Chapter 8
Use of Radiolabelled Leukocytes for Drug Evaluation in Man

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Abstract The endeavour to radiolabel or simply "label" autologous leukocytes has been a major clinical need. The endeavour to improve the labelling conditions and minimise interventional stresses and maintain cell functionality has been the driving objective. This chapter focuses on novel techniques used in this laboratory to radiolabel leukocytes, examples of the clinical indications that such labelled products might be informative, and how we can use these labelled cells in clinical situations to describe the life cycle behaviour (transit times and migratory capacity) of these labelled cells. Labelling techniques which preserve leukocyte functionality will assist in the development of new anti-inflammatory agents, anti-infectives, and indeed any drug or biologic where their clinical use may have an effect on these cells. Examples of labelling methods and clinical scenarios with imaging are described for conditions such as abscesses, ARDS, COPD, rheumatoid arthritis, inflammatory bowel disease, and vasculitis.

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8.1 Introduction

The use of radioactive isotopes to label autologous leukocytes is an established technique with numerous clinical applications. It is used for cell kinetic studies, to localise and quantify sites of inflammation and infection, and has applications in the pharmaceutical industry as a non-invasive means of testing the effects of drugs on the life cycle of these cells.

Leukocytes play a vital role in the innate immune system and form the body's primary defence against infection and parasites; they are also important mediators of inflammation. Recent studies suggest their involvement in a variety of other processes including adaptive immunity, tumour progression, and even the formation and propagation of deep venous thrombosis (Von-Bruhl et al. 2012). Using radiolabelling techniques, the migration characteristics of leukocytes can be determined in several pathological conditions including lung and inflammatory bowel disease (IBD) and infections such as solid abscesses, renal sepsis, and orthopaedic infections. Cells of the innate immune system include granulocytes (neutrophils, eosinophils, and basophils), mast cells, monocytes, macrophages, and natural killer (NK) cells. Leukocytes of the adaptive immune system provide a more specific response to antigen and include lymphocytes (B and T cells).

Autologous human leukocytes are purified from a peripheral blood collection (generally 80 mL or more) and radiolabelled with a radioactive isotope (radioisotope, radionuclide; one which is known for its specific energies applicable to the imaging system intended, i.e. planar, positron emission tomography (PET), or single-photon emission computed tomography (SPECT) systems) before being reinjected into the individual donor. A combination of peripheral blood sampling and imaging then allows the quantitative analysis of cell kinetics, distribution, and fate. The quantification of leukocyte migration can be achieved by several techniques ranging from body fluid sampling through to complex imaging modalities using gamma cameras, which provide two- or three-dimensional images of the radiation detected. More recently, SPECT has used the gamma radiation emitted from radionuclides such as technetium (Tc-99m), indium (In-111), and thallium (Tl-201) and others, to generate a three-dimensional image of radioactivity in vivo providing high-resolution digital images which can also provide quantitative assessment of regional localisation (termed region of interest, or ROI).

The use of radiolabelled leukocytes to quantify and localise infection and inflammation in vivo provides a platform to test non-invasively the effects of existing and novel drugs and is therefore highly relevant to the pharmaceutical industry. As well as continued innovation in this field, other approaches for labelling cells, particularly stem cells, include the use of superparamagnetic iron oxide (SPIO) nanoparticles in combination with magnetic resonance imaging (MRI). Cell labelling techniques include receptor-mediated endocytosis whereby a ligand is added to the surface of the SPIO nanoparticle, which then binds to the cell surface before being internalised (Gupta and Gupta 2005). Although there are several potential applications for human disease, many of the studies remain experimental in animal models.

8.2 Leukocyte Preparations and Radiolabelling Techniques

The purification of leukocytes from whole blood is divided into stages with the initial isolation of buffy coat leukocytes containing mixed leukocytes and platelets. Further purification allows the isolation of granulocytes, lymphocytes, and monocytes. It is then possible to further isolate eosinophils from granulocytes. Once purified, cells are incubated with the selected radiolabel before being re-injected into the donor.

Granulocytes prepared in the absence of autologous plasma become metabolically active and adhere to the lung capillary endothelium when re-injected causing delayed transit through the lung, low recovery in the blood, and increased liver uptake (Saverymuttu et al. 1983a). It is therefore vital to purify cells in the continuous presence of autologous plasma to minimise ex vivo activation and preserve their physiological behaviour once re-injected. Primed and activated neutrophils demonstrate shape change characterised by the formation of a broad, flattened protrusion known as a lamellipodium through changes in the actin cytoskeleton (Downey 1994). Such shape change also affects the deformability of these cells and as a consequence delays their transit through the narrow pulmonary capillary bed (Hogg 1994).

The protocol for isolating and radiolabelling leukocytes has been previously documented (Frier 1994) and is illustrated in Fig. 8.1. A wide-bore 19G butterfly is used (reduces shear stresses on the cells that can lead to activation) to withdraw up to 160 mL whole blood into a syringe pre-filled with the anticoagulant acid–citrate–dextrose (ACD), which unlike heparin does not cause leukocyte aggregation or promote adherence to plastic centrifuge tubes (Ellis 2011). Hetastarch is then added to promote erythrocyte sedimentation. After 30–60 min in the presence of hetastarch, two separate layers of cells are discernible, the lower containing erythrocytes, which is discarded, and the upper layer of leukocyte- and platelet-rich plasma (PRP). This plasma is carefully removed and centrifuged at $150 \times g$ for 5 min to generate the leukocyte pellet and a supernatant of PRP. The leukocyte pellet contains granulocytes (neutrophils and eosinophils), peripheral blood mononuclear cells (PBMCs) including monocytes, lymphocytes, and macrophages in addition to some discernible contamination with platelets and erythrocytes. The cells in this upper layer are termed "buffy coat" leukocytes. The PRP supernatant is removed and centrifuged at $1{,}500 \times g$ for 5 min to leave a platelet pellet and provide for a useful cell-free plasma (CFP) supernatant which is retained. The CFP is used to resuspend the mixed leukocyte pellet and is also used when applying selected Percoll gradients necessary to purify the granulocytes into desired enrichments of specific cell types.

Further purification of the leukocyte pellet to isolate granulocytes requires the use of discontinuous iso-osmotic Percoll gradients. Percoll is made of colloidal silica particles that have been coated in polyvinylpyrrolidone (PVP). It is mixed with sodium chloride before being diluted with autologous CFP to prepare 50, 60, and 65 % solutions. Two millilitres of each gradient solution are then gently layered, in a specific order of decreasing density, in a sterile 10 ml tube and the position of each gradient is marked.

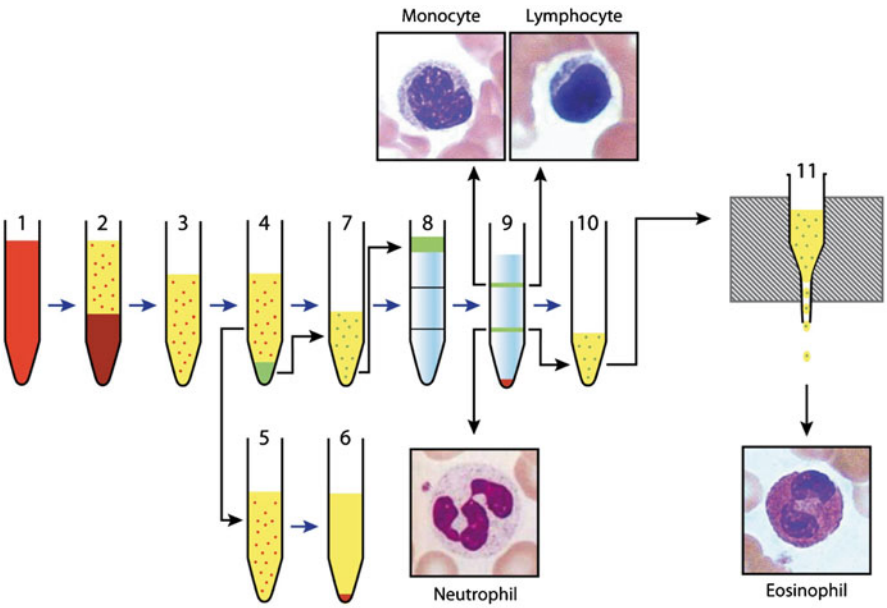

Fig. 8.1 Leukocyte isolation. (1) Whole blood in ACD. (2) Sedimentation of erythrocytes following the addition of hetastarch. Upper layer of leukocyte- and platelet-rich plasma. (3) Leukocyte- and platelet-rich plasma carefully removed from erythrocytes. (4) The generation of the leukocyte pellet (*green*) and supernatant of platelet-rich plasma following centrifugation at 150×*g* for 5 min. (5) Platelet-rich plasma supernatant is removed and centrifuged at 1,500×*g* for 5 min to generate (6) a platelet pellet (*red*) and the cell-free plasma (CFP) supernatant. (7) The leukocyte pellet is re-suspended in CFP. (8) Discontinuous Percoll gradients layered in decreasing density with leukocytes (*green*) overlaid. (9) Following centrifugation at 150×*g* for 5 min, PBMCs settle at the upper 50 % gradient interface and granulocytes at the lower 50–60 % interface. Contaminating red cells are found at the base of the tube. (10) Granulocytes are removed and re-suspended in CFP and incubated with immunomagnetic anti-CD16 antibodies. (11) Granulocytes are passed through a magnetic column which retains CD16-positive neutrophils and allows purified eosinophils to be collected

