In Vivo Tracing of Indium-111 Oxine-Labeled Human Peripheral Blood Mononuclear Cells in Patients with Lymphatic Malignancies

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The in vivo migration of [^{111}In]oxine-labeled peripheral mononuclear cells (PMNC) was studied in 20 patients with various lymphatic malignancies and palpable enlarged lymph nodes. The maximal labeling dose of 10 μCi (0.37 MBq) [^{111}In]oxine/10^8 PMNC was found not to adversely influence either cell viability or lymphocyte proliferation in vitro. For in vivo studies, 1.5×10^9 PMNC were gained by lymphapheresis and reinjected intravenously after radioactive labeling, 150 μCi (5.55 MBq). The labeling of enlarged palpable lymph nodes was achieved in three out of three patients with Hodgkin's disease and in five out of five with high-malignant lymphoma, whereas three out of seven patients with low malignant lymphoma and no patient with chronic lymphatic leukemia had positive lymph node imaging. We thus conclude that PMNC retain their ability to migrate after [^{111}In]oxine labeling and that these cells traffic to involved lymph nodes of some, but not all hematologic malignancies.

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The migration of lymphocytes from blood to spleen and their further traffic to peripheral lymph nodes is a well-described phenomenon in animals (*1–3*). Since the technical achievement of an easy labeling of white blood cells with indium-111 oxine (^{111}In*; 4), some reports have dealt with the in vivo traffic or peripheral blood mononuclear cells (PMNC) in humans as well (*5–7*). Lymphocyte traffic is not only a prerequisite for an effective immune response, but could also be involved in the spread of lymphatic malignancies. Thus, in vivo tracing of migrating human PMNC could be a valuable tool for studying the basic biology of various disorders and for a further clinical understanding of such disorders as lymphatic malignancies, graft rejection, or granulomatous inflammatory diseases in which lymphocyte traffic might play an important pathogenic role.

Cell labeling with a radioactive substance for the study of lymphocyte traffic should result in only marginal reduction in viability and proliferative responses to mitogens, and in the least adverse effects upon migratory abilities. While using a low amount of radioactive [^{111}In]oxine, we have performed in vitro studies that included the testing for viability and responses to mitogens by labeled peripheral blood mononuclear cells (PMNC) derived from ten healthy control individuals as well as in vivo investigations for the migration patterns of PMNC in 20 patients with various lymphatic malignancies.

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MATERIALS AND METHODS

Lymphocyte Isolation and In Vitro Assays for the Determination of the Optimal Dose of Radioactivity

PMNC derived from peripheral blood of ten healthy control individuals and two patients with chronic lymphatic leukemia and separated over density gradients with Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) (*8*) were incubated in 1 ml RPMI 1640- (Roswell Park Memorial Institute medium 1640, Gibco, Paisley, UK) without fetal calf serum (FCS) for 10 min at room temperature with different concentrations of [^{111}In] oxine (SGAE, Seibersdorf, Austria). Thereafter, PMNC were washed once in RPMI 1640 supplemented with 10% FCS to remove unbound [^{111}In]oxine and incubated in RPMI 1640 with 10% FCS at 37°C in a humidified atmosphere containing 5% CO_2 for varying times (24–96 hr, Table 1). Viability was determined by trypan blue exclusion test. Lymphocyte proliferation was assayed by 3H-thymidine incorporation after stim-

TABLE 1
Viability of Human Peripheral Blood Mononuclear Cells After Labeling with Various Amounts of [^{111}In]Oxine*

Time after labeling	[^{111}In]oxine/10^8 PMNC 0 μCi (0 MBq)	3 μCi (0.11 MBq)	10 μCi (0.37 MBq)	30 μCi (1.11 MBq)	100 μCi (3.7 MBq)
0 hr	99%	99%	99%	99%	99%
24 hr	99%	99%	95%	86%	81%
48 hr	95%	90%	85%	61%	42%
72 hr	95%	92%	79%	40%	12%
96 hr	88%	86%	69%	18%	8%

*Data of one representative experiment in one person are shown.

ulation with mitogens phytohemagglutinin (PHA; Wellcome, Beckenham, UK), concanavalin A (ConA; Pharmacia, Uppsala, Sweden) and pokeweed mitogen (PWM; Gibco, Grand Island, NY) using standard methods.

