Labeled Leukocyte Imaging: Dawn of an Era

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More than one hundred years after the discovery of penicillin, infection remains a major threat to humankind. In 2017, more than 8 million deaths and 400,000 years of life lost were due to infection, making it first in morbidity and third in mortality among human diseases (*1*). Diagnosing infection is challenging, and imaging studies are often used for confirmation, localization, and assessment of its extension. For nearly 50 years, beginning with ^{67}Ga, molecular imaging has played an important role in the diagnosis of infection. Suboptimal imaging characteristics, the typical 48- to 72-h delay between administration and imaging, and the inability of ^{67}Ga to differentiate infection from inflammation, tumor, and trauma inspired the search for better agents (*2*).

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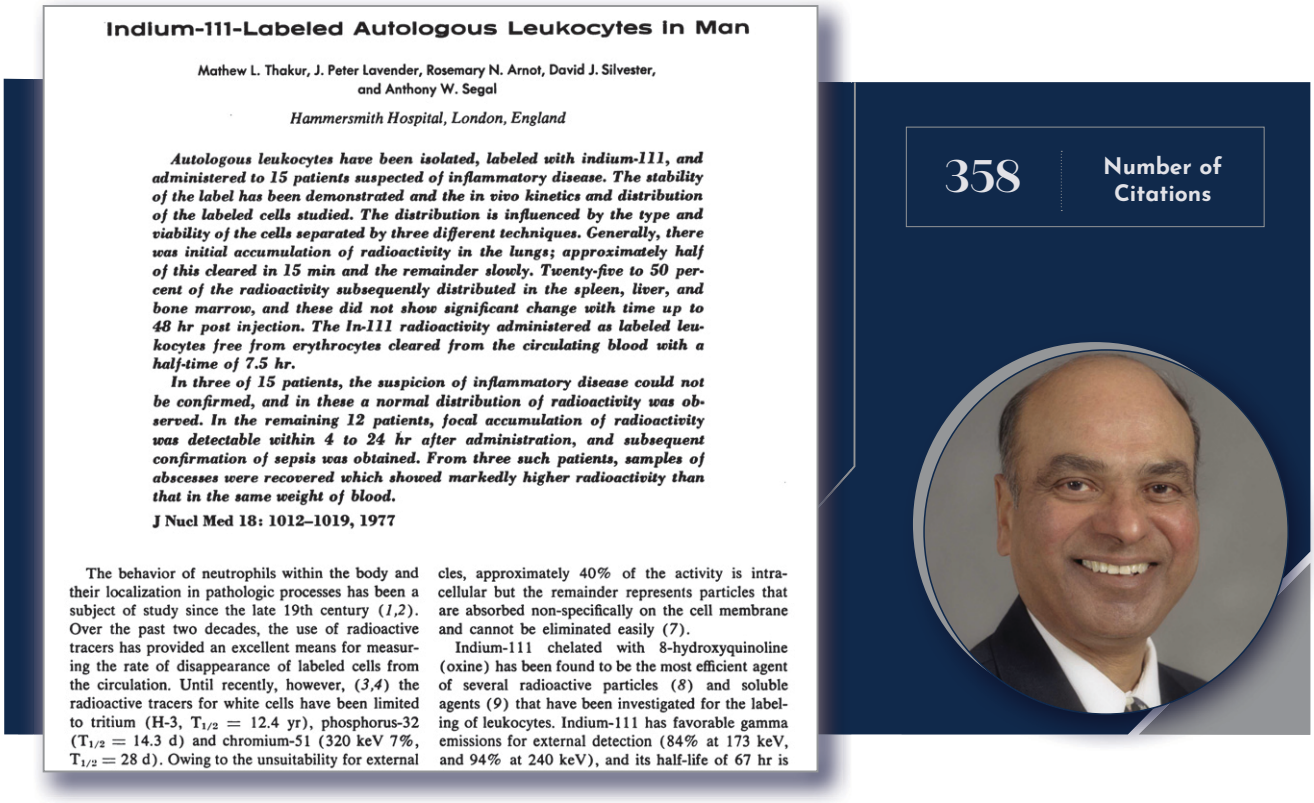
In the mid 1970s, the demonstration by Thakur et al. (*3*) that autologous leukocytes labeled in vitro with ^{111}In-oxine could image infection in humans was a seminal event. The concept on which this test is based is simple: imaging of a physiologic process, the in vivo migration of radiolabeled leukocytes. Although the concept is simple, the execution is not and depends on several factors, including the imaging characteristics of the radionuclide, the label stability, and the labeled cell viability. Tritium, ^{32}P, and ^{51}Cr, which had been used to label leukocytes, provide data on leukocyte clearance from the blood but are not suitable for studying in vivo kinetics and distribution of leukocytes and cannot be used for localizing infection. ^{111}In has γ-emissions 174 keV and 247 keV, which are suitable for imaging. Its 67-h half-life is sufficiently long to image leukocyte migration and accumulation (*3*).

