In Vivo Traffic of Indium-111-Oxine Labeled Human Lymphocytes Collected by Automated Apheresis

Elizabeth J. Read, Andrew M. Keenan, Charles S. Carter, Penney S. Yolles, and Richard J. Davey

Department of Transfusion Medicine and Department of Nuclear Medicine, National Institutes of Health, Bethesda, Maryland

The in vivo traffic patterns of autologous lymphocytes were studied in five normal human volunteers using lymphocytes obtained by automated apheresis, separated on Ficoll-Hypaque gradients, and labeled ex vivo with ^{111}In-oxine. Final lymphocyte infusions contained 1.8–3.1 × 10^9 cells and 270–390 μCi (9.99–14.43 MBq) ^{111}In, or 11–17 μCi (0.41–0.63 MBq) per 10^8 lymphocytes. Gamma imaging showed transient lung uptake and significant retention of radioactivity in the liver and spleen. Progressive uptake of activity in normal, nonpalpable axillary and inguinal lymph nodes was seen from 24 to 96 hr. Accumulation of radioactivity also was demonstrated at the forearm skin test site, as well as in its associated epitrochlear and axillary lymph nodes, in a subject who had been tested for delayed hypersensitivity with tetanus toxoid. Indium-111-oxine labeled human lymphocytes may provide a useful tool for future studies of normal and abnormal lymphocyte traffic.

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Studies of the migration and recirculation of radiolabeled lymphocytes have been essential to understanding the physiology of the lymphoid system. Such studies have defined the complex patterns of recirculation for both T and B lymphocytes, as well as the contribution of these circulation patterns to immune responses (*1*). The majority of these studies have been carried out in animal models, because the best available methodology involves invasive procedures such as cannulation of the thoracic duct or smaller efferent lymphatics, and sometimes requires killing the animal to allow autoradiography or gamma counting of organs or tissues.

The potential for carrying out in vivo studies of lymphocyte kinetics in animals and in humans was expanded with the introduction of indium-111 (^{111}In) for cell labeling. This radionuclide has several distinct advantages over chromium-51 and technetium-99m for lymphocyte labeling (*2,3*). When bound to lipophilic chelates, which allow passive diffusion across the cell membrane, ^{111}In labels cytoplasmic contents with high efficiency. Its physical half-life of 67 hr, lack of clinical toxicity at commonly used doses, and emission of two gamma photons at 173 and 247 keV with high abundance (89% and 94%, respectively) have made it a valuable cell label for in vivo human studies involving external gamma camera imaging.

Although ^{111}In has made in vivo lymphocyte kinetic studies feasible without the use of invasive procedures, several methodologic concerns remain, especially those related to the detrimental effects of the labeling process, both radiologic and physical, on the lymphocytes. In vivo animal studies and in vitro studies of human lymphocytes have demonstrated impairment of lymphocyte function as the concentration of the ^{111}In label is increased, and several reports have recommended a limit of 20 μCi of ^{111}In per 10^8 lymphocytes to ensure adequate viability and function of the labeled lymphocytes throughout several days of a kinetic study (*4–8*). This study was designed to examine the traffic of normal human peripheral blood lymphocytes using a larger number of cells than previously attempted, in an effort to minimize the effects of the radioactive label on the cells. In addition, we incorporated whole-body counting, improved imaging techniques, and quantitative measurement of organ uptake by computer-assisted image analysis, in order to delineate as precisely as possible the traffic patterns of labeled lymphocytes.

METHODS

Human Subjects

Five normal volunteer subjects, four males and one female, were studied using a protocol approved by our institutional review board and the National Institutes of Health Radiation Safety Committee. All subjects had previously donated blood and undergone automated hemapheresis procedures without difficulty and underwent standard blood donor screening immediately prior to this study. None of the subjects had any acute or chronic illnesses or abnormalities of the immune

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For reprints contact: Elizabeth J. Read, MD, Department of Transfusion Medicine, Bldg 10, Rm. 5D-56, National Institutes of Health, Bethesda, MD 20892.

system, and none were taking any medications known or suspected to interfere with lymphocytes or the immune system. All subjects gave informed consent. On the day before his apheresis procedure, one subject (Subject 5) received a subcutaneous injection of tetanus toxoid (TT) on the right forearm and of keyhole limpet hemocyanin (KLH), an antigen to which he had never had prior exposure, on the left forearm.