Patients

Twenty patients (ten males, ten females; mean age 59.0 ± 13.9 yr, range 25–82 yr) with lymphomas of various histology (duration of disease: 1 mo–15 yr) were studied (Table 3). The histologic diagnosis was obtained by lymph node biopsy in all patients. Ten patients had not received radiation or chemotherapy until the time of investigation, the other ten patients who had undergone different treatment protocols including radiation and/or chemotherapy were studied at least 4 wk after termination of their last treatment. Informed consent was obtained from all patients studied.

Controls

Three patients with active Crohn's disease without enlarged peripheral lymph nodes were investigated as disease controls. Healthy normal control individuals were not tested for ethical reasons.

Lymphapheresis for In Vivo Studies

In order to fulfill the requirement of using a low radioactive dosage, i.e., 10 μCi (0.37 MBq)/10^8 PMNC and to get adequate gamma-camera images at the same time, it was necessary to obtain a large number of lymphocytes. Therefore, an IBM 2997 blood cell separator was used to collect the buffy coat, followed by a depletion of contaminating platelets by repeated low-velocity centrifugation (200 g for 20 min) in saline and a separation step of PMNC by centrifugation over a Ficoll-Hypaque gradient, as described above. After these purification steps, which were carried out under strict sterile conditions in laminar air flow, the number of PMNC and platelets were assessed using a Coulter counter; in addition, the viability of PMNC was assayed by trypan blue exclusion and a smear for the determination of neutrophil contamination was prepared. Cells purified by the described method consisted of more than 98% PMNC (75–85% lymphocytes, 15–25% monocytes, and 1–2% granulocytes), but were—despite extensive washings—contaminated with platelets to varying degrees ranging from ten to 0.5 platelet(s) per mononuclear cell.

PMNC Labeling for In Vivo Migration Studies

1.5×10^9 PMNC were labeled in a volume of 1 ml saline by adding 150 μCi (5.5 MBq) of [^{111}In]oxine and incubated at room temperature for 10 min. In order to remove unbound ^{111}In, PMNC were washed once in RPMI 1640 with 10% FCS and resuspended in 0.9% saline. Aliquots were drawn for the determination of labeling efficiency (always between 92% and 96%), for the assessment of viability as well as the number of PMNC and platelets after the labeling process. The used lots of RPMI 1640 and FCS were found to be free of endotoxin, as assessed by the limulus lysate assay (*9*).

Heat-Damaged Labeled PMNC

After labeling PMNC in the described manner, the lymphocyte suspension was incubated in a water bath at 56°C for 1 hr. Thereafter, the number and viability (>75%) of PMNC were determined.

In Vivo Studies

For in vivo studies, 1.5×10^9 autologous PMNC labeled with 150 μCi (5.5 MBq) [^{111}In]oxine were injected into the cubital veins of the patients who were lying in supine position under the gamma camera. In six patients with low platelet contamination of labeled PMNC, serial blood samples were drawn via an indwelling vein canula on the other forearm prior to and at 5, 15, 30, 45, and 60 min intervals after reinjection of PMNC. Further samples were obtained in variable intervals during the next 3 days. All samples were gamma-counted at the end of the investigation. Results were expressed as percent of radioactivity present in the respective sample, as compared to the one taken 5 min after reinjection.