The label must be stable, and the labeled cells must be viable. Significant radionuclide elution from the cells would confound the interpretation of the test because it would not be possible to differentiate accumulation of labeled leukocytes from accumulation of other radiolabeled complexes or free radionuclide. Once inside the leukocyte, ^{111}In binds to intracellular components



Indium-111-Labeled Autologous Leukocytes in Man

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Autologous leukocytes have been isolated, labeled with indium-111, and administered to 15 patients suspected of inflammatory disease. The stability of the label has been demonstrated and the in vivo kinetics and distribution of the labeled cells studied. The distribution is influenced by the type and viability of the cells separated by three different techniques. Generally, there was initial accumulation of radioactivity in the lungs; approximately half of this cleared in 15 min and the remainder slowly. Twenty-five to 50 percent of the radioactivity subsequently distributed in the spleen, liver, and bone marrow, and these did not show significant change with time up to 48 hr post injection. The In-111 radioactivity administered as labeled leukocytes free from erythrocytes cleared from the circulating blood with a half-time of 7.5 hr.

In three of 15 patients, the suspicion of inflammatory disease could not be confirmed, and in these a normal distribution of radioactivity was observed. In the remaining 12 patients, focal accumulation of radioactivity was detectable within 4 to 24 hr after administration, and subsequent confirmation of sepsis was obtained. From three such patients, samples of abscesses were recovered which showed markedly higher radioactivity than that in the same weight of blood.

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The behavior of neutrophils within the body and their localization in pathologic processes has been a subject of study since the late 19th century (*1,2*). Over the past two decades, the use of radioactive tracers has provided an excellent means for measuring the rate of disappearance of labeled cells from the circulation. Until recently, however, (*3,4*) the radioactive tracers for white cells have been limited to tritium (H-3, $T_{1/2}$ = 12.4 yr), phosphorus-32 ($T_{1/2}$ = 14.3 d) and chromium-51 (320 keV 7%, $T_{1/2}$ = 28 d). Owing to the unsuitability for external cles, approximately 40% of the activity is intracellular but the remainder represents particles that are absorbed non-specifically on the cell membrane and cannot be eliminated easily (*7*).

Indium-111 chelated with 8-hydroxyquinoline (oxine) has been found to be the most efficient agent of several radioactive particles (*8*) and soluble agents (*9*) that have been investigated for the labeling of leukocytes. Indium-111 has favorable gamma emissions for external detection (84% at 173 keV, and 94% at 240 keV), and its half-life of 67 hr is

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with minimal elution; up to 90% of the activity is retained intracellularly at 22 h. Finally, the labeled leukocytes must be viable; leukocyte viability was about 75% (*3*). To put the work of Thakur et al. (*3*) into proper perspective, in the decades since its publication, among the several radionuclides (including positron emitters) investigated for labeling leukocytes, only ^{99m}Tc has yielded a clinically useful agent (*2*).

Compared with the ideal molecular infection imaging agent, which should be safe, available, rapidly completed, and sensitive and specific for infection, labeled leukocyte imaging has several weaknesses. The in vitro labeling process requires handling of human blood products, with its associated hazards for personnel and patients. The procedure is labor-intensive and time-consuming and can be performed only by trained individuals. Consequently, in most institutions, the test is available only during routine working hours.

The issue of sensitivity and specificity is not straightforward. Although labeled leukocyte imaging is used for infection imaging, it is in reality host-response imaging, in which the presence of infection is implied by patterns of labeled leukocyte accumulation. In the usual clinical scenario, most leukocytes labeled are neutrophils; the test is therefore most sensitive for detecting those infections in which the primary cellular response is neutrophilic, such as bacterial infections. The procedure is less sensitive for detecting those infections in which the predominant cellular response is not neutrophilic, that is, tuberculosis and some opportunistic infections (*2*).

Labeled leukocyte imaging is specific for leukocyte-mediated inflammatory processes but is not specific for infection and will be positive in any inflammatory process mediated by neutrophils. For example, the test cannot reliably differentiate septic arthritis from an active inflammatory process such as rheumatoid arthritis (*2*).

Why then does labeled leukocyte imaging—a procedure that is not without some risk, is not ubiquitously available, and is not specific for infection—still have a preeminent position in molecular imaging of infection? The answer is that, to date, no better agent has come along. Numerous attempts have been made to develop in vivo leukocyte labeling methods using antigranulocyte antibodies, antibody fragments, and peptides, none of which have stood the test of time. Generally they are sensitive, but specificity is variable and they have their own safety issues, including human antimurine antibody development precluding repeat use, and severe adverse events occurring shortly after administration. Attempts to develop infection-specific agents such as radiolabeled antibiotics, vitamins, and antimicrobial peptides have had only modest success, and none have ever made it into routine nuclear medicine practice (*2*).

The one agent that has gained widespread acceptance for imaging of infection is ^{18}F-FDG. Though not specific, the test is rapidly completed and provides high-resolution images. Furthermore, ^{18}F-FDG is especially useful for those indications for which labeled leukocyte imaging is of limited value, such as tuberculosis, spondylodiscitis, and fever of unknown origin. ^{18}F-FDG, however, is a complement to, not a replacement for, labeled leukocyte imaging (*2*).