The leukocytes, suspended in CFP, are carefully overlaid onto the gradients and the tube is centrifuged at 150×*g* for 5 min. Following centrifugation, several distinct layers are visible and different cell types found at each of the three gradient interfaces. At the upper 50 % interface, the less dense platelets and PBMCs including lymphocytes and monocytes can be recovered and either used for research or discarded. The denser granulocytes settle at the 50–60 % interface and may be carefully withdrawn using a wide-bore pipette tip to minimise shear force damaging the cells. Contaminating erythrocytes may be visible at the base of the tube. The granulocytes are re-suspended in 3 mL buffer containing 0.5 % CFP and centrifuged at 150×*g* for 5 min. The supernatant is withdrawn and the granulocytes again re-suspended gently in 3 mL buffer containing 0.5 % CFP.

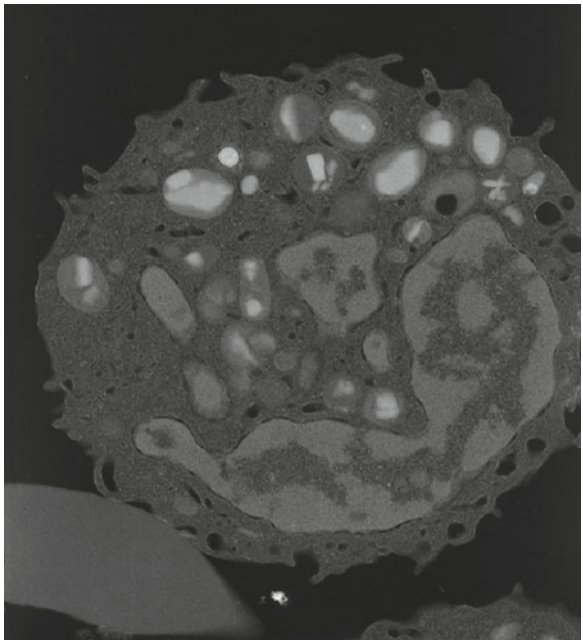

Fig. 8.2 Electron micrograph of a purified eosinophil showing the absence of cell surface CD16 antibody-coated immune-magnetic beads, which will only stain positive with neutrophils

If collection is desired, eosinophils can then be purified from the granulocyte fractions by negative selection using immunomagnetic anti-CD16 antibodies which retain CD16-positive neutrophils when passed through a magnetic column (Hansel et al. 1991) (Fig. 8.1). Purified eosinophils will therefore not stain positive for CD16 (Fig. 8.2).

Following isolation, cells are re-suspended in CFP and radiolabelled by incubation with an appropriate radioisotope, and at a desirable specific radioactivity for the intended imaging parameters of interest, for at least 15 min. The choice of radioisotope is dependent on its half-life and photon abundance required for the duration of the study without exposing the subject to excess radiation to stay within required limits of absorbed dose. The total effective dose received by subjects is dependent on the radionuclide used, whether additional imaging such as CT is incorporated into the protocol, and the organs exposed. 8 MBq of In-111 for example is used to label purified eosinophils used in planar gamma camera imaging, which gives an effective dose of 3.6 mSv to the subject. 200 MBq of Tc-99m used to label cells to be imaged with SPECT/CT gives an effective dose of 2 mSv. A low-dose CT thorax, if imaging the chest, will contribute an additional 3 mSv to the dose the subject receives.

The radioisotope in its ionic form cannot permeate the cell membrane and must therefore be complexed to a lipophilic chelator (ligand) with high affinity that results in an in vivo stable complex to allow the passive diffusion of the ligand–radioisotope complex into the cells of interest. This ligand must reach its target tissue without significant binding to plasma proteins and must not, at the dose of ligand extracted, either be toxic to its target cell or result in toxemias to the individual.

Early pioneer imaging of inflammation with Cr-51-labelled leukocytes was limited by this isotope's low labelling efficiency, high elution rate from the labelled cells and the infection locale, and poor emission characteristics of gamma radiation (high-energy gamma rays), all of which led to suboptimal imaging (Froelich and Swanson 1984). Its long half-life of 27.7 days also led to the use of other isotopes being favoured. Subsequent techniques involved the use of Ga-67-citrate to directly label inflammatory processes. Following injection, Ga-67-citrate binds to circulating transferrin (as an iron analogue) and extravasates within inflammatory sites due to increased vascular permeability where it binds to lactoferrin released from leukocytes or to siderophores produced by bacteria (Froelich and Swanson 1984). Following intravenous administration, 10–25 % of Ga-67-citrate is excreted by the kidneys within 24 h. There is slower excretion via the gastrointestinal tract and the remainder of the radioisotope localises predominantly in the liver and skeleton (Hoffer 1980). Such background tissue uptake resulting in increasing intra-abdominal activity limits the use of this radionuclide in the assessment of intra-abdominal collections. Ga-67 can be used favourably if the radionuclide is complexed such that the mechanism of distribution is not iron analogue related. Ga-67-citrate also binds to other lesions such as neoplasms which have bleeding and necrosis which have likely increases in transferrin deposition, thus limiting its specificity (Peters and Saverymuttu 1987). The long physical half-life of Ga-67-citrate of 78 h and the fact that only 10–25 % is excreted renally in the first 24 h, and thence elimination is primarily faecal, also lead to a higher radiation dose to the patient.

The most commonly used isotopes currently are In-111, which has a half-life of 67 h, and Tc-99m, which has a half-life of 6 h. In-111 was first complexed to 8-hydroxyquinolone (oxine) to radiolabel leukocytes (McAfee and Thakur 1976). The complex forms a neutral and lipophilic compound (3:1 with indium), which diffuses readily across the cell membrane. Indium then disassociates and binds to intracellular components whilst some of the oxine leaves the cell leaving the leukocytes stably labelled (Thakur et al. 1977). In-111-oxine also binds to plasma transferrin and in order to obtain high labelling efficiency cells must be removed from plasma before labelling, which risks their activation. In contrast to Ga-67-citrate, there is no renal or bowel excretion making indium scintigraphy a useful tool for the localisation of intra-abdominal inflammation or infection. Tropolone was first used as a ligand for indium in 1982 and may be incubated with cells in autologous plasma at lower concentrations of leukocytes (Danpure et al. 1982; Danpure and Osman 1988). It has since been used widely to label leukocytes, platelets, and erythrocytes and is certainly now the chelate of choice for neutrophil, eosinophil, and mixed leukocyte studies using In-111.

Technetium bound to hexamethylpropyleneamineoxime (HMPAO) was originally used to assess cerebral perfusion but has since been adapted to radiolabel leukocytes. Tc-99m-HMPAO forms a neutral lipophilic complex, which passively diffuses into the cell. It is then converted into a hydrophilic form that is unable to cross the cell membrane, thereby trapping the technetium within the cell (Ellis 2011). Despite some initial elution of the label from the cells and a degree of

non-specific bowel activity arising from excretion via the renal and hepatobiliary tract, the use of ^{99m}Tc-HMPAO allows for higher resolution images and a reduction in the radiation dose delivered to the patient relative to In-111 (Robins et al. 2000). These techniques lead to indiscriminate labelling of cells although ligands may vary in their affinity for different cell types (Puncher and Blower 1994). It is therefore important that the cells of interest are purified prior to the radiolabelling process.