Gamma Camera Imaging

A large field-of-view gamma camera (Searle Radiographics Inc., Netherlands) connected to a data processor (PDP 11/34, Digital Equipment Int. Ltd., Galeway, Ireland) was used. Data were stored on disks for further computer processing and analysis. The gamma camera was equipped with a high-sensitivity collimator (Searle) and the lower energy peak of ^{111}In (173 keV) was selected as the energy window. In some patients, a sequential image was recorded during the first hour after reinjection of labeled PMNC that consisted of 12 frames with 300 sec each, with the collimator placed over the lungs, heart, spleen, and liver. Routine static gamma camera images with an acquisition time of 300 sec were taken 1, 24, 48, 72, and 96 hr after reinjection and consisted of three views: (a) head, neck, shoulder, and axillary region; (b) thoracic and upper abdominal region including spleen and liver; and (c) lower abdominal region including inguinal, iliac and paraaortal lymph nodes, but excluding liver and spleen.

Assessment of the Spleen/Liver Index

Counts over defined and constant regions of interest located in either of these two were measured, and the final result was calculated as a ratio of counts (i.e., spleen/liver).

RESULTS

In Vitro Experiments

Viability. The viability of PMNC of three healthy persons labeled with different [^{111}In]oxine doses followed by an incubation period of varying length was determined by trypan blue exclusion test (Table 1). These data show that the higher the [^{111}In]oxine dosage

TABLE 2
Mitogen-Induced Proliferation of [^{111}In]Oxine Labeled Peripheral Blood Mononuclear Cells
(Culture Period: 6 Days)

Mitogen	[^{111}In]Oxine/10^8 PMNC 0 μCi (0 MBq)	[^{111}In]Oxine/10^8 PMNC 10 μCi (0.37 MBq)
PHA (1:100)	57.886 ± 7.011*	52.041 ± 3.527
ConA (60 μg/ml)	42.637 ± 7.637	41.583 ± 6.036
PWM (1:25)	43.788 ± 7.306	40.884 ± 6.666

* Proliferation was assessed by 3H-thymidine incorporation following stimulation with the respective mitogen. Mean dpm ± s.d. of optimal mitogen concentrations obtained in one representative experiment performed in triplicate are given.

used for labeling, the lower was the number of viable cells recovered after culture. Similar data were obtained with PMNC derived from two patients with chronic lymphatic leukemia (data not shown). Extrapolating from these data, we concluded that not more than 10 μCi (0.37 MBq)/10^8 PMNC should be used for cell labeling in order to avoid impairment of cell viability during a time period of 4 days.

Proliferation. Labeling of PMNC derived from healthy control individuals with 10 μCi (0.37 MBq) [^{111}In]oxine per 10^8 PMNC did not influence adversely lymphocyte proliferation in response to the three mitogenic lectins PHA, PWM, or ConA, as compared to unlabeled cells (Table 2).

In Vivo Data

Heat-damaged PMNC. One week after having studied them with undamaged autologous cells, four patients were restudied using autologous-labeled cells damaged by incubation at 56°C for 1 hr. This procedure did not reduce viability (>75% by trypan blue exclusion test) in vitro, but resulted in a trapping of radioactivity by the lungs for more than 6 hr and a prominent imaging of the liver that was probably a result of phagocytosis of damaged cells by the reticuloendothelial system. In these studies, an almost complete accumulation of radioactivity was found in the lungs during the first 3 hr followed by a shift of radioactivity into the spleen and liver which was complete by the end of the observation period, i.e., 72 hr after reinjection of radiolabeled cells. In contrast, in the two patients with lymphoblastic lymphoma studied with heat-damaged cells, the spleen/liver index did not change over time during 72 hr, as seen in the same patients restudied with undamaged cells.

Kinetic studies. These studies were performed in six patients (Fig. 1). During the first hour after reinjection of a preparation of labeled cells with low platelet contamination, a rapid decline of blood radioactivity was observed to values between 45% and 80% of those measured 5 min after reinjection. In three patients suffering from low-malignant lymphoma, a late peak of counts in peripheral blood was seen after 28 to 36 hr. In contrast, three patients with chronic lymphatic leukemia showed a continuous decrease in count rates without the occurrence of a further peak.

Spleen/liver index. The spleen/liver index recorded 30 min after reinjection of labeled cells correlated well (r = 0.83) with the PMNC:platelet ratio in the cell preparation: the lower the platelet contamination, the higher was the spleen/liver index.