More than 4 decades after its introduction, in vitro labeled leukocyte imaging still occupies a preeminent role in molecular imaging of infection. Considering the rapidity with which modern-day diagnostic imaging tests are developed, this is a remarkable feat.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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In three of 15 patients, the suspicion of inflammatory disease could not be confirmed, and in these a normal distribution of radioactivity was observed. In the remaining 12 patients, focal accumulation of radioactivity was detectable within 4 to 24 hr after administration, and subsequent confirmation of sepsis was obtained. From three such patients, samples of abscesses were recovered which showed markedly higher radioactivity than that in the same weight of blood.

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The behavior of neutrophils within the body and their localization in pathologic processes has been a subject of study since the late 19th century (*1,2*). Over the past two decades, the use of radioactive tracers has provided an excellent means for measuring the rate of disappearance of labeled cells from the circulation. Until recently, however, (*3,4*) the radioactive tracers for white cells have been limited to tritium (H-3, $T_{1/2}$ = 12.4 yr), phosphorus-32 ($T_{1/2}$ = 14.3 d) and chromium-51 (320 keV 7%, $T_{1/2}$ = 28 d). Owing to the unsuitability for external detection of the radiation from these nuclides, it has been impossible to study the in vivo kinetics and distribution of the labeled cells.

Gallium-67 (*5*) and technetium-99m (*6*), both of which can be monitored externally, have been incorporated into leukocytes. These have been used to locate abscesses but are unsuitable for recording the kinetics or total-body distribution of cells. In the case of Ga-67, only 6% of the activity is incorporated within the cell (*5*) and its use has been limited. In the case of Tc-99m sulphur colloid particles, approximately 40% of the activity is intracellular but the remainder represents particles that are absorbed non-specifically on the cell membrane and cannot be eliminated easily (*7*).

Indium-111 chelated with 8-hydroxyquinoline (oxine) has been found to be the most efficient agent of several radioactive particles (*8*) and soluble agents (*9*) that have been investigated for the labeling of leukocytes. Indium-111 has favorable gamma emissions for external detection (84% at 173 keV, and 94% at 240 keV), and its half-life of 67 hr is ideal for in vivo studies. Lipid-soluble In-111 oxine diffuses through the cell membrane, and the In-111 binds to cytoplasmic components and remains in association with labeled cells (*10*). This offers additional advantages over above-mentioned tracers, which are known to elute from the labeled cells in vivo. The autologous In-111-labeled cells have been investigated for their viability and ability to take part in pathologic processes in the dog (*7,11*) and have been used successfully for the location of abscesses in a limited number of patients (*12*). The purpose of the present communication is to report the distribution of autologous labeled leukocytes in man, the radiation dosimetry, the in vivo stability and viability of the labeled cells, and their ability to concentrate in inflammatory lesions.

MATERIALS AND METHODS

1. Patients. Fifteen patients thought to be suffering from inflammatory disease were studied. Their diagnoses were based on standard investigative techniques. The clinical findings and details are given in Table 1.

2. Preparation of indium-111 oxine. Indium-111 was procured as chloride solution in 0.04 *M* HCl*. A suitable quantity of the radioactivity was drawn from the stock and transferred into a sterilized 30-ml conical glass tube to which were added 1 ml water for injections and 0.2 ml of 0.3 *M* sodium acetate buffer, pH 5.5. The contents were mixed and 50 μl of oxine solution in ethanol (1 mg/ml) were added, mixed, and the complex formed was extracted in two equal volumes of chloroform, which were then combined and evaporated to dryness in a boiling-water bath. The residue was dissolved in 50 μl of absolute ethanol to which were added 150 μl of sterile normal saline.

3. Separation of cells. Fifty milliliters of venous blood were drawn into a sterile disposable plastic syringe containing preservative-free heparin (300 IU). In four preparations, 1.5 ml of 2% methyl cellulose (W/V, 600 cp) in isotonic saline were added to the blood under sterile conditions, mixed gently, and the syringe held vertically for 45 to 60 min. In the remaining preparations the erythrocytes (RBC) were allowed to sediment spontaneously during 30 to 40 min. The leukocyte-rich plasma was transferred to 15-ml conical sterile tubes and centrifuged at 450 g for 5 min. The platelet-rich plasma was removed and the cells in the pellet (with contaminating RBC) were gently suspended in normal saline, centrifuged, and the step repeated to wash the cells.