Factors influencing labelling efficiency (LE) of cells include the presence or the absence of plasma, concentration of ligand mixed with the radionuclide, pH of the labelling medium, incubation time, temperature, and cell concentration (Danpure and Osman 1988). Labelling cells in buffered saline can achieve high LE but again risks activating the cells. In contrast, labelling with In-111-troponolate or Tc-99m-HMPAO in plasma protects the cells whilst maintaining satisfactory LE (Ellis 2011). LE increases with cell concentration but falls if high volumes of plasma are used during the incubation as there is competition between plasma and cells for In-111-troponolate. Labelling 1×10^8 granulocytes in 1 ml of 90 % plasma gives an LE of 90 % (Danpure and Osman 1988). Radiolabelling for at least 15 min at room temperature and a pH in the range 7–7.6 are the optimal conditions for both LE and maintaining the viability of the cells. As both oxine and tropolone form a 3:1 complex with indium, an excess of ligand is required to saturate the radionuclide. An excess of HMPAO is also required for the formation of technetium complexes.

Once the cells of interest have been radiolabelled they are centrifuged and the supernatant containing unbound radionuclide is discarded to minimise the uncomplexed radioisotope from being co-injected along with the desired radiolabelled cells (Peters and Saverymuttu 1987).

Paramount to obtaining the in vivo outcomes is the retention of functionality and viability of the purified radiolabelled cells. Successful in vivo functionality must be correlated with in vitro functionality. In vitro functional assessment includes determining the ability of the cells to phagocytose (e.g. granulocytes) and to replicate (e.g. lymphocytes) (Ellis 2011). Another in vitro assessment of viability involves staining cells with trypan blue, which only permeates the cell membrane of non-viable cells and provides a way to quantify the percent viability. In vivo assessment is considered a better marker of leukocyte function, and includes observing the circulating characteristics of radiolabelled cells and the 45-min recovery, which is the percentage of radiolabelled cells injected that are still present in the freely circulating blood pool at 45 min post injection. Activated or damaged cells demonstrate a far slower transit through the lung and increased uptake in the liver (Saverymuttu et al. 1983a) and hence give rise to a much reduced 45-min recovery.

Once re-injected, the radiolabelled cells distribute within the circulating blood volume. Counts derived from subsequent small-volume blood samples are then corrected to a blood volume calculated according to the subject's height, weight, and gender. An individual's drug history is also important as agents such as antibiotics or corticosteroids may influence the chemotaxis of leukocytes to areas of infection and inflammation (Ackrill 2011). Corticosteroids reduce the transcription of cytokines (e.g. interleukins (IL)-4, -5, -13, chemokines, such as IL-8, regulated upon activation, normal cell expressed, and secreted (RANTES), eotaxins, and adhesion

molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin (Barnes et al. 2003), which are important in the recruitment of leukocytes to areas of inflammation through rolling of the leukocytes on the vascular endothelium). Another factor which may affect the initial isolation and labelling process includes the erythrocyte sedimentation rate (ESR), which can vary in disease states. Leukocytes are also susceptible to mechanical damage during the isolation process by way of shear forces experienced in passage through needles, shaking, and centrifugation and these steps should be strictly controlled to reduce this variable.

8.2.1 Buffy Coat Leukocytes

Buffy coat leukocytes were the cells initially used to establish the technique of leukocyte radiolabelling. They have the advantage of being relatively easy to isolate from whole blood with minimal activation when isolated in plasma. They were used to determine normal leukocyte migration and kinetics in vivo and can localise to sites of focal infection such as abscesses and osteomyelitis (Palestro et al. 2006) as well as focal inflammation in conditions including rheumatoid arthritis (Peters and Saverymuttu 1987).

The effect on cell labelling, trafficking, and abscess or infection localisation by existing or novel non-specific anti-inflammatory drugs (NSAIDs) in vivo could be tested using buffy coat leukocytes.

8.2.2 Granulocytes

Both neutrophils and eosinophils are important mediators of infective and inflammatory processes. Neutrophils, which represent 50–60 % of circulating leukocytes, and eosinophils play a role in airway inflammation and also asthma (Busse et al. 1992) (Sampson 2000). Granulocytes are also important in the pathogenesis of other conditions such as bronchiectasis (Currie et al. 1987), pneumonia, chronic obstructive pulmonary disease (COPD) (Ruparelia et al. 2011), IBD (Cheow et al. 2005), systemic vasculitis (Jonker et al. 1992), and acute respiratory distress syndrome (ARDS) (Ware and Matthay 2000).

In the presence of ongoing neutrophilia, a mixed leukocyte preparation may be sufficient for the detection of focal infection and inflammation. The localisation of low-grade infections or inflammation, such as chronic osteomyelitis, however is enhanced if purified granulocytes are used (Peters and Saverymuttu 1987). Other situations in which pure granulocytes are superior to mixed leukocytes include intravascular sepsis (as it avoids detecting signal from contaminating labelled platelets and erythrocytes in the circulation), neutropenic patients, and if quantitative studies are required such as In-111-granulocyte excretion in IBD (Peters 1994).

Radiolabelled granulocytes can also be used to assess the effects of drugs on the circulating physiology of these cells and their impact on the conditions described. This technique would be particularly useful, for example, if specific anti-neutrophil strategies are being tested.

8.2.3 Eosinophils

Eosinophils account for only 1–3 % of circulating leukocytes and higher volumes of peripheral whole blood are required to isolate them. Eosinophilic inflammation is the pathological hallmark of asthma and allergic inflammation. Eosinophilia is present and pathogenic in a wide range of conditions including parasitic infection, cancer and organ-specific diseases affecting the lung- allergic bronchopulmonary aspergillosis (ABPA), eosinophilic pneumonia; the gastrointestinal tract- IBD, radiation enteritis; the skin- eczema and cellulitis and the vasculitic condition eosinophilic granulomatosis with polyangiitis (EGPA).

The radiolabelling of autologous human eosinophils remains experimental at this stage but is now an active area of research with significant applications to the pharmaceutical industry. A greater understanding of eosinophil kinetics and trafficking in health, and in conditions such as asthma, will likely allow for lower "N" studies (fewer animals or patients) in proof of concept (POC) studies to quantify the impact of novel therapeutics on allergic inflammation.

8.2.4 Lymphocytes

The radiolabelling of lymphocytes has been severely limited due to the extreme sensitivity of these cells to radiotoxicity, both when the whole body is irradiated as well as when directly radiolabelling the cells. This is especially notable in studies of replication. Whole-body irradiation results in lymphopenia, which is thought to be secondary to loss of a predominant T-cell population, although all populations are affected (Campbell et al. 1976). Initial studies suggested that In-111-oxine-labelled lymphocytes could be used to track the migration of the cells to lymphoid organs of healthy individuals and those with Hodgkin's disease (Lavender et al. 1977). However, subsequent studies have demonstrated that radiolabelling with In-111-oxine impairs lymphocyte function with reduced H-3 thymidine incorporation suggesting impeded cell proliferation (Segal et al. 1978) and alters the normal migration of these cells. This results in increased activity in the spleen and liver, and minimal activity in the lymph nodes (reduction in cells with POS migratory functionality), suggesting the sequestration of damaged cells (Chisholm et al. 1979). Chisholm et al. have also demonstrated a dose-dependent reduction in the ability of the HeLa S3 cell line to proliferate and form colonies as a result of the radiation emitted from In-111. Caution should also be recognised in that the chelating agent itself that is used to complex indium (oxine) may also lead to direct lymphotoxicity (Segal et al. 1978).

Although less cellular damage occurs when lymphocytes are radiolabelled with Tc-99m-HMPAO (Ellis 2011), studies have suggested that labelling mixed leukocytes with Tc-99m-HMPAO may still cause chromosomal abnormalities in the lymphocyte fraction (Ilknur et al. 2002).

8.2.5 Monocytes

Circulating monocytes represent 5–10 % of circulating leukocytes and have the capacity to migrate to areas of inflammation where they differentiate over a period of days into tissue macrophages and dendritic cells. The half-life of human monocytes in the blood is 3 days (Whitelaw 1972); and it is suggested that this short half-life can be explained by the continuous replenishment of the macrophage and dendritic cell population, although some organ macrophages may renew their populations independently from blood monocytes, e.g. alveolar macrophages (Tacke et al. 2006). It has been demonstrated in patients with rheumatoid arthritis that monocytes may be purified from 100 mL of whole blood by positive selection using CD14 cell surface marker reagents (Van Hemert et al. 2007; Bennink et al. 2008). Radiolabelled monocytes have also been used to investigate the pathogenesis of atherosclerosis (Dorffel et al. 2001) and acute myocardial infarction (Leuschner et al. 2012). There have again been concerns about the cytotoxicity and possible inhibition of monocyte differentiation into tissue macrophages by radiolabelling with tropolone or oxine. The use of tropolone as a chelator, rather than oxine, however, allows for the use of higher concentrations of In-111 in labelling suggesting that oxine is an important source of cytotoxicity (Van Hemert et al. 2009).