Gamma camera imaging. A typical picture arose

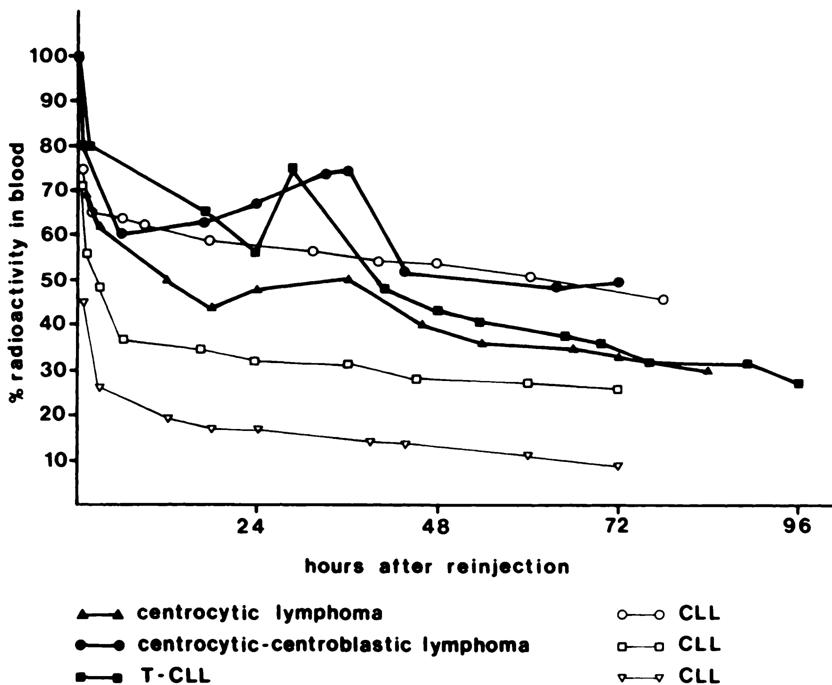

FIGURE 1
Blood kinetic studies of [^{111}In]oxine labeled PMNC in patients with various lymphatic malignancies. Percent radioactivity was calculated as the remaining radioactivity in whole blood relative to the amount measured 5 min after reinjection of labeled cells.

after reinjection of labeled cells. A transient imaging of the lungs was seen during the first 15 min followed by a predominant distribution to the spleen and a moderate activity over the liver and the cardiac region. A slight shift of radioactivity to the liver occurred during the next days, as reflected by a decreasing spleen/liver index. In some patients (see below), radioactivity accumulated in regions corresponding to palpably enlarged lymph nodes that were easily visualized under the gamma camera after a time interval of 18 to 36 hr (Figs. 2 and 3). At that time, a faint imaging of the pelvic bone and the vertebral column was observed in almost all patients indicating either traffic of labeled cells through the bone marrow compartment or phagocytosis of radioactive material by the reticuloendothelial system. Prolonged gamma camera studies for several additional days did not change this picture, except in one patient with prolymphocytic leukemia in whom positive imaging of axillary lymph nodes was detected only after an interval of 4 days.

Imaging of palpable lymph nodes (Table 3). In 11 out of a total of 20 patients with various lymphatic malignancies, an accumulation of radioactivity over palpable and enlarged lymph nodes could be seen. In nine other patients with lymph node enlargement, no accumulation of labeled PMNC was found in these regions. As also shown in Table 3 a successful imaging was associated with certain histologic lymphoma types: all patients with high-grade malignant lymphomas (five out of five) and all patients with Hodgkin's disease (three out of three) had positive lymph node imaging. In contrast, only three out of seven patients with low-grade malignant lymphomas (two of the three patients with centrocytic lymphoma and one patient with T-CLL) had lymph nodes visible by gamma camera imaging. No association was found, however, between the presence of a positive lymph node imaging and a prior administration of chemo- or radiotherapy. No accumulation of radioactivity was encountered in palpable lymph nodes of five patients with CLL and in three patients with centrocytic-centroblastic lymphoma. As shown in Table 4, no obvious association was observed between the presence of malignant cells in the circulation and the accumulation of labeled PMNC in enlarged lymph nodes.