For one patient, leukocyte-rich plasma obtained after spontaneous sedimentation of RBC was centrifuged (450 g, 5 min) and residual erythrocytes in the cell pellet were removed by hypotonic lysis during

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TABLE 1
Case History, Diagnosis, and Observation in In-1 II Leukocyte Studies

Patient No.	Age	Sex	Diagnosis	Observations
1	23	M	Abscess in right paracolic guter 21 days after ileo-colic resection for Crohn's disease. Resolved with antibiotic therapy.	Accumulation of radioactivity in area of abscess.
2	55	F	Wound abscess after biliary tract surgery. Resolved with antibiotic therapy.	Focus of radioactivity in area of wound abscess 22 days post-operative.
3	79	F	Chronic rheumatoid arthritis. Increased activity in knees.	Focal accumulation of radioactivity in knees. Radiolabeled neutrophils isolated from joint cavity.
4	48	M	Persistent pyrexia 5 days after negative laparotomy for possible appendix abscess. Further surgery after scan revealed abscess.	Focus of radioactivity between incision and iliac crest. Radioactivity concentrated in pus from abscess cavity.
5	16	M	Bacterial endocarditis on congenitally stenosed aortic valve.	Patchy aggregates of radioactivity in chest, one of which was possibly related to position of aortic valve.
6	76	F	Biliary colic. No evidence of sepsis.	Normal distribution of radioactivity.
7	52	M	Paralyzed left hemidiaphragm. Initially thought to be left subphrenic abscess.	Elevated left diaphragm widely separated from spleen. No abnormal localization of radioactivity.
8	22	F	Bilateral nephrectomies in past for chronic pyelonephritis. Suspected pelvic abscess.	Accumulation of radioactivity at pentazoline injection site in buttocks. No evidence of abscess.
9	54	M	Crohn's disease with inflammatory mass in right iliac fossa. Surgery performed later revealed a chronic abscess.	Focal increased activity in right iliac fossa (Fig. 4).
10	68	F	Resected volvulus of small bowel followed by peritonitis. Resolved with antibiotics and drainage.	Multiple focal areas of increased activity throughout the abdomen. Activity disappeared after drainage of pus (Fig. 3).
11	65	M	Pyrexial illness with jaundice. Shown to have staphylococcal septicaemia. Multiple soft-tissue abscesses aspirated subsequent to scan.	Multiple focal regions of high activity in soft tissues of shoulders, thighs and legs (Fig. 2).
12	30	F	Intra-abdominal sepsis following bowel resection and leaking anastomosis. Associated with acute renal failure.	Focal increased activity in left paracolic gutter and pelvis.
13	19	F	Crohn's disease with tender mass in right iliac fossa.	Minimal increase of activity in right lower abdomen.
14	40	F	Acute myeloid leukaemia with fever and cellulitis of leg.	Minor increase of activity in both legs.
15	66	M	Diverticulitis with perforation and local peritonitis in pelvis and left paracolic gutter. Scan performed 3 wks post operation.	Focal increase in left paracolic gutter below spleen.

60 seconds using 2.5 ml of water for injections; the solution was then made isotonic by the addition of 2.5 ml of 1.8 M sterile NaCl solution. The cells were centrifuged and supernatant was rejected.

The leukocyte-rich plasma (after spontaneous sedimentation of RBC) from one patient was centrifuged through Ficoll/sodium metrizoate† gradient (*13*), erythrocytes were removed by hypotonic lysis, and the pure pneutrophils thus obtained were washed once in normal saline.

4. Cell labeling and administration. The cells obtained by all preparations were finally suspended in 5 ml normal saline, transferred to a sterile polythene syringe and the In-111 oxine solution added drop-wise. After incubation for 15 min at room temperature, a small aliquot was removed to assess labeling efficiency; 5 ml of plasma were added to syringe, and total radioactivity measured with a calibrated high- pressure ionization chamber. The labeled leukocytes (300-900 μCi) were then administered intravenously by fast-running saline drip. The residual radioactivity in the syringe was measured. A reference solution of In-111 was also prepared with a known amount of radioactivity for use in subsequent quantitative procedures.

5. Analysis and viability of the labeled cells. Three separate batches of the leukocytes separated by the technique using methyl cellulose sedimentation and Ficoll/sodium metrizoate gradient were subjected to analysis of cell type and viability. The leukocytes were smeared, stained and counted differentially for neutrophil, lymphocyte and monocyte content. Red cells in samples obtained after methyl cellulose sedimentation were also counted.

TABLE 2
Cell Separation, Labeling, and Viability

Average of three results	Methylcellulose sedimentation				Ficoll/sodium metrizoate		
	Neutrophils	Lymphocytes	Monocytes	Red cells	Neutrophils	Lymphocytes	Monocytes
In preparation (%)	68	26	9	24	97	1	2
Radioactivity (%)	80	15	—	5	98	—	—
Viable cells (%)	75	—	—	—	45	—	—

In order to estimate the radioactivity associated with each of the different cell populations resulting from the methyl cellulose sedimentation technique, a further separation of these cells was performed using the Ficoll/sodium metrizoate gradient method. Radioactivity associated with neutrophils, lymphocytes and red cells was measured.

The viability of neutrophils resulting from the above two techniques of cell separation was assayed by their ability to phagocytose zymozan particles (2 μ diam) impregnated with nitroblue tetrazolium *(14)* (Table 2).