Collection and Preparation of Peripheral Blood Lymphocyte Suspensions

Peripheral blood mononuclear cells were collected using a two-armed automated lymphocytapheresis procedure on the Cobe 2997 blood cell separator (Cobe Laboratories, Lakewood, CO). Acid citrate detrose, solution A (ACD-A, Fenwal Laboratories, Roundlake, IL) was used for anticoagulation. For each subject, 3.0–6.0 liters of blood was processed to collect a total of 3–4 × 10^9 mononuclear cells and 200 ml of autologous plasma. Further processing of the mononuclear cell collections was carried out using strict sterile technique under a laminar flow hood. Cell suspensions were layered onto Ficoll-Hypaque density gradients (LSM, Organon Teknika, Bethesda, MD) in 50-ml conical tubes (Falcon, Becton Dickinson, Lincoln Park, NJ), and centrifuged at 600 × g for 25 min at 20°C. Mononuclear cells at the interface were collected, pooled, and washed twice in phosphate buffered saline (PBS, M.A. Bioproducts, Whittaker, Walkersville, MD) to remove contaminating platelets and residual Ficoll-Hypaque. No attempt was made to remove monocytes from the cell suspensions. Autologous plasma was centrifuged at 1600 × g for 10 min to remove remaining cellular material prior to its use in the labeling procedure.

Radiolabeling of Lymphocyte Suspensions

To each lymphocyte suspension, 400 μCi ^{111}In-chloride (Medi-Physics, Richmond, CA) and 50 μg purified oxine (JT Baker Company, Phillipsburg, NJ), previously dissolved in ethanol at a weight/volume concentration of 1 mg/ml, were added. (Since this study was completed, our technique has been modified to use the commercially available ^{111}In-oxine preparation (Amersham, Arlington Heights, IL); comparable results have been obtained.) After the volume was brought up to 20 ml with PBS, the suspension was incubated for 15 min at room temperature, with gentle rocking. Following the incubation, labeled lymphocytes were washed twice with and then resuspended in autologous plasma to a final volume of 20 ml. The final labeled lymphocyte suspension was filtered through a 140-micron, 16-gauge filter needle (Monojet, Sherwood Medical, St. Louis, MO) immediately prior to infusion.

In Vitro Functional Assays

For the in vitro studies, lymphocytes from seven normal donors were collected, separated, and labeled by methods similar to those used for the in vivo studies, except that the total number of cells labeled was lower (~10^8), and the labeling volume was 2 ml. Lymphocytes were incubated at concentrations of 30 μCi (1.11 MBq) and 50 μCi (1.85 MBq) per 10^8 cells. Proliferation of the lymphocytes was assayed by ^{3}H-thymidine incorporation following mitogen or antigen stimulation using standard methods. Cultures with concanavalin A (Con A) and phytohemaglutinin (PHA) were assayed after 3 days, and those with pokeweed mitogen (PWM) and TT after 5 days. Proliferation in response to the mitogenic or antigenic stimulus was expressed as a percentage of the value obtained for the unlabeled control lymphocytes.

Preparation of Standards and Measurement of Labeling Efficiency

Standards (STD) for gamma counting were prepared in triplicate by transferring a weighed 50-μl aliquot of the labeled cell suspension to a counting vial and adding normal saline to a total volume of 1.0 ml. Standards of the cell-free supernatant (CFS-STD) were prepared in a similar manner by obtaining weighed 50-μl aliquots of the supernatant following hard centrifugation of a 1.0-ml aliquot of the labeled lymphocyte suspension. The fraction of the total activity in the infused cell suspension that was cell-associated (%CAAI) was calculated as follows:

$$\%\ \text{Cell-associated activity of infused suspension (\%CAAI)} = \frac{(\text{cpm in STD} - \text{cpm in CFS-STD})}{\text{cpm in STD}} \times 100\%.$$

White blood cells (WBCs) and red blood cells (RBCs) were counted electronically (Model M430, Coulter Electronics, Hialeah, FL), as were platelets (Model P260, Coulter Electronics, Hialeah, FL), on each cell suspension prior to and following labeling. White cell differential counts were determined from Wright's stained blood smears. Mononuclear cell viability was assessed by trypan blue dye exclusion on slide preparations of cells.

Radioactivity of the lymphocyte suspension during the incubation (total added activity), and after the labeling procedure (final activity), as well as radioactivity of the supernatant, were measured by a dose calibrator. Labeling efficiency was calculated as follows:

$$\text{Labeling efficiency} = \frac{\text{Final activity } (\mu\text{Ci})}{\text{Total added activity } (\mu\text{Ci})} \times 100\%.$$