8.3 Methods for Quantifying Leukocyte Transit Time/Migration

The radiolabelling isotopes discussed in the previous section (i.e. In-111, Cr-51, Tc-99m) are unstable isotopes that emit energy in the form of gamma radiation when they decay to a more stable state. The radiation is readily detected with scintillation instruments in the form of probes, well counters, whole-body counters (WBC), and gamma cameras. These instruments have two principal components in common: the first is the scintillation crystal, commonly thallium-doped sodium-iodide (NaI(Tl)), which converts the energy of gamma photons into visible light; the second is the photomultiplier tube, which converts the energy of the visible light to electrons, and accelerates these through a high-voltage cascade to multiply the weak original signal to create a measurable electrical signal. Other crystals such as bismuth geranium oxide crystals have been utilised in other systems such as PET and have used photomultiplier tubes to generate high-pixel images.

8.3.1 Skin Patches

Micropore filters placed on skin abrasions have been used to capture radiolabelled granulocytes migrating to such sites, which can then be quantified and imaged (Pinching et al. 1990).

Skin abrasions were made on healthy volunteer's forearms using a dental drill. Micropore filters were placed on the abrasion and autologous In-111-tropolonate granulocytes injected at different time points between 4 and 20 h following the abrasion. Filter papers were changed at regular intervals and the radioactivity absorbed was measured by a well counter. The rise and fall of the pad radioactivity can be taken to represent granulocyte migration into the abrasion as plasma exudation of In-111 as cell-free circulating indium is persistently low. The ratio of radioactivity detected on the filter paper compared to blood cell-bound radioactivity was used to quantify the granulocyte presence in the abrasion. Abrasions of all ages demonstrated peak radioactivity levels 2–4 h following re-injection with the older abrasions having higher peaks. Physical exercise reduced the migration of labelled granulocytes to the abrasion due to diverted blood flow from the skin to exercising muscles and demargination of granulocytes at the sites of abrasion.

The ability to quantify granulocyte migration into skin abrasions supports the use of these non-invasive techniques to assess inflammatory diseases affecting the skin. It may also be used to assess the effect of treatment with local or systemically delivered drugs on the migration of granulocytes to the skin.

8.3.2 Scintillation Probe Counting

Scintillation probes, as body surface or as isolated tissue counters, containing a single, small NaI(TI) crystal and photodiode have been used in the detection of radioactivity in vivo, for example during surgical procedures where modest isolation of a target tissue may be procured allowing for improved target-to-non-target ratios of counts. These kinds of probes generally comprise a handheld modestly shielded detector (focused probe) coupled to an electronic display unit (e.g. analogue-to-digital counter or rate meter).

8.3.3 The Whole-Body Counter and Body Fluid Sampling

A WBC system allows assessment of total body radioactivity to be measured and quantified, and theoretically provides a way to study whole-body retention of radiolabelled products. The patient lies supine between highly sensitive anteriorly and posteriorly placed scintillation detectors containing sodium iodide crystals.

Fig. 8.3 Imaging modalities. (**a**) Planar gamma camera; (**b**) whole body counter; and (**c**) SPECT/CT

These detectors move and record radioactivity counts from head to toe. The WBC chamber is made with pre-1945 steel with 3 mm lead shielding on the inner walls from which no detectable isotopes are emitted during counting. Such shielding minimises background interference and allows the administration of ultra-low radiation doses to the patient (Fig. 8.3b).

This technique has been used to detect whole-body granulocyte distribution in healthy individuals and in those with bronchiectasis, asthma, and COPD where measuring the radioactivity in the sputum at the same time demonstrates the migration of granulocytes into the airway and loss from the body (Szczepura et al. 2011). Whole-body counting has also been used in patients with IBD (Carpani de Kaski et al. 1992) and is an accurate alternative to measuring the radioactivity of stool samples collected over 4 days. Loss of granulocytes through faeces correlates with disease activity in IBD (Saverymuttu et al. 1983c).

8.3.4 Planar Imaging

The first scintillation gamma camera was introduced in 1958 and is known as the Anger gamma camera (Anger 1958) (Fig. 8.3a). Visible light produced in a large-area (unitised) scintillation crystal reaches several photomultipliers arranged in a precise array behind the crystal, resulting in varying total signal strengths across the array depending on the photon annihilation location within the crystal and thus a depreciative electronic signal in photomultipliers away from the immediate scintillation point. The "centre of mass" (maximal photonic translation) of these signals is calculated to give the position of interaction of the original gamma photon. A collimator comprising multiple narrow, parallel holes in a thick sheet of lead limits detection to photons travelling at near-perpendicular trajectories toward the crystal. Gamma photons not in such line with the collimator holes are scattered to lower energies or absorbed by the lead. Thus, unimpeded and detected photons bear a spatial relationship to the "source" of the radiation, and hence an image can be constructed. The image, however, is a projection of photons from a 3-D object translated as a single 2-D plane view of the overlaying and underlying organ systems within the field of view.

Static planar images have been used to identify and quantify sites of infection and inflammation in numerous conditions including bronchiectasis, COPD, asthma, IBD, and rheumatoid arthritis where reduction in radioactivity in the joints can be demonstrated following effective treatment. Using a combination of whole-body counting, leukocyte scintigraphy, and body fluid sampling, there is an improvement in the appearance of the In-111-leukocyte scan and a reduction in the faecal loss of labelled granulocytes following treatment of Crohn's patients with oral fluticasone (Carpani de Kaski et al. 1991). Such techniques could therefore be used to assess the efficacy of new anti-inflammatory treatments in other conditions.

8.3.5 Dynamic Planar Imaging

Dynamic (imaging over time) gamma camera imaging entails the acquisition of multiple image frames generally in a short space of time (seconds to minutes), which for example allows the assessment of the initial distribution and subsequent margination of labelled leukocytes. The radioactivity is quantified through digital acquisitions and regions of interest (ROI) organ time–activity curves by drawing defined ROI over the organs of interest and recording their respective counts per pixel within the ROI. The use of short-lived radioisotopes requires these ROI collections to be decay-corrected with respect to the radionuclide used.

This technique has been used to assess the normal distribution of ex vivo radiolabelled and re-injected leukocytes, granulocytes, eosinophils, monocytes, and lymphocytes in healthy individuals and has led to knowledge about the in vivo kinetics of circulating as well as marginated cell pools as well as sites of cell destruction.

Dynamic imaging can also be used to assess lung transit time and alterations of normal cell kinetics and pools in disease states. For example, when In-111-troponolate-labelled granulocytes are re-injected into patients with IBD and imaged dynamically with a gamma camera it has been observed that these cells can localise in sites of inflammation within 10 min post injection (Saverymuttu et al. 1983b).

8.3.6 Single-Photon Emission Computed Tomography

As previously described in Sect. 8.3.4, planar scanning can only generate two-dimensional images, in which it can be difficult to distinguish specific organ borders or uptake within overlying structures within the ROI. The revolution of emission tomographic imaging allows for three-dimensional imaging of the distribution of radioactivity in vivo and thus specific organ uptake and potentially distribution information within an organ or a region of uptake (e.g. infection).

In SPECT, one or more gamma camera heads rotate around the patient and acquire planar images from several angles, which are then mathematically reconstructed to form cross-sectional (bread-slice) images through the body providing now volume pixels, or voxels, defined by the slice thickness of the system, of count information (Hutton 2011) (Fig. 8.3c). Sequential SPECT has been used to quantify the migration of labelled granulocytes into regions of the lung of patients with COPD whereby collecting data at regular intervals allows the calculation of granulocyte flux into the lung (Ruparelia et al. 2011). Sequential SPECT has also been used to quantify disease activity in IBD (Weldon et al. 1995b) and can theoretically be applied to other conditions characterised by inflammation.

SPECT has recently been coupled to X-ray CT, which can provide more quantitative data by allowing for correction of photon attenuation (loss of signal by scatter and absorption by tissue density) in the subject, and also allowing for imaging a merged anatomical (CT) and functional (SPECT) co-registration making it easier to determine the actual location of anatomic structures relative to the site of photon origination (radiolabelled cell localisation). SPECT/CT has been used in combination with either Ga-67- or In-111-labelled leukocyte scintigraphy to provide this additional co-registration information aiding diagnosis, precise localisation, and better assessment of disease or extent of infection (Bar-Shalom 2006). The use of SPECT/CT following the re-injection of autologous Tc-99m-granulocytes has also led to more accurate diagnoses of left-ventricular-assist-device infection (Litzler et al. 2010).