6. In vivo kinetics of labeled autologous leukocytes. In four patients In-111 labeled autologous leukocytes, with contaminating RBC, were administered and the distribution of radioactivity was recorded continuously for the first 60 min by means of a large-field gamma camera placed posteriorly covering the bases of the lungs, liver, and spleen. The uptake of radioactivity in the lungs, liver, and spleen was recorded with a computer system. In one patient a similar study was also carried out with Tc-99m sulphur colloid (Figs. la, lb).

Five to six samples of heparinized blood were taken from 5 min to 24 hr after the administration of radioactivity, and the percentage of the administered radioactivity per gram of blood in each sample was measured. The samples were spun for 15 min at 1800 g and the radioactivity associated with plasma and cells measured. The results were expressed as a percentage of the total radioactivity in each sample (Table 3).

Urine from two patients was collected for the 24 hr after the administration of labeled cells; the radioactivity was measured and expressed as a percentage of the administered dose.

7. Quantitation of the radioactivity in the liver, spleen, and bone marrow. Five patients were scanned using a whole-body scanner at various intervals up to 48 hr after the administration of labeled pure polymorphonuclear leukocytes and leukocytes with and without contaminating RBC. Data were recorded on a paper tape and the radioactivity in the liver, spleen, and a small volume of bone marrow were computed as percentages of the administered radioactivity (*15*) (Table 4). On one occasion, the results of in vivo quantitations were confirmed by in vitro measurements of radioactivity in the spleen of a neutropenic patient.

RESULTS

Cell labeling and viability. Using 50 μg of oxine, 75-95% of the In-111 activity is chelated at pH 5.5, of which greater than 95% is incorporated within the cells following 15 min of incubation at room temperature. Some preparations were tested for bacterial contamination and found to be sterile, and no adverse reactions were noted in the patients.

Table 2 indicates that the cells separated by the methyl cellulose sedimentation technique are mixed and are contaminated with red cells, which carry approximately 5% of the activity. The cells separated by the Ficoll/sodium metrizoate technique are 97% polymorphonuclear leukocytes and carry greater than 98% of the radioactivity. The viability of these cells, however, is only 45% compared with 75% viability of (mixed) cells, which have not undergone the trauma of further purification. The in vivo stability of the label, measured over 22 hr (Table 3), shows that at least 90% of the radioactivity remains in association with cells separated by either technique.

The urinary excretion of two patients within 24 hr after administration amounted to 0.035% and 0.05% of the administered radioactivity.

In vivo distribution of radioactivity. A graphic representation of the initial dynamics of the cells is shown in Fig. la. Following the injection of cells the radioactivity is immediately distributed throughout the lungs; half

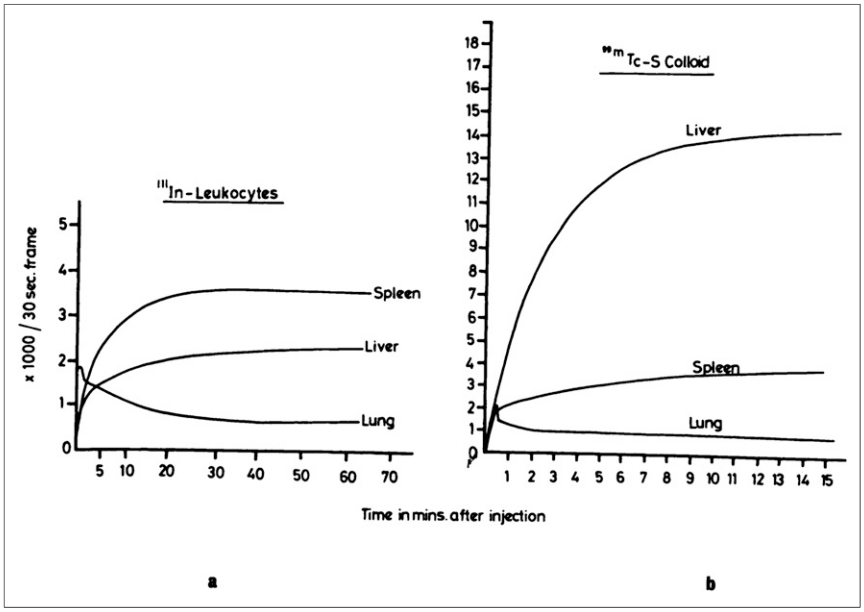

FIGURE 1. Graphical representation of the initial dynamics of (a) In-111-labeled leukocytes and, for comparison, (b) Tc-99m sulphur colloid. It is apparent from (a) that splenic uptake of In-111 radioactivity, unlike that of Tc-99m, is greater than hepatic uptake, and that half of the In-111 radioactivity in the lungs clears in the first 15 min; the rest slowly. By 4 hr after injection most of radioactivity clears from normal lungs.