Infusion of Lymphocyte Suspensions and Post-Infusion Blood Sampling

Labeled lymphocyte suspensions were infused over 1 min through a 19-gauge steel butterfly needle into a peripheral vein, followed by a normal saline flush of 20 ml. Serial blood samples were collected from a separate venipuncture site at 5 and 30 min and at 1, 2, 24, 48, 72, and 96 hr postinfusion. For each sample, 7 ml of peripheral blood were drawn through a 19-gauge needle into a syringe and transferred immediately into a tube containing potassium EDTA at a 0.15% (w/v) concentration. Gamma counting was carried out on the standards prepared in triplicate from the injected cell suspension, and on duplicate whole-blood aliquots and a single cell-free plasma aliquot from each blood sample, using an Auto Gamma 5000 gamma counter (Packard Instrument Company, Sterling, VA). Gamma counting parameters were set to include both gamma emissions as well as the summation peak of ^{111}In and to give a counting error (sigma) of <2%. Cell-associated radioactivity was calculated and expressed in cpm/

volume of whole blood for each time point as follows:

$$\text{Cell-associated activity (cpm/ml blood)} = \text{(cpm/ml whole-blood aliquot)} - \text{(cpm/ml plasma aliquot} \times (1 - \text{Hct})).$$

The recovery of cell-associated radioactivity at each time point was also expressed as a fraction of the injected cell-associated radioactivity by using the following formula:

$$\text{Percent recovery of cell-associated activity} = \frac{\text{cell-associated activity (cpm/ml blood)}}{\text{cpm/ml STD} \times \text{dilution of STD} \times \%\text{CAAI}} \times \frac{\text{blood volume (ml)} \times 100\%}{\text{injected volume (ml)}}.$$

In addition, the recovery of plasma-associated radioactivity was expressed as a fraction of the total injected radioactivity by using the following formula:

$$\text{Percent recovery of plasma-associated activity} = \frac{\text{cpm/ml plasma} \times (1 - \text{Hct})}{\text{cpm/ml STD} \times \text{dilution of STD}} \times \frac{\text{blood volume (ml)} \times 100\%}{\text{injected volume (ml)}}.$$

Blood volume was calculated for each subject by a standard formula based on height and weight (*9*).

Whole-Body Counting

Whole-body retention of ^{111}In was measured with a total-body counter designed to detect millicurie levels of activity. This system consisted of a 12.7 × 10.2 cm thallium-activated sodium iodide scintillation detector located 230 cm above a flat stretcher. Subjects were positioned so that their umbilicus was located directly beneath the center of the detector. The system was relatively insensitive to changes in tracer distribution within the body due to the large distance between the detector and the subjects. The detector output was connected to a multi-channel analyzer and all pulses with energies between 156 and 280 keV (a range which included both of the major photopeaks of ^{111}In) were integrated for each measurement. Decay-corrected counts obtained at sequential time points were expressed as percentages of the total initial activity.

Gamma Camera Imaging and Computer-Assisted Image Analysis

Gamma camera imaging was carried out at ~2 hr after injection and then daily for four days using a jumbo field-of-view General Electric MaxiCamera 500 (General Electric, Milwaukee, WI) equipped with a medium-energy parallel-hole collimator for static imaging and a diverging collimator for whole-body scanning. Twenty percent windows were centered over the 173 and 247 keV photopeaks of ^{111}In. Anterior and posterior whole-body scans and 10-min regional scintiphotos of the anterior chest and the anterior abdomen, including the pelvis, were recorded on film and computer.

Regions of interest (ROIs) were manually drawn around computer-digitized images of both lungs, the liver, the spleen, and bilateral lymph node groups in the axillary and inguinal areas from the anterior regional scintiphotos. Radioactive counts in each region were decay-corrected for comparison of tracer uptake at serial time points. Correction for background activity was not necessary due to the rapid blood clearance immediately following tracer injection. Liver and spleen uptakes were assumed to be underestimated by a factor of two due to tissue attenuation, based on the half-value layer of ~15 cm in water for the gamma photons of ^{111}In. Attenuation correction was not used for counts in the axillary and inguinal lymph nodes due to their relatively superficial location, and it was not used for lung counts due to the large volume of air in lung tissue. Counts per minute in individual organs were expressed as percentages of the total injected activity derived from whole-body images. The whole-body counts per minute calculated to the time of injection were assumed to represent the total injected activity (100%).

RESULTS

In Vitro Functional Studies

Results of the in vitro functional studies are presented in Table 1. In seven experiments representing lymphocytes of seven different donors, mean lymphocyte proliferation following mitogenic stimulation (Con A, PHA, PWM) was reduced significantly to ~50% ($p < 0.01$) after labeling at 30 μCi (1.11 MBq) per 10^8 cells, and was further reduced to ~30% ($p < 0.01$) after labeling at 50 μCi (1.85 MBq) per 10^8 cells. In vitro proliferation following stimulation with the TT antigen was more severely impaired at both labeling concentrations. These data suggested that labeling for the in vivo studies be carried out at concentrations well below 30 μCi (1.11 MBq) per 10^8 cells, if possible.