Dynamic SPECT is also possible and involves the rapid acquisition of image frames to create dynamic images of physiological or pathophysiological processes. It is important to note that the accumulation of a single-slice SPECT image is far greater in time than the acquisition time of a CT scan and thus very fast functional dynamic images are difficult to acquire. Very rapid functional dynamic imaging is better accomplished with PET (Sect. 8.3.7).

8.3.7 Positron Emission Tomography

An alternative method of functional scintillation tomographic detection is with PET scanners, which have a ring (single or multiple) of detectors (original PET systems had as few as 280 crystals in one ring (Derenzo 1980) to modern systems of more than 16 rings with over 32,000 detectors) around the patient for coincidence detection of the two 511 keV gamma photons. These gamma photons are created by the annihilation of nuclear emitted positrons which interact with the outer shell electrons causing release of two photons in opposite (180°) orientation (Hutton 2011). Radioligands such as F-18-fluorodeoxyglucose (F-18-FDG) is a widely used reagent for PET scanning of cardiac and neurologic conditions. The absence of a collimator as needed in planar imaging makes the technique more sensitive than SPECT. The detectors are again linked to photomultipliers, which convert the gamma signal into a digitised light signal that is used as in SPECT to reconstruct the tomographic image. The ability to localise lesions with PET is, as seen with SPECT, aided if coupled to CT, which also allows absolute quantification of the activity again by providing a mechanism for attenuation correction. Original PET systems used a ring of Ge-68, which is also a position isotope, as a low count rate tool (relative to X-ray CT) to correct for structural/anatomical attenuation.

Retrospective studies have suggested a role for F-18-FDG PET in the investigation of fever of unknown origin (FUO) and in patients with suspected focal infection or inflammation (Bleeker-Rovers et al. 2004). A recent review summarises the utility of PET in the investigation of malignancies, the diagnosis of chronic osteomyelitis (particularly of the axial skeleton) and infections associated with prostheses, and opportunistic infections in AIDS (Zhuang and Alavi 2002). The same review describes the use of PET to monitor disease activity in sarcoidosis and to detect vasculitis, and inflammation in IBD. F-18-FDG is also able to discriminate between infection and rejection in lung transplantation patients (Jones et al. 2004) and uptake has also been shown to be greater in the lungs of COPD patients but not in asthmatics (Jones et al. 2003).

Despite its advantages, it has been shown that elevated uptake of F-18-FDG by neutrophils detected by PET is a post-migratory event (likely related to metabolic activity of the locale) and may not always correlate with the extent of neutrophilic infiltration to the site of inflammation (Jones et al. 1997). Neutrophil migration and activation (although not NADPH oxidase activity) is thought to be responsible for increased F-18-FDG uptake. Another disadvantage of PET is that because no suitable metabolically stable PET radiolabels have yet been developed, it does not allow the quantification of cell trafficking to sites of inflammation in the same way that leukocyte scintigraphy with lower energy isotopes has performed. Furthermore, although the PET signal is thought to represent increased granulocyte glucose uptake, it is likely that other metabolically active cells at the site of inflammation contribute to the signal as noted above.

8.4 Biodistribution and Intravascular Lifespan of Leukocytes

8.4.1 Granulocytes

Granulocytes are formed from pluripotent stem cells in the bone marrow before being released into the circulation. As neutrophils mature from CD34-positive myeloid progenitors, they acquire new surface markers such as CD16b, CD35, and CD10 (Elghetany 2002). Early work with the diisopropylfluorophosphate (DFP^{32})-labelled granulocytes demonstrated that 50 % of the cells re-injected into healthy volunteers disappear rapidly from the circulation with a half-life of 6.6 h (Mauer et al. 1960). Following re-injection, radiolabelled cells are distributed in dynamic equilibrium between circulating and marginated granulocyte pools (Athens et al. 1960) (Fig. 8.4a). These two pools form a larger pool known as the total blood granulocyte pool (TBGP), which is twofold greater than that calculated from the

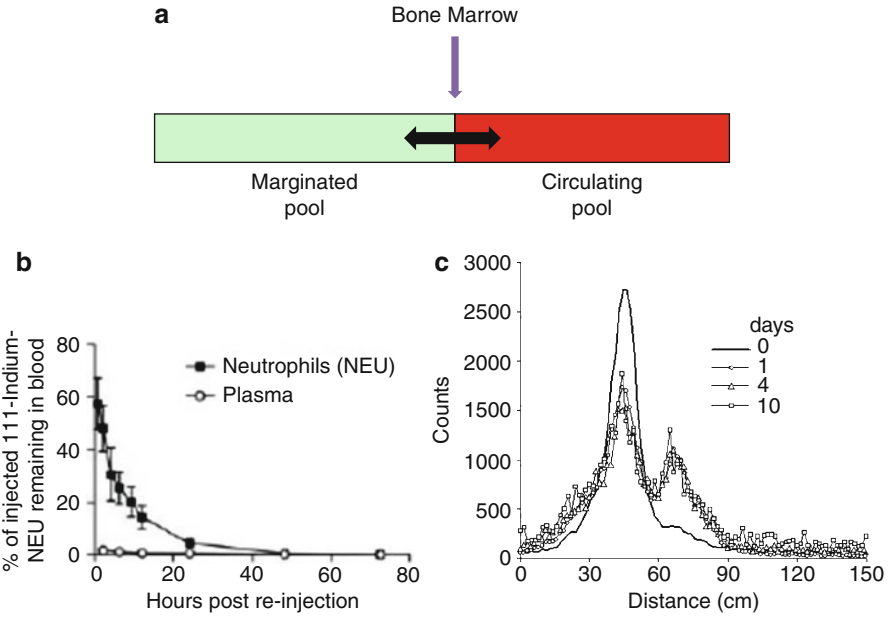

Fig. 8.4 Biodistribution and intravascular lifespan of granulocytes. (**a**) Schematic representation of the biodistribution of granulocytes between the bone marrow, marginated, and circulating pools. (**b**) Graph indicating the exponential decline in radiolabelled neutrophils from the circulation (Farahi et al. 2012). (**c**) Whole-body count profiles at increasing distances in cm from the head (*left*) to feet (*right*) in a normal subject at 45 min (day 0), 24 h, and 4 and 10 days following re-injection of In-111-labelled neutrophils. The *tallest peak* represents radioactivity in the liver and spleen and the *smaller peak* corresponds to radioactivity within the pelvic bone marrow. Physiological redistribution of labelled neutrophils from the liver and spleen to the bone marrow is demonstrated by 24 h following re-injection (Ruparelia et al. 2011)

peripheral blood granulocyte count and blood volume alone. Since the original work, In-111-granulocytes have been shown to have a mean intravascular residence time (MIRT) of 10 h with a half-life of 7 h (Saverymuttu et al. 1985b). It is thought that the marginated pool equilibrates with the circulating pool over a period of approximately 30 min and so use of the 45-min blood recovery value provides a sensitive biomarker of the functional viability of these cells (Peters 1994). As 50 % of the TBGP is immediately distributed to the marginated pool, the 45-min recovery will always be less than 50 % and in most studies has been shown to approximate 35–40 % for both In-111- and Tc-99m-HMPAO-granulocytes.

There is evidence that a large proportion of the marginated pool lies within the spleen (hence a dosimetry concern). Within 5 min of the re-injection of labelled granulocytes, the marginated pool accounts for 54 % of the total blood granulocyte pool (Peters et al. 1985a). Of this early marginated pool, the granulocytes are distributed between the spleen, liver, lung, and the remainder of the body. Whilst liver and splenic uptake increases with time, this uptake appears to be reversible suggesting a dynamic transit of granulocytes through these organs (Peters et al. 1985b). Hence adrenaline and exercise are both able to mobilise granulocytes from the marginated into the circulating pool, while in opposition, prednisolone increases the size of both pools, and endotoxin causes a shift of granulocytes from the freely circulating into the marginated pool (Athens et al. 1961). Following the injection of adrenaline, the use of Cr-51-labelled granulocytes demonstrates a fall in splenic activity and a corresponding rise in peripheral blood activity (McMillan et al. 1968). This illustrates the dynamic movement of granulocytes in the spleen and suggests that the initial margination seen in the spleen is physiological rather than a result of cell activation or damage (Peters and Saverymuttu 1987).