TABLE 3
In Vivo Stability of In-111 Leukocytes (Percentage of Radioactivity Associated with Plasma)

Time after injection	Patient			
	A.L.	C.C.	V.B.	C.G.
5 min	9.8	5.5	4.5	5.2
1 hr	—	4.0	5.2	4.6
2 hr	8.9	—	—	—
4.4 hr	8.6	—	—	—
6 hr	—	9.2	—	—
7 hr	—	—	5.9	—
8.45 hr	8.3	—	—	—
19.25 hr	8.4	—	—	4.8
20 hr	—	4.3	5.4	—
22 hr	—	—	—	4.6

of this clears within about 15 min. By 4 hr after injection, no detectable radiosactivity was noted in the normal lungs. There is a rapid accumulation of radioactivity in both liver and spleen, with the counting rate per unit area relatively higher in spleen. For comparison, the distribution of radioactive colloid in these three organs, measured similarly, is given in Fig. lb. This indicates a higher uptake in the liver than in the spleen.

The localization of cells in inflammatory sites is not usually apparent during the initial 60 min but may be visible at 4 hr. The relative accumulation in inflammatory lesions, however, is much more marked by 24 hr and thereafter appears to remain unaltered.

Results of quantitation of radioactivity in organs are given in Table 4. The patient given pure polymorphonuclear leukocytes (patient No. 5) shows a blood clearance half-time of 4.5 hr. One patient (No. 1) who received mixed cells free of erythrocytes had a clearance half-time of 7.5 hr. The remaining patients given mixed cells showed slower blood clearances. The radioactivity in the liver and spleen of all patients shows no significant change with times up to 48 hr post injection, but the uptake in bone marrow appears to increase. The uptake in the region of bone considered for the radioactivity computation was small, however, and this increase may not be significant.

Radiation dosimetry. Radiation doses to the spleen and liver were calculated (*16*), assuming that the uptake of radioactivity was instantaneous and had an infinite biologic half-time, as is indicated by the above distribution data. For 1 mCi administered, the maximum radiation dose to the spleen (20% uptake and 12% in the liver) would be 18 rads, and the minimum (6% spleen 12% liver) 6 rads. The liver would receive 1–5 rads and the whole-body radiation dose would be approximately 0.5 rads.

CLINICAL RESULTS

Focal accumulation of radioactivity was detected in 12 of 15 patients. In the remaining three there was a normal distribution of radioactivity, and subsequent follow-up failed to show evidence of focal sepsis. In nine patients with suspected sepsis within the abdominal wall, positive scans were obtained. Operative confirmation was obtained in five of these cases, in three of which pus

TABLE 4
Percentage of Injected Radioactivity in Liver, Spleen, and Bone Marrow at Various Times After Administration of Labeled Leckocytes

Patient No.	Organ	Time after administration (hr)				Half blood clearance time (hr)
		0.5	**3-5**	**22-28**	**44-48**	
1*	Spleen	13	13	13		7.5
	Liver	39	36	37		
	Lower spine	0.7	0.8	1		
2†	Spleen		9	8	8	17
	Liver		14	15	17	
	Lower spine		0.9	1.4	2	
3†	Spleen		22	19		12
	Liver		12	12		
	Lower spine		0.9	1.1		
4†	Spleen				15	21
	Liver				7	
	Lower spine				1	
5‡	Spleen				6	4.5
	Liver				34	
	Lower spine				0.8	

*White cells without contaminating RBC.
†White cells with contaminating RBC.
‡Pure polymorphonuclear leukocytes.

recovered showed ten to 25 times more radioactivity than in an equal weight of blood.

In the other patients with various disseminated lesions, it was noted that acute focal sepsis was accompanied by marked localization of radioactivity (as in Case #1, below). Mild inflammatory lesions caused by intramuscular injection and indolent cellulitis demonstrate obvious but less dramatic localization of white cells.

CLINICAL HISTORIES

Case 1. A 65-year-old man suffered a febrile illness progressing to jaundice and joint pains. Following admission to hospital 14 days after the onset of his illness, he was found to have splenomegaly, with polymorphonuclear leukocytes at 19,300. During admission he developed cellulitis of the left shoulder, and tender and swollen left thigh and right knee effusions. Positive blood cultures showed *Staphylococcus aureus.* On the twenty-first day of his illness he received 600 μCi of In-111 autologous leukocytes. A whole-body scan 24 hr after infusion of labeled cells (Fig. 2) showed activity in liver and enlarged spleen, with multiple areas of increased soft-tissue activity in shoulders, thighs, and legs. There is also a small area of increased activity in the left lung. Aspiration of soft tissues over the scan-positive areas produced pus cells. The patient made a good recovery on antibiotic treatment.

Case 2. A 68-year-old woman was admitted to hospital with abdominal pain 17 days before scanning. Surgery revealed a small-bowel volvulus with peritonitis. The patient improved initially but then developed probable septicaemia and severe peritonitis. At the time of the scan the patient was still pyrexial but was improving on antibiotics. Fig. 3 shows gamma-camera views of the anterior abdomen 24 hr after infusion of white cells. It demonstrates focal accumulation in a number of sites in the abdomen. A scan performed at 48 hr showed considerable diminution of radioactivity in these areas, this being associated with drainage of pus. Material recovered from the drainage showed about 15 times the radioactivity of an equal weight of blood.

