 1 to 4 hours after autotransfusion and onwards (Fig. 1) are taken to indicate an equilibrium between labelled and unlabelled lymphocytes leaving and entering the blood lymphocyte pool. Thus, whereas in the 4 groups of aleukaemic patients (N-, untreated and irradiated HD- and ST-patients) the vast majority of autotransfused

lymphocytes rapidly emigrated from the blood despite increased B cell fractions in the irradiated patients, considerably higher proportions of the neoplastic B lymphocytes remained intravascularly in the leukaemic NHL-patients. The prolonged blood transit time of both types of leukaemic B lymphocytes may be caused by:

1. Defective migration ability of neoplastic NHL-cells either because of their B cell nature or of their cellular abnormality;
2. Limited passage capacity of postcapillary venules;
3. Restricted entrance into and traffic through pathologically enlarged lymph nodes and spleen showing effacement of normal architecture.

Since the studies on peripheral lymph, exhibiting no or very few B cells in all patients, exclude any influence of pathological alteration of lymph nodes and spleen, the first explanation is more likely than the third one; in addition, thoracic duct lymphocyte recovery was normal in an aleukaemic NHL-patient despite splenomegaly and generalized lymphadenopathy (6).

The second explanation that a restriction or limitation may exist concerning how many cells can pass through the walls of the postcapillary venules per time unit, must be considered, since the total number of emigrating B lymphocytes was considerably higher in the 2 groups of leukaemic NHL-patients. However, akin to normal situation, also in CLL lymph nodes a constant ratio was found between the lymphocyte numbers in the vessel lumen, in the endothelial wall of the postcapillary venules and in the perivascular sheet irrespective of the blood lymphocytosis; an increase in blood lymphocytosis was correlated with a proportionate rise of emigrating lymphocytes (21).

Thus, the first explanation seems to be the most likely (3). But since the irradiated patients exhibited, in particular, normal blood lymphocyte transit times despite enlarged B cell fractions, the prolonged blood transit times of both types of leukaemic B lymphocytes indicate, that this intravascular retention cannot be related to the B cell characteristics

but must be attributed to (a) cellular alteration(s) of the leukaemic B cells, possibly associated with neoplastic transformation processes. An extended intravascular sojourn has been found also for other types of leukaemic leukocytes (22–23). Therefore, a prolongation of the respective blood transit time seems to be a generally altered parameter of the circulation kinetics of leukaemic leukocytes.

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Discussion

Godal: Could it not be that you have different kinds of B-cells normally circulating and that they then – these CLL-cells belong to let us say a subgroup of normal B-cells with these characteristics. Can you exclude that?

Bremer: Making it short, this cannot be excluded. We have expected that patients with increased B-cell percentages in the blood will have a slower than-normal disappearance of their labelled blood lymphocytes. Taking the recovery values to compare the different groups of patients, the normals exhibited initial recovery values of about 34%, which then went down very rapidly. Since B-cells do not emigrate as well as T-cells from the blood, we therefore expected higher recovery values within one to four hours after autotransfusion in the irradiated patients with Hodgkin's disease or seminoma testis. In these irradiated patients we expected recovery values between those of the two groups of leukaemic lymphoma patients and the normal controls. But this was not the situation, since their recovery values were similar to or even lower than those of the normal patients. Only the lymphocytes of the leukemic patients with CLL and of the other leukemic lymphoma patients behaved as expected; they showed increased recovery values. Therefore, we concluded that the B-cell nature per se does not modify to any significant extent the migration or the intravascular blood transit times of blood B lymphocytes, but that the high recovery values in the leukemic lymphoma patients are due to the neoplastic nature of these leukemic B lymphocyte populations; their neoplastic nature may be expressed by an alteration of the cell surface possibly resulting in increased rigidity of membrane fluidity, altered electric surface charge or something like this.

Ford: I fully agree with Dr. Bremer's interpretation. Some evidence that normal B lymphocytes recirculate from blood to thoracic duct lymph in man has come from the Lyon group (Revillard et al., 1968). They found that approximately $15 \pm 5\%$ of human TDL bore B cell markers and this proportion remained almost constant as the total output of lymphocytes fell during thoracic duct drainage. No doubt almost all of the B lymphocytes were recirculating across the high endothelial venules within lymph nodes since you have shown that very few B cells are

present in afferent lymph. I am assuming that this finding holds good for peripheral lymph from all tissues.

Godal: Well, I am only playing a devil's advocate, because we have done quite a lot of studies on the membrane function in chronic lymphocytic leukemia (see Int. J. Cancer 21: 561, 1978) and so compared to the great majority of circulating B-cells they are very aberrant in the sense that they do not cap a variety of surface antigens, there is no increased influx of rubidium in response to B-cell mitogens like anti-beta-2-microglobulin and so on. They do not respond to a number of mitogens except A23187, which probably bypass the membrane event in the mitogenic stimulation. So it would fit very well with our data.

Olszewski: Do the leukemic cells incorporate more label than normal lymphocytes, are they more fragile, can it in some way affect your searchings?

Bremer: The uptake of ^3H -cytidine differs from cell to cell; but on the average, the activity of CLL lymphocytes in incorporating ^3H -cytidine as well as ^3H -uridine is 10 times higher than that of the normal lymphocytes.

Olszewski: If these cells incorporate more of the isotope and then if you calculate total activity per same number of cells and refer to the controls, the readings might in some way be inadequate.

Bremer: This should not alter the data because we exposed the autoradiograms until a labelling index of about 100% is reached in normal and CLL lymphocytes; on the average, the labelled lymphocytes had a quite high number of about 50 grains. Furthermore, control studies have shown that the viability of the lymphocytes is not altered during the in vitro labelling, and there are no more data pointing to a rapid sequestration or destruction of a significant quantity of the autotransfused labelled lymphocytes. Thus, there is no evidence for an in vivo labelling of additional lymphocytes, which may be prevented also by washing off the excess of extracellular free radioactivity of the blood plasma before retransfusion.