Intravascular transit time is estimated at 10 min in the spleen and 2 min in the liver; the persistent radioactive signal detected in the spleen beyond this represents both pooled and destroyed (or damaged) granulocytes (Peters 1998). Inflammatory conditions lead to a fall of activity detected in the spleen as granulocytes are diverted to sites of inflammation (Peters et al. 1985b). Although the liver normally represents a smaller part of the marginated pool, there is increased uptake if granulocytes are activated or damaged, which suggests that the destruction of granulocytes occurs within the reticuloendothelial system.

Granulocytes have a rapid transit through the lung under normal physiological circumstances (Saverymuttu et al. 1983a) and a mean transit time of between 20 s in the capillary blood (Doershuk 2000). Others have reported longer capillary transit times as much as 3–5 min (Peters 1998). There has been considerable debate regarding the size of the pulmonary granulocyte pool: it has been suggested that marginated granulocytes within the lung exist in two pools, one in which the granulocytes are moving quickly at the speed of erythrocytes and another pool in which granulocytes are met with obstruction as they do not deform as readily as erythrocytes as they travel through the vasculature (Peters 1998). For example, fully dilated capillaries have a diameter of 7.5 ± 2.3 μm whereas neutrophils have a diameter of 6.8 ± 0.8 μm; larger neutrophils are therefore restricted by smaller capillaries causing a 60–100-fold slower transit through the pulmonary capillary system relative to erythrocytes, which travel through in less than a second (Hogg 1994).

The same review suggests that these slower moving neutrophils increase their concentration within the pulmonary circulation relative to erythrocytes, and form the marginated, neutrophil pulmonary pool. Inhaled platelet-activating factor (PAF) leads to marked pulmonary sequestration of ^{111}In-labelled neutrophils, which again illustrates the capacity for activated neutrophils to be retained in the lung due to cytokines or other bioactive molecules (Tam et al. 1992).

In addition to dynamic movement of granulocytes within the spleen, a similar process of margination occurs in the bone marrow which has an intravascular transit time for granulocytes of 10 min (Ussov et al. 1995). A method using H-3 thymidine has provided insight into bone marrow neutrophil kinetics (Dancy et al. 1976). This study suggests a daily generation of 0.85×10^9 post-mitotic neutrophils per kg body weight within the bone marrow. Furthermore, the paper demonstrates that this post-mitotic pool of neutrophils contains adequate number of maturing and storage neutrophils to maintain the turnover of 0.85×10^9/kg/day for 6.6 days, therefore demonstrating a bone marrow transit time of just under 7 days. This is consistent with the observed neutropenia resulting from total body radiation where the nadir of cell counts in the peripheral circulation is 5–7 days post irradiation.

Using a WBC, the approximate distribution of In-111-labelled granulocytes can be detected using extremely low administered activities of the radioisotope (Szczepura et al. 2011). Neutrophils have a 45-min recovery time of approximately 40 % (due to their distribution between the marginated and freely circulating intravascular pools) and are then removed from the circulation in a mono-exponential manner (zero order) with final localisation in the liver, spleen, and bone marrow, which is evident at 24 h, reflecting their sites of destruction (Fig. 8.4b, c).

Tc-99m-HMPAO-granulocytes have identical kinetics through the lung, liver, and spleen as In-111-granulocytes. The half-life, however, of Tc-99m-HMPAO-leukocytes is lower at 4 h than that of In-111-granulocytes, which is thought to be an artefact, and represents Tc-99m elution from the circulation. Correction of such elution by in vitro stability experiments demonstrates a half-life approximating 6 h (Peters 1994). Non-specific bowel, urinary, and hepatobiliary activity is seen due to the excretion of hydrophilic complexes of Tc-99m-HMPAO and this must be taken into consideration in the analysis of abdominal scans performed in IBD or to localise abdominal collections. Early and sequential imaging is therefore important in IBD to avoid such non-specific bowel activity, which is not usually seen until at least 2 h following re-injection.

8.4.2 Eosinophils

To date there has been only one published study examining the kinetics of circulating radiolabelled eosinophils in healthy individuals (Farahi et al. 2012). Two early papers used intravenous injection of tritiated thymidine (H-3 thymidine) in patients with eosinophilia following surgical treatment for oesophageal cancer, bladder cancer (Parwaresch et al. 1976), glioblastoma, and chronic lymphocytic leukaemia

(Steinbach et al. 1979). Two patients with eosinophilia of unknown aetiology have also been studied although in one case, sarcoidosis was suspected (Herion et al. 1970). In Herion's paper leukocytes were separated by dextran sedimentation followed by hypotonic lysis of erythrocytes and then labelled with sodium radiochromate (Cr-51) before being re-injected. In-111-oxine-labelled eosinophil-enriched cells have also been used to assess eosinophil kinetics in one patient with Churg–Strauss syndrome and one with hypereosinophilic syndrome (Yamauchi et al. 1989). In the latter studies eosinophils accounted for only 87 % of the "eosinophil-enriched cells" that were purified and labelled, and neither technique isolated cells in autologous plasma as is our practice. Other disadvantages of the experimental methods used in these studies include the poor image resolution derived with the use of Cr-51, the potentially cytotoxic effect of In-111-oxine, and the risk of leukocyte clumping induced by heparin.

More recently however, eosinophil intravascular half-life has been determined using human mixed leukocytes that have been purified in autologous plasma and radiolabelled with In-111-tropolonate before re-injection. Subsequent blood sampling followed by isolation of "ultra-pure" eosinophils has indicated an intravascular residence time for these cells of approximately 25 h (Farahi et al. 2012). These investigators also studied purified eosinophils (prepared by discontinuous Percoll gradients and CD16-negative selection) radiolabelled with In-111-troponolate. In preliminary studies, the use of dynamic and static planar gamma camera imaging demonstrated rapid eosinophil transit through the lungs and margination in the liver and spleen (and to a far lesser extent bone marrow) with evidence of some recirculation of eosinophils from the liver (Farahi et al. 2012). This technological advance and knowledge about the circulation of eosinophils in healthy subjects provide an opportunity for pharmaceutical companies to test the impact of new drugs on eosinophil kinetics. Given the important role of eosinophils in allergic inflammation, this has wide applications in common conditions such as asthma and other conditions characterised by eosinophilic inflammation.

8.4.3 Lymphocytes

Lymphocyte kinetic studies have been limited by the use of suboptimal radionuclides such as Cr-51 and concerns about the lymphotoxicity of the oxine ligand used for In-111-oxine. It has been suggested that restricting the dose of radionuclide (either lower counts or higher specific radioactivity) allows lymphocytes to behave physiologically and unencumbered by the presence of oxine (Chisholm et al. 1979). Although In-111-oxine has been used to assess lymphocyte trafficking in man (Wagstaff et al. 1981), the amount of In-111-oxine label per 10^8 lymphocytes used in this study exceeds the level that has been shown to be lymphotoxic, particularly after 24 h. Lymphocytes were purified using a cell separator in the absence of autologous plasma. This produced lymphocytes that were greater than 95 % pure and 90 % viable. Following re-injection, serial blood samples demonstrated an initial

rapid clearance from the blood followed by a rise at 3–4 h which plateaued for 4–8 h before a second fall. Gamma camera imaging indicated a lack of uptake in the lungs and a distribution of activity between the liver and spleen by 4 h leading to the initial rapid clearance. There was a subsequent reduction in the splenic signal, which correlated with an increase in lymph node activity suggesting the recirculation of lymphocytes from the spleen into the circulation and thence to the lymph nodes. Heat-damaged lymphocytes demonstrate altered kinetics with significant lung and liver uptake and low activity in lymph nodes relative to controls suggesting that the In-111-oxine-labelled lymphocytes were behaving relatively physiologically.

8.4.4 Monocytes

Tritiated thymidine has been used to study monocyte kinetics in seven healthy individuals and this demonstrated a circulating half-life of 71 h (Whitelaw 1972), considerably longer than neutrophils. The use of Tc-99m-HMPAO-labelled monocytes to assess their biodistribution when re-injected into patients with rheumatoid arthritis has also shown initial margination in the lung followed by the re-distribution to the liver, spleen, and bone marrow (Bennink et al. 2008).

8.5 Migration Characteristics of Leukocytes

Following re-injection, the migration characteristics of labelled autologous leukocytes can clearly be seen to alter in pathological conditions such as abscesses and other systemic inflammatory processes. The pathological situations which involve leukocyte migration can be separated broadly into three categories according to the migratory behaviour of these cells: firstly, conditions in which leukocytes migrate to sites of infection and inflammation and remain localised such as abscesses; secondly, conditions in which leukocytes travel to such sites but continue to migrate into the lumen of the airway or the bowel such as bronchiectasis, COPD, and IBD; and thirdly, conditions such as community-acquired pneumonia (CAP) where re-injected leukocytes fail to migrate to the inflamed site because of early termination of such migration in the disease history.