TABLE 1
Mitogen- and Antigen-Induced Proliferation in Normal Human Lymphocytes: Effect of Labeling with Two Different ^{111}In Concentrations*

	Labeling concentration (^{111}In activity per 10^8 lymphocytes)		
Mitogen or Antigen	0 μCi	30 μCi	50 μCi
Concanavalin A	100	53.3 ± 23.9	31.6 ± 15.8
Phytohemagglutinin	100	51.3 ± 18.5	32.6 ± 16.1
Pokeweed mitogen	100	56.4 ± 31.7	32.9 ± 14.4
Tetanus toxoid	100	14.7 ± 27.6	4.0 ± 6.3

* Lymphocytes obtained from each of seven normal donors were separated, incubated with ^{111}In-oxine at 30 and 50 μCi (or none) for 15 min, washed, and placed in culture. Lymphocyte proliferation was measured by ^{3}H-thymidine incorporation after stimulation with the mitogen or antigen in 3-day (Con A, PHA) or 5-day (PWM, TT) cultures. Results are expressed as a percentage of the proliferative response measured in unlabeled control lymphocytes (0 μCi). This table presents the means ± s.d.s for the seven experiments. Mitogen- and antigen-induced proliferative responses were significantly lower in lymphocytes labeled at 30 and 50 μCi than unlabeled controls ($p < 0.01$); 50 μCi led to significantly greater impairment than 30 μCi ($p < 0.01$) for all but tetanus toxoid.

TABLE 2
Labeling Characteristics of ^{111}In-oxine Labeled Lymphocyte Suspensions in Five Normal Subjects

Subject	% labeling efficiency	% viability	% cell-bound activity	Total activity (μCi)	Activity (μCi) per 10^8 lymphs
1	81	98	99	320	18
2	88	98	98	350	11
3	98	96	100	390	17
4	80	99	99	319	14
5	72	99	97	270	13
$\bar{X}$	84	98	99	330	14.6
s.d.	9.8	1.5	1.1	44	2.9

In Vivo Studies

Cell Collection and Characteristics of Lymphocyte Suspensions. The absolute lymphocyte count in the peripheral blood of the five normal subjects ranged from 1,078 to 1,683 per microliter. Lymphocyte concentrates containing $3.8 \pm 0.8 \times 10^9$ (range $3.2–5.1 \times 10^9$) leukocytes (92% lymphocytes, 7% monocytes, and 1% granulocytes) were collected by processing 3.0–6.0 liters of the subject's blood in the automated blood cell separator. Leukocyte counts in the labeled lymphocyte suspensions ranged from 1.8 to 3.1×10^9 ($X \pm$ s.d. = $2.3 \pm 0.5 \times 10^9$, of which 90%–100% were lymphocytes, 0%–10% were monocytes, and <1% were granulocytes.

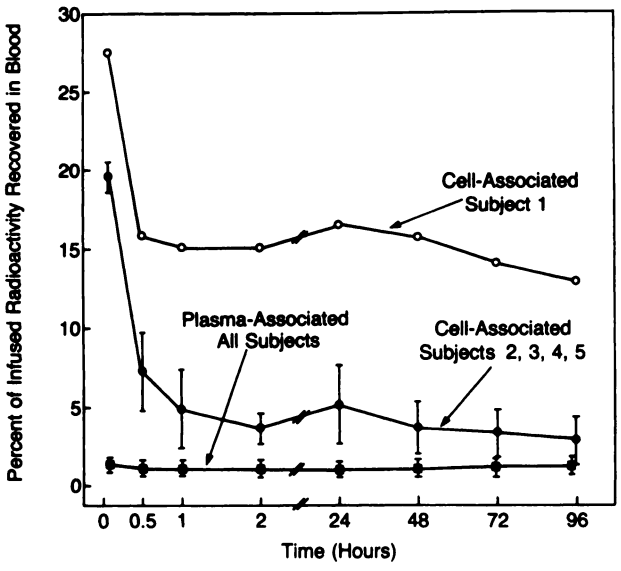

FIGURE 1
Clearance of cell-associated radioactivity and plasma-associated radioactivity following infusion with ^{111}In-labeled autologous lymphocytes. Plotted points on the top curve (open circles) represent recoveries of cell-associated radioactivity in Subject 1. These were substantially higher at all time points than for the other four normal subjects, whose data is presented (as mean ± s.d.) below (closed circles). Recovery of radioactivity in the plasma-associated portion of blood, presented as mean ± s.d. for all five normal subjects (closed squares), consistently represented <2% of the infused radioactivity.

Final lymphocyte suspensions were contaminated with $<10^8$ erythrocytes and $<10^8$ platelets except in Subject 1, whose injected suspension of 1.8×10^9 leukocytes contained 6×10^8 erythrocytes.