Case 3. A 48-year-old man with a previous history of appendectomy was admitted with pain in the right iliac fossa. A limited laparotomy failed to reveal disease, but symptoms and fever persisted. Scanning with labeled white cells was performed 7 days after admission and showed an accumulation of radioactivity in the right iliac fossa (Fig. 4). A repeat laparotomy 21 days later revealed a retrocaecal abscess. Pus removed from it showed approximately 20 times the radioactivity of the same weight of blood.

DISCUSSION

The present work demonstrates the ability of isolated cells to accept a radioactive label efficiently while maintaining viability, and to take part in pathologic processes after being reintroduced into the body. The "homing" chemotactic mechanisms of polymorphonuclear leukocytes can thus be used for non-invasive diagnostic purposes, and the in vivo distribution of the cells can be studied. Previous radioactive labels used for this purpose (Tc-99m and Ga-67) have proved unsatisfactory for reasons discussed previously. The present technique has the advantage of high yields combined with minimal trauma to the cells. The indium radioactivity—which is known to bind to the cytoplasmic components in the cells—truly represents the in vivo distribution of the cells as long as they remain viable. The half-life of In-111 is favorable to the biologic events that can be observed externally by conventional instruments owing to the suitability of the gamma emission of the radionuclide. Following the intravenous infusion of labeled leukocytes, radioactivity is distributed throughout the lungs, from which half is then cleared in about

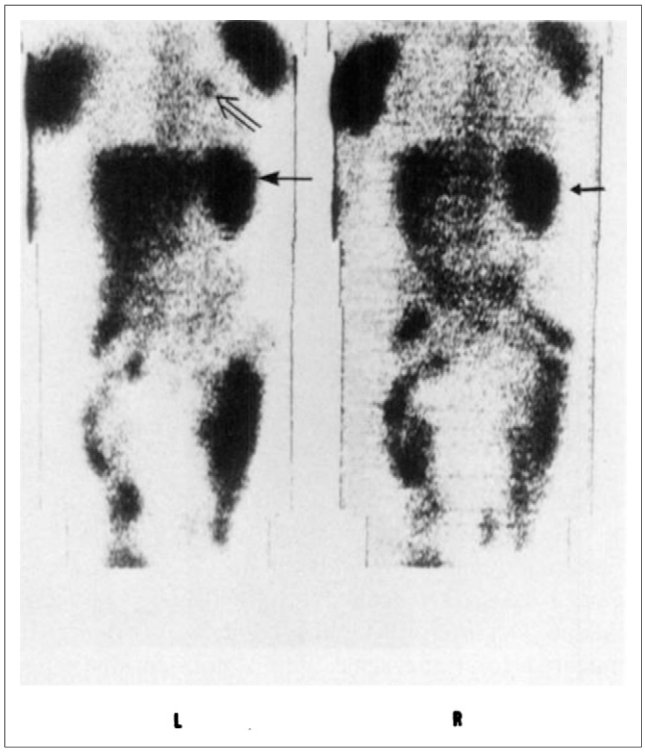

FIGURE 2. Case 1. Anterior (L) and posterior (R) whole-body scans 24 hr after administration of In-111 leukocytes. Radioactivity is seen in normal liver and in enlarged spleen (solid arrow). Abnormal accumulation of radioactivity is seen in soft tissues over shoulders, thighs, right knee, right groin and also in posterior left lung field (open arrow).

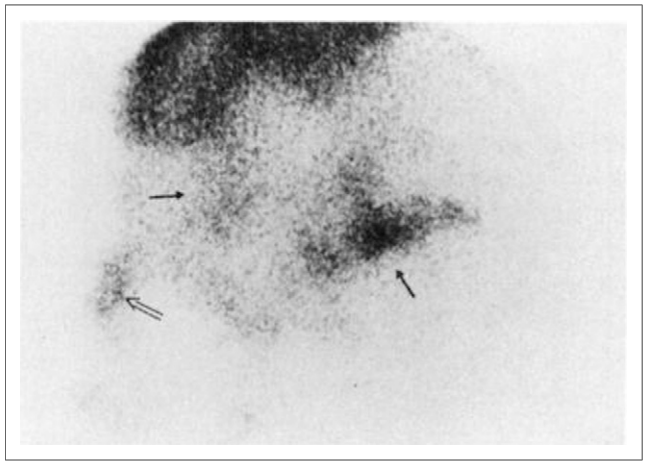

FIGURE 3. Case 2. Anterior gamma-camera view of abdomen 24 hr after infusion of labeled leukocytes. The radioactivity at upper left indicates lower border of liver. Arrows at center indicate pus collections within abdominal cavity. Increased radioactivity in the right abdominal wall (open arrow) represents cellulitis involving soft tissues in this region.