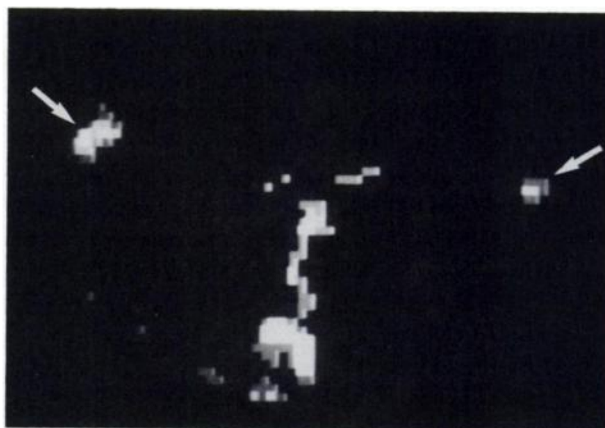

FIGURE 3
Axillary lymph node imaging (single arrows) in a patient with low grade malignant lymphoma.

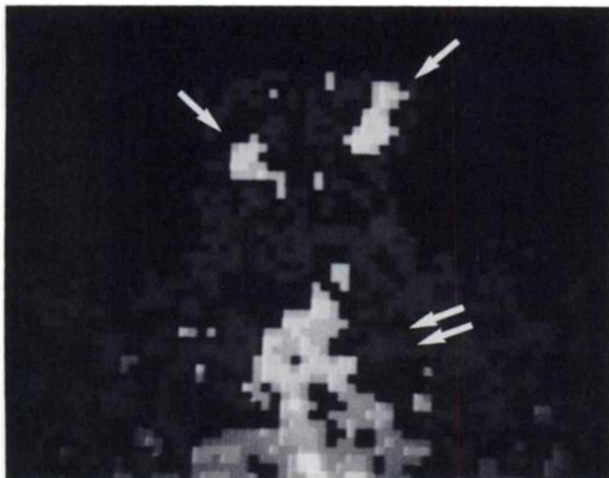

FIGURE 2
Cervical lymph node imaging (single arrows) in a patient with high grade malignant lymphoma. According to results from computed tomography, the radioactivity detected over the mediastinum (double arrows) did not correspond to enlarged lymph nodes. The accumulation of radioactivity corresponds most probably to the reticuloendothelial system.

Imaging of enlarged nonpalpable lymph nodes. Of the 11 patients with positive imaging of palpable lymph nodes, seven also had enlarged lymph nodes at sites not accessible to palpation, but ascertained by ultrasound scanning and/or computerized tomography. The enlarged lymph nodes were located at the mediastinum, the porta hepatis, paraaortally, and along the iliac vessels. The latter were also seen by the described method in four patients in whom a positive imaging of palpable lymph nodes at other body sites had been achieved. In contrast, enlarged lymph nodes located in the mediastinum or at the porta hepatis were not discernible with certainty because of the radioactivity accumulated over the spine or the liver.

Imaging of nonenlarged lymph nodes in control patients. No accumulation of radioactivity was found in nonenlarged lymph nodes of three patients with active Crohn's disease who were investigated as disease controls.