Labeling characteristics of the lymphocyte suspensions are presented in Table 2. Mean labeling efficiency was 84%. Viability ranged from 96% to 99%. Total activity injected ranged from 270 to 390 μCi (9.99–14.43 MBq), with a mean of 330 μCi (12.2 MBq), and mean activity per 10^8 lymphocytes was 14.6 (0.54 MBq). In no case did the activity exceed 20 μCi (0.73 MBq) per 10^8 lymphocytes. Percent cell-associated activity ranged from 97%–100%, indicating that only minimal free ^{111}In was being infused with the labeled lymphocyte suspensions.

Blood Clearance of Labeled Lymphocyte Suspensions. The blood clearance of the labeled lymphocytes is illustrated in Figure 1. Because the clearance data for Subject 1 appeared to be quantitatively different from the other four subjects, that individual's clearance is graphed separately from the others. Mean (± s.d.) intra-

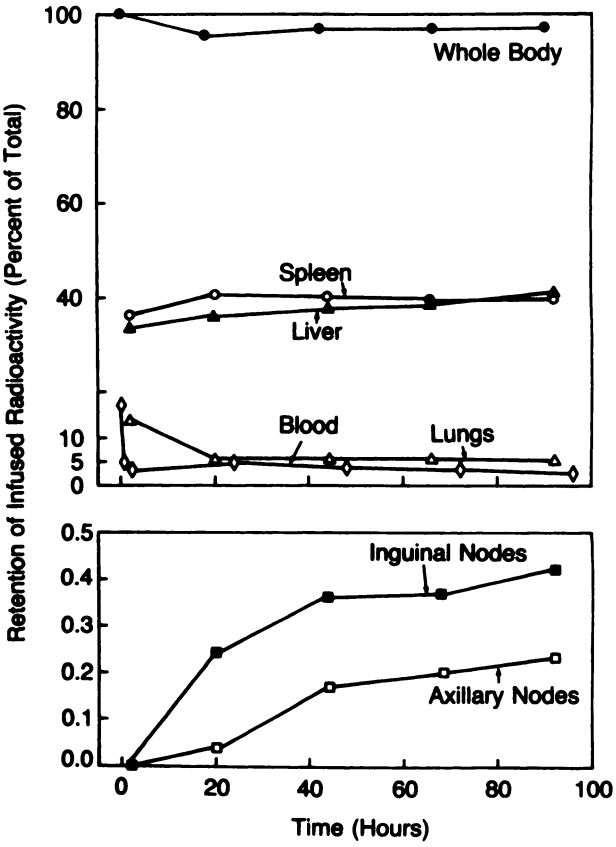

FIGURE 2
Quantitation of whole-body retention, blood clearance, organ retention, and lymph node retention of ^{111}In-oxine labeled autologous lymphocytes in four normal subjects. Whole-body retention of ^{111}In was measured by whole-body counting. Blood clearance was quantitated by gamma counting of whole blood and plasma aliquots of blood samples. Organ and lymph node retention were quantitated from computer-assisted analysis of counts from gamma camera images.

vascular recovery of cell-associated radioactivity for the four subjects was 17.2% ± 0.9% at 5 min, 7.3% ± 2.5% at 30 min, 4.9% ± 2.5% at 1 hr, 3.7% ± 0.9% at 2 hr, 5.1% ± 2.4% at 24 hr, and then declined steadily to 2.8% by 96 hr following infusion. The intravascular recovery for Subject 1 was 27.8% at 5 min, 16.2% at 30 min, 15.1% at 1 hr, 15.1% at 2 hr, 16.6% at 24 hr, 15.5% at 48 hr, 14.1% at 72 hr, and 12.8% at 96 hr. This clearance curve resembles that of the four other normal subjects, but the curve is shifted in the direction of greater activity at each time point, suggesting the presence of an additional population of labeled cells with a relatively longer intravascular survival. Recovery of labeled cells at 24 hr was higher, for all subjects, than the recovery at 2 hr. Thereafter, the intravascular recovery of labeled lymphocytes declined steadily. In all subjects, radioactivity recovered in the plasma at all time points consistently represented less than 2% of total activity infused.

Whole-Body Retention of ^{111}In. Whole-body radioactivity measurements showed that more than 95% of the injected activity was retained during the four-day period of study in all three subjects assayed. These data are presented graphically in Figure 2, along with organ retention and blood clearance data.