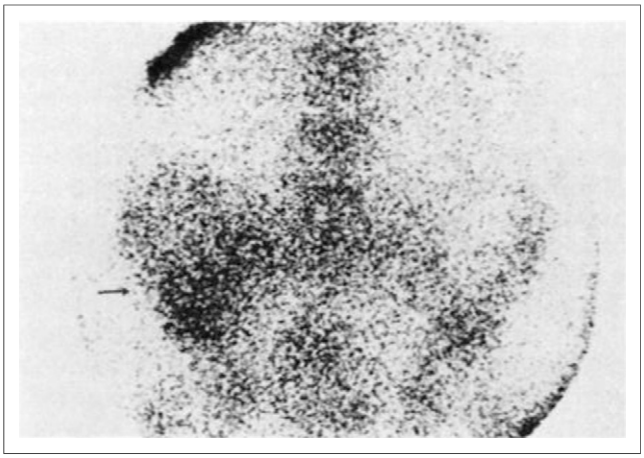

FIGURE 4. Case 3. Gamma-camera view of lower abdomen, obtained 24 hr after infusion of labeled leukocytes. Area of increased radioactivity is seen below right iliac crest, representing increased radioactivity within walled-off retrocaecal abscess.

15 min. This phenomenon may represent the natural physiology of polymorphonuclear leukocytes in the lung (*17*). Subsequent accumulation of 25 to 50% of administered radioactivity in liver and spleen appears to represent margination of the viable labeled cells as well as removal of damaged cells or cell fragments. The distribution of radioactivity between the liver and spleen seems influenced by the type of preparation. Cells with lowered viability give high levels of activity in the liver, whereas a preparation with higher viability but contaminated with red cells and lymphocytes results in high levels of radioactivity in the spleen. The mixed cells free of erythrocytes showed a clearance half-time of 7.5 hr, which agrees with the figures obtained by other workers (*4*). However a further factor that may influence these results is the patient's own physiologic response to infection.

The percentage of radioactivity found in plasma may be an overestimation, since not all leukocytes could be depleted from plasma without destruction. However, this gives a good indication that at least 90% of the circulating radioactivity remains in association with the cells and represents their true in vivo distribution. A small proportion of radioactivity was always found in bone marrow, but no attempts were made to ascertain whether it were due to In-111 transferrin or to migration of cellular components.

The radiation dose of 6 to 18 rads to the spleen, and 1 to 5 rads to the liver, per mCi administered is high compared with the majority of diagnostic procedures. This, however, can be reduced in two ways: first by administering cells of adequate viability and purity, and second by administering considerably less than 1 mCi of radioactivity, provided a patient's condition would allow increased examination time to obtain images of desirable quality. In some cases it has been possible to obtain a high quality image by injecting as little as 300 μCi.

Along with the clinical results, the relatively high sensitivity of the technique should be mentioned. In only three of this small group of patients was there acute and active inflammatory disease as evidenced by marked leukocytosis and fever. In these patients localization of radioactivity was striking. It appeared from the scans that a considerable proportion of the 50–75% of circulating cells was accumulated in the inflammatory lesion. In patients with no marked systemic symptoms there was clearly detectable localization of radioactivity. The in vivo viability of the labeled cells and the stability of the label are attested by the behavior even in minor inflammatory processes such as those associated with intramuscular injection sites and by association of the radioactivity with neutrophils separated from synovial fluid from knees of a patient with rheumatoid arthritis (Table 1).

The leukocytes obtained by simple sedimentation are perhaps the most viable cells but are mixed (polymorphs, lymphocytes and monocytes) and contaminated with red cells. Since most of the radioactivity in leukocytes separated by this technique is associated with polymorphonuclear leukocytes, the mixed nature of the cells is acceptable for abscess location. Nevertheless, two further problems remain. First, these mixed cells cannot be used to study the kinetics of a specific cell type and, second, the contaminating red cells prolong circulation of the activity and therefore add to the splenic radiation dose. When the polymorphonuclear leukocytes are separated by Ficoll/sodium metrizoate technique, followed by elimination of erythrocytes by lysis, two effects are observed. First, their circulation half-time is decreased to 4.5 hr and, second, the hepatic uptake is increased. It seems likely that this increased uptake relates to the damaged cells entering the reticuloendothelial system. A technique described for isolating pure polymorphonuclear leukocytes in a single-step separation (*18*) failed in our laboratory, and it may be a worthwhile exercise to label and study polymorphonuclear leukocytes isolated by a laser-beam technique (*19*). The difficulty, however, does not arise in the case of platelets and lymphocytes, which can be isolated relatively free from other cells and labeled with In-111 without affecting their viabiilty (*20,21*).

CONCLUSION

The accumulation of radiolabeled neutrophils within areas of inflammation has obvious clinical importance. Of more fundamental importance, however, is the principle that different cellular constituents of the blood can be isolated, labeled with In-111, reinjected, and their subsequent fate determined externally. It is hoped that this technique will provide much needed information on the movements of cells in health and disease.

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FOOTNOTE

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