DISCUSSION

In the present paper, we describe the migratory behavior of ^{111}In-labeled PMNC in patients with various

TABLE 3
Characteristics of Patients Included in the Study and Results Obtained by ^{111}In Labeling of Their PMNC

Patient no.	Age (yr)	Sex	Diagnosis	Location of enlarged lymph nodes Palpable	Nonpalpable	Results of ^{111}In PMNC scans
1	25	F	Hodgkin's disease	Cerv., axill.	Mediast.	Cerv., axill.
2	54	M	Hodgkin's disease	Ing.	Iliac.	Ing., iliac.
3	41	M	Hodgkin's disease	Ing.	Iliac.	Ing., iliac.
4	81	F	Prolymph. leukemia	Axill.	Mediast.	Axill.
5	82	F	Ki-I lymphoma	Cerv.	—	Cerv.
6	62	F	Immunoblastic lymphoma	Cerv.	—	Cerv.
7	55	F	Centroblastic lymphoma	Cerv.	—	Cerv.
8	53	F	Centroblastic lymphoma	Cerv., ing.	Iliac.	Cerv., iliac.
9	56	M	T-CLL	Axill.	—	Axill.
10	54	M	Centrocytic lymphoma	Cerv.	Mediast., port. hep.	Cerv.
11	57	M	Centrocytic lymphoma	Cerv., ing.	Retroperit., port. hep.	Cerv., ing.
12	64	M	Centrocytic lymphoma	Cerv., ing.	Retroperit., mediast.	Negative
13	38	M	Centrobl.-centrocyt.l.	Axill.	Mediast.	Negative
14	78	F	Centrobl.-centrocyt.l.	Cerv., axill.	Retroperit.	Negative
15	54	M	Centrobl.-centrocyt.l.	Cerv.	Mediast.	Negative
16	45	M	CLL	Cerv., axill., ing.	—	Negative
17	62	F	CLL	Axill.	Retroperit.	Negative
18	66	F	CLL	Cerv., ing.	—	Negative
19	76	M	CLL	Ing.	Retroperit.	Negative
20	71	F	CLL	Cerv., ing.	—	Negative

lymphatic malignancies. Such studies seem to be of particular interest for a better understanding of lymphocyte traffic and may eventually result in beneficial therapeutic approaches in lymphatic malignancies. Moreover, the modulation of the immune response in nonmalignant diseases could be reached by the interference with recirculating regulatory and effector lymphocytes. Thus, the study of lymphocyte traffic in lymphomas could constitute a model for further investigations.

We report that radioactivity first accumulated predominantly over the spleen followed by a redistribution to areas corresponding to enlarged lymph nodes after reinjection of ^{111}In-labeled PMNC in 11 out of the 20 investigated patients after an interval of 18 to 36 hr. In an effort to further analyze this phenomenon, it was found that the migration of PMNC to peripheral lymph nodes was associated with certain histologic lymphoma types: three out of three patients with Hodgkin's disease, five out of five patients with high malignant lymphomas, but only three out of seven patients with low malignant lymphomas and none of the five patients with CLL had positive lymph node imaging. This is in contrast to other studies (*10*) in which enlarged lymph nodes in patients with CLL could easily be seen, perhaps as a result of the higher amount of total radioactivity injected in patients included in the latter reports. A similar explanation could be valid for our disease controls without enlarged lymph nodes in which no accumulation of radioactivity sufficient for positive imaging was encountered. It is rather unlikely that the only use of the lower energy peak of radioactive indium might have resulted in a lower sensitivity of detection. When considering our results it is intriguing, nevertheless, that reinjection of a constant number of PMNC labeled with the same amount of [^{111}In]oxine into patients with various histologic lymphoma types resulted in a divergent behavior, thus suggesting a varying migration pattern. It has to be taken into account that—in contrast to the other lymphomas studied—PMNC of patients with CLL are predominantly malignant that could influence their migratory behavior. Thus, also blood kinetic studies showed a divergence between patients with CLL and those with other low-malignant lymphomas. In accordance with other authors (*6,7*), also our patients with CLL had a steady decrease of radioactivity counts in peripheral blood over time, whereas the other patients typically showed a second rise of blood radioac-

TABLE 4
Results of [^{111}In]Oxine Imaging of Enlarged Lymph Nodes in Relation to the Presence of Malignant Cells in the Circulation