Gamma Camera Imaging. Representative gamma camera images are presented in Figure 3. In all subjects, gamma camera images showed early distribution of tracer to lungs, liver, and spleen, with slight body background activity at 2 hr postinjection. The greatest amount of uptake was measured in the liver and spleen at all time points. Lung uptake cleared substantially during the first 24 hr of the study. Bone marrow activity was observed at low levels as early as 2 hr postinfusion, but increased slightly over the next 48 hr, and could be seen in the ribs, spine, and pelvis. Lymph node uptake in the axillary and inguinal regions was observed on all 24-hr images, and showed a steady rise over the first 72 hr. Quantitative measurements of uptake are shown in Figure 2, along with quantitation of blood clearance and whole-body retention. Retention of the label in the liver and spleen over the 96-hr postinfusion period rose slightly above the initial uptake measured at 2 hr. Quantitation of uptake in lymph nodes showed that the major increase in uptake of the label occurred in the 48 hr following cell infusion, but that uptake continued to rise through the final scan at 96 hr.

Subject 5, who had received a subcutaneous TT skin test on the right forearm and a KLH skin test on the left forearm one day prior to apheresis and infusion, showed uptake at the right forearm skin test site and in the ipsilateral epitrochlear and axillary lymph nodes within 24 hr of injection (Fig. 4). Uptake in the right axillary node region was greater than on the left side at all time points and appeared sooner after injection. No uptake was seen in the left forearm or left epitrochlear at any time during the study. These findings on gamma images corresponded to clinical evidence of a typical delayed hypersensitivity skin response on the right forearm, accompanied by tenderness and enlargement of the right epitrochlear, but not axillary, lymph node.

DISCUSSION

A major issue raised by in vivo studies of radiolabeled lymphocytes is how well the traffic patterns of the labeled cells, which are subject to physical and radiologic damage from the collection, separation, and labeling procedures, represent the traffic of unlabeled, unmanipulated cells. Previous studies have documented a variety of detrimental effects of ^{111}In labeling on lymphocytes. Impairment of the in vitro proliferative re-

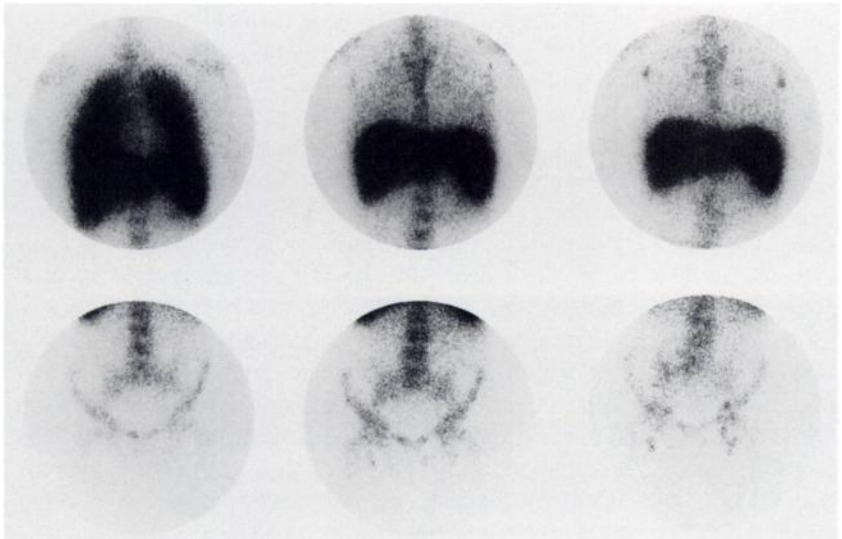

FIGURE 3
Gamma camera images of the chest and abdomen in a normal subject following infusion of ^{111}In-oxine labeled autologous lymphocytes. At 2 hr, uptake of cells is detected in the spleen, liver, and lungs. By 24 hr, the lung activity has cleared. Uptake of labeled cells in the axillary and inguinal lymph nodes is visualized on the 24- and 72-hr images.