8.5.1 Solid Abscess

Ga-67 citrate has been used previously to localise abscesses, but despite high sensitivity, it lacks specificity due to transferrin and other iron-binding sources which gallium also follows. Furthermore, renal and gastrointestinal excretion of radiogallium leads to non-specific bowel activity, which makes the detection of

intra-abdominal collections difficult. The delay of 24–72 h before the result of such a scan is available is also generally clinically unacceptable.

In-111-leukocytes have been used since the late 1970s for the localisation of abscesses and results are generally interpreted within 24 h; although faster than Ga-67, not all NM Departments offer this service and the time requirement still remains a disadvantage relative to other imaging modalities such as computed tomography (CT) although CT is non-specific and relates only to anatomical density discrimination or tissue/organ displacements. Intra-abdominal abscesses are identified with the use of a planar gamma camera as focal activity outside the normal distribution with intensity greater than that of the liver (Peters and Saverymuttu 1987). Only 33 % of abscesses are identified within 1–4 h of re-injection but 95 % are true positives (TP) at 18–24 h. The use of In-111-tropolone-labelled granulocytes can however generate false-positive (FP) images in conditions such as IBD, haematomas, tumours, non-rejecting renal allografts, and uncomplicated bowel anastomosis (Peters and Saverymuttu 1987).

In-111-labelled leukocytes would be the modality of choice in the investigation of an abscess in the gastrointestinal tract as ultrasound, and to some extent CT images, may be difficult to interpret because of overlying bowel gas and the distinction between an abscess and thickened or dilated loops of bowel. Comparison between In-111-troponolate-granulocytes, Tc-99m-HMPAO-leukocytes, and ultrasound scanning for the detection of intra-abdominal abscess has shown that Tc-99m-HMPAO scanning is at least as accurate as In-111-troponolate scanning and that both have a greater sensitivity and specificity than ultrasound scanning, although scintigraphy is less effective at detecting or discriminating hepatic abscesses (Weldon et al. 1995a, b). The physiological uptake of labelled leukocytes in the liver makes the detection of hepatic abscesses more difficult; however, sequential imaging may demonstrate a gradual increase in radioactivity within a septic focus whereas the physiological liver uptake falls between 1 and 4 h (Cousins et al. 2003a). Figure 8.5 demonstrates the use of In-111-labelled granulocytes to localise a communicating abscess.

The use of In-111-labelled autologous granulocytes is also able to detect pulmonary (Saverymuttu et al. 1985a) and brain (Peters et al. 1980) abscesses. Tc-99m-labelled leukocytes have also been used to detect brain abscesses (Grimstad et al. 1992) and both techniques may help distinguish infective or malignant lesions at this site.

8.5.2 Intrathoracic Disease

Re-injected radiolabelled autologous leukocytes usually demonstrate a rapid transit through the lungs unless the cells have been activated in vivo (for example, in graft-versus-host disease (GVHD) or septicaemia) or damaged ex vivo. Leukocyte trafficking in the lung however is altered under several disease conditions, such as in ARDS, COPD, bronchiectasis, and lobar pneumonia.

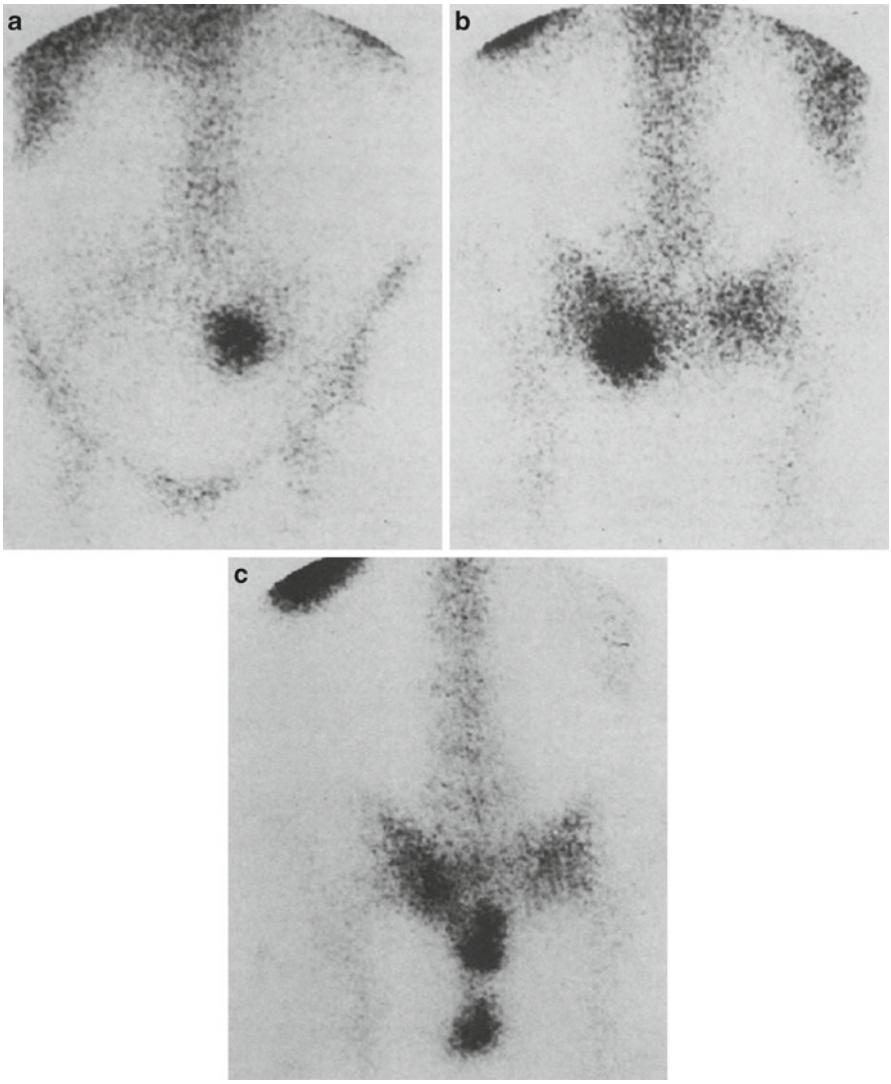

Fig. 8.5 In-111-granulocyte scans taken 3 h following injection. (**a**) Anterior planar camera view and (**b**) posterior planar camera view of an abscess in the left iliac fossa anterior to the left sacro-iliac joint. (**c**) The same individual scanned at 24 h indicating residual activity at the site of the abscess but also new activity in the distal colon because of a communicating abscess (Peters 1994)

8.5.3 ARDS

Neutrophils play a vital role in the pathogenesis of acute lung injury (Ware and Matthay 2000). The histological appearance of the lungs of patients with ARDS demonstrates the presence of neutrophils in the lung interstitium, alveolar space,

and vascular compartment, as well as structural damage to the endothelium and epithelium. These anatomical distributions and structural alterations are postulated to affect the barrier function of the alveolar capillary endothelium leading to lung injury (Downey et al. 1999). Granulocytes dual-labelled with both In-111 and Tc-99m have been used to determine pulmonary granulocyte kinetics in individuals with systemic inflammation characterised by increased soluble E-selectin levels, a marker of vascular activation (Ussov et al. 1999). Individuals with systemic inflammation demonstrate a larger pulmonary granulocyte pool but of note, these cells do not migrate into the interstitium unless the pulmonary endothelium is also activated. The re-injection of In-111-labelled autologous granulocytes into patients with ARDS demonstrates pulmonary retention even in the context of concurrent steroid treatment (Warshawski et al. 1986).

8.5.4 COPD

Neutrophils are abundant in the airways and lung parenchyma of individuals with COPD and are believed to be involved in the pathogenesis of the condition. The assessment of neutrophil kinetics in the lungs of patients with COPD has been determined using In-111-troponolate- and Tc-99m-HMPAO-labelled granulocytes combined with WBC and SPECT, which measures neutrophil clearance from the blood into the lung parenchyma (Ruparelia et al. 2011). At 45 min post re-injection, WBC data demonstrates a peak of radioactivity within the liver and spleen region followed by a second peak in the pelvic bone marrow. By 24 h, the peak within the liver and spleen is reduced whereas the bone marrow peak is higher, consistent with the known physiological re-distribution of neutrophils (Fig. 8.4c). The mean whole body loss of In-111-tropolonate in COPD patients over 7 days is less than 10 % and similar to healthy non-smokers; by contrast currently smoking COPD patients displayed a greater whole body In-111-tropolonate cell loss (Fig. 8.6b). SPECT images also demonstrate significant time-dependent and quantifiable migration of neutrophils into the lungs of patients with COPD (Figs. 8.6c and 8.7a) and this signal offers considerable promise as a marker of drug efficacy in this condition.