Diagnosis	No of patients with: Circulating malignant cells	Positive imaging	Imaging and circulating malignant cells
High malignant lymphoma (n = 5)	3	5	3
Low malignant lymphoma (n = 7)	3	3	2
Hodgkin's disease (n = 3)	0	3	0
Chronic lymphatic leukemia (n = 5)	4	0	0

tivity starting between 6 and 24 hr after reinjection (*5,7,10,11*). In patients with CLL, even prolonged blood kinetic studies lasting 72 hr did not reveal a second peak, suggesting that the B-cells in CLL could either take longer than 3 days to traverse the spleen (*1*) or remain sequestered in it. Interestingly, one patient with T-CLL and enlarged peripheral lymph nodes had a distinct second peak in blood radioactivity as soon as 24 hr after reinjection of the labeled PMNC, corresponding to data of other authors (*12*). This points to the well-known fact that B-cells have a different migratory behavior than T-cells (*1,2*) with the T cells' spleen transit time being considerably shorter (*13*).

When interpreting our results, it is necessary to distinguish between shifts in radioactivity to different body regions as a result of the physiologic traffic of lymphocytes and technical artifacts produced by damaged PMNC that have lost their normal migratory abilities. Cell damage can result from inappropriate in vitro handling of cells or by the radiation effect on the cells in vivo (*14,15*). In order to assess the effect of possible physical damage of PMNC on some functional parameters, we have carried out in vitro and in vivo experiments: Dye exclusion tests of PMNC labeled with different dosages of [^{111}In]oxine and incubated for various times clearly showed that a labeling dose of higher than 10 μCi (0.37 MBq)/10^8 PMNC intolerably reduced cell viability. This effect was most prominent at >48 hr after labeling when cells were expected to have left the spleen and to have migrated to peripheral lymph nodes (*1*). Among other parameters indicating an alteration in PMNC function, lymphocyte responses to mitogenic lectins are valuable indicators of a physiologic behavior after labeling (*16*): PMNC labeled with 10 μCi (0.37 MBq)/10^8 PMNC that was also used for in vivo studies showed no loss of proliferative capacity in response to either of the three tested lectins.

The next experiment produced an in vivo evidence for the result of cell damage. Serial gamma-camera imaging of patients injected with viable, but heat-damaged cells showed a completely different picture than the one obtained in the same patients who were studied using undamaged cells a few days later, in that heat-damaged PMNC accumulated in the lungs for a long time followed by a predominant accumulation of radioactivity over the liver. In contrast to the situation found with nondamaged PMNC, heat-damaged PMNC accumulated only faintly over the spleen. The different in vivo distribution of radioactivity seen with damaged and nondamaged cells suggests that the described method of [^{111}In]oxine labeling of PMNC probably does not alter the migratory behavior. Similar conclusions have been drawn by other authors who used both, animal models and investigations in man (*5–7,16–18*).

Furthermore, it is interesting to note that we were not able to achieve an accumulation of radioactivity in all involved sites in every patient with high-grade malignant lymphoma. This fact could have such explanations as a divergent blood supply in differing anatomic locations or differences in host responses to the disease.

Another possible pitfall in the interpretation of in vivo migration studies of PMNC is the extent of contamination with platelets that are also enriched during the cell separation procedure. When correlating the platelet contamination per single PMNC with the ratio of radioactivity over spleen and liver accumulating 30 min after reinjection of labeled cells, we have found that the number of platelets highly influenced the relative distribution of radioactivity between these two organs. Although the volume of a platelet (*19*) is approximately only 1/300th of a lymphocyte (*5*), the presence of a high number of platelets could affect the measured blood kinetics and the spleen:liver distribution of radioactivity during the first phase of the study. In contrast, in patients with lymphomas other than CLL the variation in distribution of various cell types within the reinjected PMNC populations was rather small thus excluding this to be a reason for the observed differences in migratory behavior.