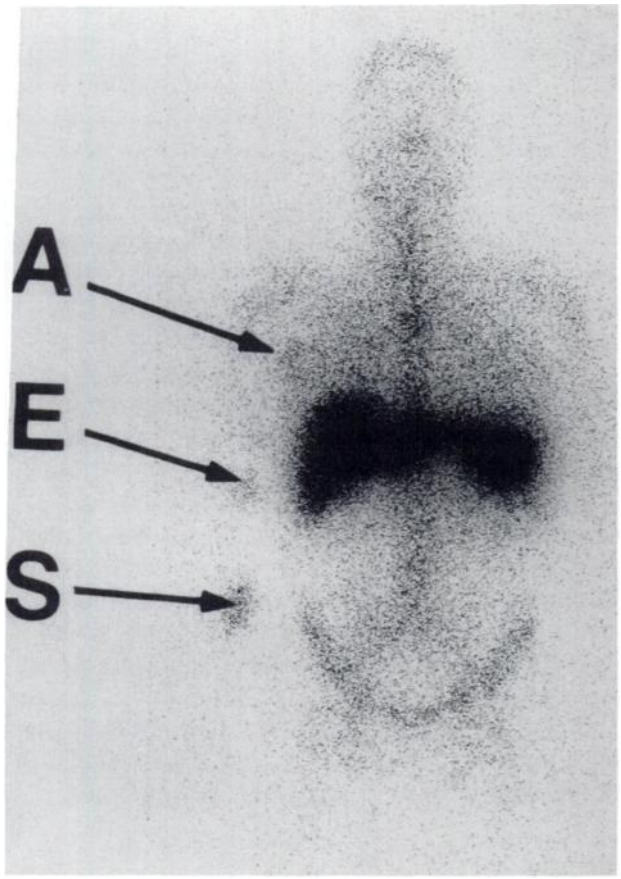

FIGURE 4
Whole-body gamma camera image following infusion of ^{111}In-oxine labeled autologous lymphocytes in a normal subject who had a TT skin test placed on the right forearm and a control skin test on the left forearm one day prior to lymphocyte infusion. This scan, taken 24 hr after lymphocyte infusion, demonstrates the accumulation of labeled lymphocytes at the tetanus skin test site and its associated epitrochlear and axillary lymph nodes.

sponse to mitogen and antigen stimulation has been demonstrated with increasing concentrations of ^{111}In, for both animal and human lymphocytes (*7,8*). The results of our own in vitro studies are consistent with those of others and show that lymphocytes labeled at 30 and 50 μCi (1.1 and 1.8 MBq) per 10^8 lymphocytes have significant impairment, but by no means complete ablation, of the proliferative response to mitogenic and antigenic stimuli.

Whether and how well lymphocyte functional damage detected by in vitro studies predicts changes in their in vivo migratory behavior or other functions is unclear. In previous normal human studies, infusion of lymphocytes labeled at concentrations higher than 20 μCi per 10^8 cells resulted in their apparent homing to normal lymph nodes over a 48-hr period (*10–12*). However, studies in rats that used sampling through a thoracic duct cannula and gamma counting of tissues from killed animals showed significant impairment of thoracic duct lymphocytes to recirculate to thoracic duct lymph and to migrate to normal lymph nodes were observed when the labeling concentration exceeded 20 μCi of ^{111}In per 10^8 lymphocytes (*5,*).

Current concepts of the mechanisms of lymphocyte homing or trafficking would suggest that a distinction be made between the migratory characteristics and the proliferative functions of lymphocytes. The migratory characteristics of lymphocytes appear to be dependent on interactions between cell surface receptors on circulating lymphocytes and complementary receptor molecules located in the high endothelial venules of lymphoid organs, and possibly in nonlymphoid tissues when inflammation is present (*13*). One cannot assume that a labeling procedure that impairs the proliferative response would necessarily interfere with the cell surface molecules necessary for cell-cell interactions. For example, radiation most likely exerts its biologic effects by damaging DNA in chromosomes, as well as the nuclear membrane (*14*). Proliferation, which relies on intact DNA, would appear to be more susceptible to radiation-induced damage than would migratory functions dependent on cell surface structures. Therefore, although a high degree of impairment on in vitro proliferative assays may indicate that the labeling procedure is too harsh or that the level of radioactivity will result in cell death within days of labeling, all such assays should be interpreted with caution, and in relationship to the nature of the in vivo phenomenon under investigation.

In spite of the uncertain relationship between in vitro and in vivo lymphocyte studies, we aimed to minimize potential problems associated with radiosensitivity by using automated lymphapheresis to collect large numbers of peripheral blood lymphocytes in order to satisfy our goal of maintaining a labeling dosage below 20 μCi (0.74 MBq) per 10^8 lymphocytes. Our labeled lymphocytes had a mean of 14.6 μCi, and never more than 20 μCi, of ^{111}In per 10^8 cells. They not only accumulated over the 96 hr of the study in normal lymph nodes, but also were demonstrated to migrate, over the same time period, to the site of a delayed hypersensitivity response and its draining lymph nodes, suggesting that the trafficking function of at least a portion of these cells was not significantly impaired. This is the first in vivo demonstration in humans that radiolabeled lymphocytes can function an immune response to an antigenic challenge, although it is not unexpected in view of a previous study showing ^{111}In-labeled lymphocytes functioning in vivo in a sheep model (*15*).

Differences in methodology unrelated to the dose of radioactivity may also be responsible for differences in results from one trafficking study to the next. It is notable that our normal blood clearance curve for labeled autologous lymphocytes has lower recoveries at all time points than the normal curves published by Wagstaff and Lavender, which show initial recoveries

of 40% and 50%, respectively, and plateaus in the range of 20% (*10–12*). Subject 1, whose lymphocyte suspension was collected from a smaller blood volume and contained more red cells had blood clearance more like the clearance curve reported by other investigators than that of the other four normal subjects in our study. Although the difference between our data and Wagstaff's could be attributed to differences in physical damage to the labeled lymphocytes or in red cell or platelet contamination of the cell suspensions, another possibility is that the mononuclear cell suspensions we studied had a different composition than those of Wagstaff or Lavender. In our department, the percentage of B-lymphocytes in lymphocyte concentrates collected by automated lymphapheresis procedures on normal subjects ranges from 5% to 10%, substantially lower than the 20% B-lymphocytes in the normal subject reported by Wagstaff (*11*). In addition, although monocytes always represented <10% of the content of the labeled lymphocyte suspensions, the contribution of monocytes to the trafficking measurements may be overrepresented, by virtue of their larger size. Another difference is our collection of a larger number of lymphocytes ($3–5 \times 10^9$) by means of an automated lymphapheresis procedure that differed from Wagstaff's because it involved apheresis by a continuous, rather than intermittent, flow procedure and a larger total volume of blood processed. These automated apheresis procedures are not known to cause any harmful effects on lymphocytes, and are not likely to differ in this regard from other techniques that involve simple phlebotomy and centrifugation of freshly drawn venous blood to obtain lymphocytes, as Lavender used (*10*). However, it is possible that the larger volume processed and continuous flow of our automated apheresis procedure could have mobilized and collected a larger proportion of lymphocytes from organs and tissues, as opposed to simply collecting those that were in circulation. If this were the case, one might expect both a lower recovery of labeled cells in the blood and also a different pattern of organ uptake. Other factors in the preparation of the cells, such as the media in which they were suspended or the density gradient medium (Ficoll-Hypaque) used for separation, were not notably different from those used in other studies, but should be considered as possible factors that could affect the function of the labeled lymphocytes.

Our observation of transient uptake of labeled lymphocytes is comparable to the lung uptake reported by both Thakur and Weiblen in studies of radiolabeled autologous human granulocytes (*16,17*) where lung activity was maximal immediately following infusion, but no longer present on the 4-hr images. Some investigators have argued that the lung trapping of autologous leukocytes is purely a function of cell damage caused by the separation and labeling procedures (*11,18*). However, because the pulmonary circulation has pressures that are significantly lower than pressures in the systemic circulation and a capillary bed that is subject to transient narrowing during expiration, it should not be surprising that some portion of any cell infusion, especially a large one administered over a short period of time, would be temporarily retained in the pulmonary circulation (*19*). The transient nature of this trapping in the case of both autologous granulocytes and lymphocytes suggests that whatever damage is present may be mild enough to be overcome by the majority of the cells within several hours. The lack of lung uptake in Wagstaff's studies is curious and could be due to infusion of lower numbers of cells, a more prolonged infusion time, differences in imaging techniques, or a lower degree of cell damage.

Although we did not investigate the long-term fate of ^{111}In in these studies, our data indicate that ^{111}In, when infused as ^{111}In-labeled lymphocytes, is retained almost completely in the body, a finding that confirms the results of others (*3*). Other studies have shown that the elution of ^{111}In from lymphocytes is low (*3*) and that there is no in vivo relabeling of lymphocytes or other cells by free ^{111}In (*15*). The ^{111}In from labeled lymphocytes is transferred over a seven-day period to noncirculating radioresistant cells, presumably by phagocytosis in the spleen, lymph nodes, and other reticuloendothelial sites representing their final destination (*5*). However, our inability to distinguish cell-bound from non-cell-bound ^{111}In in areas other than in the blood always raises the possibility that free ^{111}In might be contributing to specific organ uptake. Free ^{111}In binds avidly to plasma transferrin, and is cleared from the circulation with a half-life of 6–10 hr, accumulating primarily in bone marrow and liver (*20*). However, the absence of substantial amounts of free ^{111}In in our infused suspensions as well as the low and continuously decreasing level of plasma-bound ^{111}In recovered in the blood suggest that ^{111}In from infusion or from intravascular cell lysis was not contributing significantly to estimates of organ and tissue retention.

We are currently applying the methods used in this study to investigate the in vivo traffic of autologous lymphocytes in autoimmune disease, allogeneic monocytes in patients with severe infections, and autologous tumor-infiltrating lymphocytes cultured and stimulated ex vivo prior to labeling and reinfusion (*21*). Another group has recently applied similar methods to studies of patients with Hodgkin's and non-Hodgkin's lymphomas (*22*). Design and interpretation of such studies require careful attention to the methods of cell collection, composition and viability of the cell suspension, presence of free ^{111}In in the suspension, and the manner and timing of cell infusion. Further improvements in cell separation technology, labeling techniques, and imaging techniques are likely to contribute to the utility

of these studies in defining the normal and abnormal kinetics of lymphoid cells.

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