In conclusion, our study showed that it is possible to obtain a sufficient number of PMNC and to label them with [^{111}In]oxine without a significant loss of viability or proliferative capacity. In addition, the difference in migration between heat-damaged and nondamaged lymphocytes suggests that the traffic of [^{111}In]oxine labeled PMNC is not significantly altered by the labeling procedure. The migratory pattern of PMNC derived from patients with various lymphatic malignancies may mirror a divergent biologic behavior, as it has also been demonstrated in connection with other parameters including cell surface markers, chromosome deviations, the speed and tendency to dissemination, and responses to therapeutic measures.

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REFERENCES

1. Ford WL. Lymphocyte migration and immune response. *Prog Allerg* 1975; 19:1–59.
2. Sprent J. Recirculating lymphocytes. In: Marchalonis JJ, ed. *The lymphocyte: structure and function.* New York: Marcel Dekker, 1977.
3. Rannie GH, Thakur ML, Ford WL. An experimental comparison of radioactive labels with potential application to lymphocyte migration studies in patients. *Clin Exp Immunol* 1977; 29:509–514.
4. McAfee JG, Thakur ML. Survey of radioactive agents

for in vitro labeling of phagocytic leukocytes. I. Soluble agents. *J Nucl Med* 1976; 17:480–487.

5. Lavender JP, Goldman JM, Arnot RN, Thakur ML. Kinetics of indium-111 labelled lymphocytes in normal subjects and patients with Hodgkin's disease. *Br Med J* 1977; 2:797–799.
6. Wagstaff J, Gibson C, Thatcher N, et al. A method for following human lymphocyte traffic using indium-111 oxine labelling. *Clin Exp Immunol* 1981; 43:435–442.
7. Wagstaff J, Gibson C, Thatcher N, Ford WL, Sharma H, Crowther D. Human lymphocyte traffic assessed by indium-111 oxine labelling: clinical observations. *Clin Exp Immunol* 1981; 43:443–449.
8. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968; 21 (suppl 97):77–80.
9. Yin ET, Galanos C, Kinsky S, et al. Picogram-sensitive assay for endotoxin: gelation of Limulus polyphemus blood cell lysate by purified lipopolysaccharide and lipid A from gram-negative bacteria. *Biochim Biophys Acta* 1972; 261:284–290.
10. Wagstaff J, Gibson C, Thatcher N, Crowther D. The migratory properties of In-111-oxine labelled lymphocytes in patients with CLL. *Br J Haematol* 1981; 49:283–291.
11. Scott JL, Davidson JG, Marino JV, McMillan R. Leukocyte labelling with Cr-51. III. The kinetics of normal lymphocytes. *Blood* 1972; 40:276–282.
12. Miller RA, Coleman CN, Fawcett HD, et al. Sezary syndrome: a model for migration of T lymphocytes to skin. *N Engl J Med* 1980; 303:89–92.
13. Yamauchi K, Nagao T, Sugihara M, Suzuki Y. Kinetics of indium-111-labelled T-lymphocytes in a patient with adult T-cell leukaemia. *Scand J Haematol* 1983; 30:353–355.
14. Chrisholm PM, Danpure HG, Healey G, Osman S. Cell damage resulting from the labeling of rat lymphocytes and HeLa S3 cells with In-111 oxine. *J Nucl Med* 1979; 20:1308–1311.
15. Sparshott SM, Sharma H, Kelly JD, Ford WL. Factors influencing the fate of 111-indium-labelled lymphocytes after transfer to syngeneic rats. *J Immunol Meth* 1981; 41:303–320.
16. Issekutz T, Chin W, Hay JB. Measurement of lymphocyte traffic with indium-111. *Clin Exp Immunol* 1980; 39:215–221.
17. Dutcher JP. Labeled cells in patients with malignancy. *Semin Nucl Med* 1984; 14:251–261.
18. Schroeder S, Caughran M, Jochelson M, et al. Imaging of lymphoid structures with indium-111-labeled lymphocytes. *Invest Radiol* 1983; 18:87–93.
19. Hardeman MR. Cell isolation techniques: a critical review. In: Thakur ML, Ezekowitz MD, Hardeman MR, eds. *Radiolabeled cellular blood elements*. New York—London: Plenum Press, 1984.