8.5.5 Bronchiectasis

The re-injection of In-111-troponolate-granulocytes followed by gamma camera imaging and whole-body counting has been used to assess granulocyte kinetics in patients with bronchiectasis (Currie et al. 1987). This demonstrates the migration of granulocytes to actively inflamed bronchiectatic areas of the lung within the first 24 h. These labelled cells then continue to migrate into the airway lumen and are expectorated in the sputum (Fig. 8.6a). Patients with severe bronchiectasis can lose

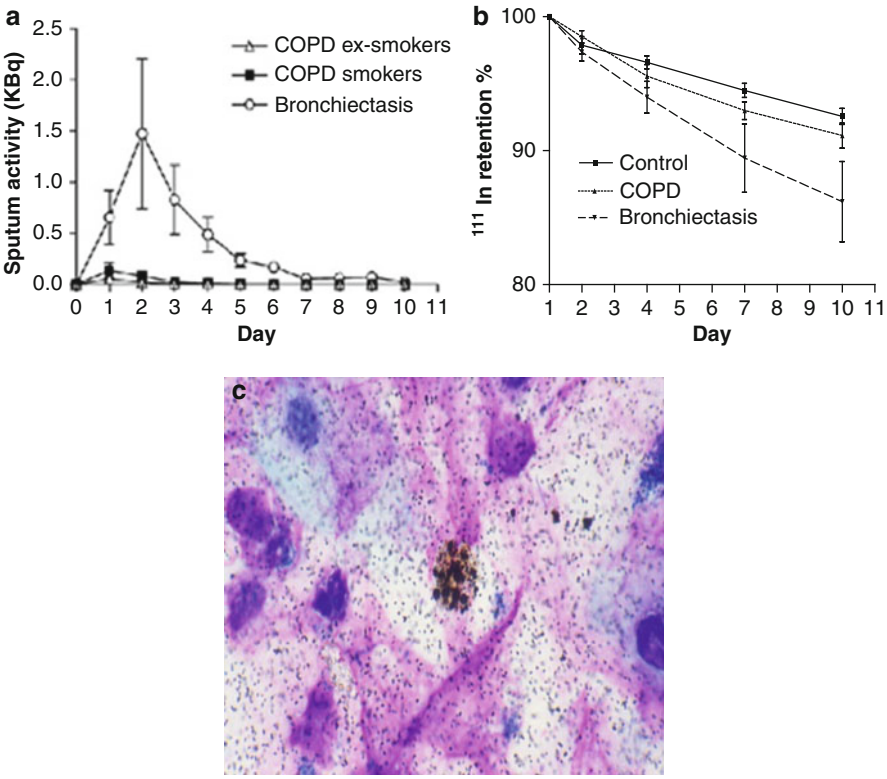

Fig. 8.6 Migration of In-111-labelled neutrophils into the sputum of patients with COPD and bronchiectasis. (**a**) The radioactivity of expectorated sputum in each 24-h period. (**b**) The percent of In-111 retention over a 10-day period in healthy volunteers (controls) and in patients with COPD and bronchiectasis. A higher level of In-111 loss from the body is demonstrated in those with bronchiectasis and COPD compared to controls. (**c**) Autoradiograph of an ^{111}In-labelled neutrophil in the expectorated sputum of a patient with COPD (Ruparelia et al. 2011)

>50 % of the re-injected labelled neutrophils in the sputum (Currie et al. 1987). As a consequence, radioactivity may be detected in the gastrointestinal tract of patients with severe bronchiectasis due to swallowed sputum containing In-111-labelled cells. In-111 uptake however is not present in all segments of bronchiectatic lung demonstrated on CT and such areas presumably reflect focally quiescent disease. The use of In-111-labelled granulocytes followed by WBC scanning demonstrates that bronchiectatic patients with active disease have a higher 7-day whole body loss of In-111, approximating 25 %, compared to less than 10 % in those with quiescent disease, which is similar to the loss seen in healthy non-smokers (Ruparelia et al. 2011) (Fig. 8.6b).

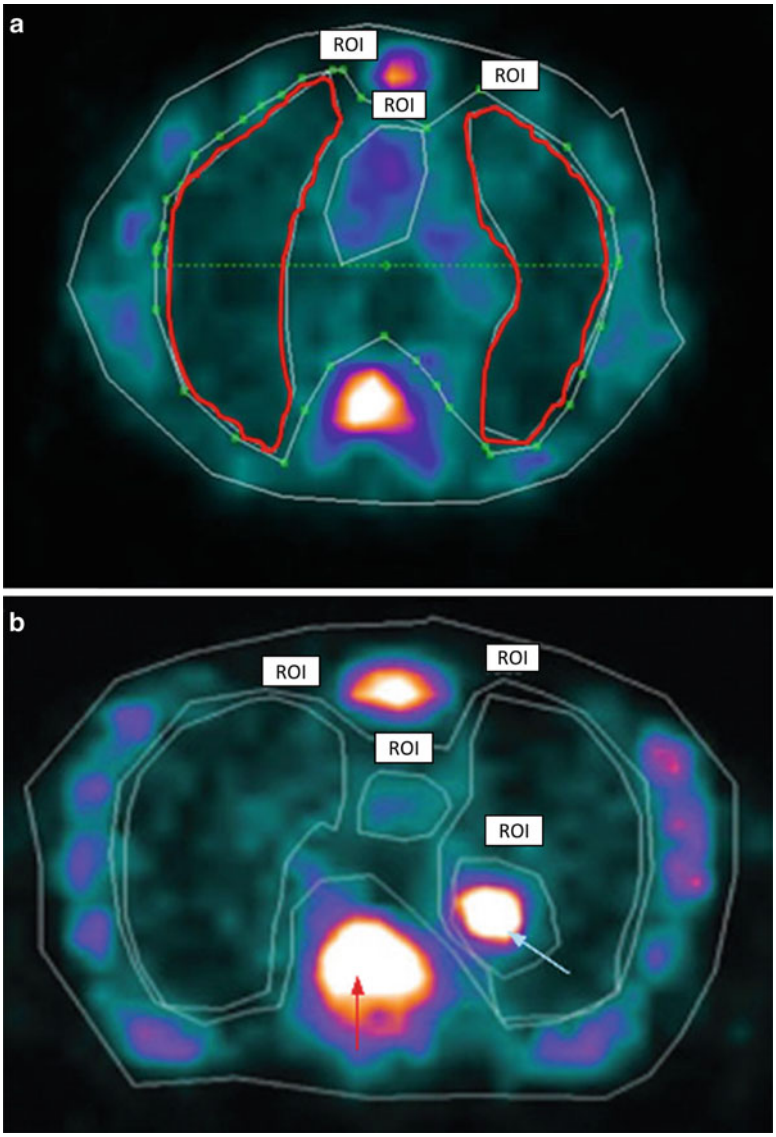

Fig. 8.7 (**a**) SPECT image demonstrating increased Tc-99m-labelled neutrophil uptake in the lung parenchyma of COPD patients. Regions of interest highlighted over the lungs (*red outline*), heart, and bone marrow (ribs, sternum, and vertebral body) (Ruparelia et al. 2011). (**b**) SPECT image indicating focal uptake of Tc-99m-labelled neutrophils in the left lower lobe of the lung (*blue arrow*). The *red arrow* indicates physiological uptake in the vertebral bone marrow) (Ruparelia et al. 2009)

8.5.6 *Lobar Pneumonia*

Although In-111-labelled leukocytes can be used to detect and localise abscesses in the lung and elsewhere, no signal is detected by gamma camera imaging in patients with uncomplicated lobar pneumonia. This is likely related to the extremely early (less than 6 h) termination of granulocyte migration in this disease (Saverymuttu et al. 1985a). The injection of Tc-99m-labelled autologous neutrophils followed by SPECT imaging in a patient with COPD, who was clinically well at the time of injection, incidentally demonstrated focal uptake in the lung at 4 hours after injection (Ruparelia et al. 2009) (Fig. 8.7b). Within 48 h the patient under study developed symptoms of dyspnoea, fever, and purulent sputum and clinical signs of consolidation confirmed by chest radiograph along with a positive sputum culture. This represents the capacity of these techniques to identify a pneumonic focus at a very early time point in the natural history of this condition before neutrophil migration has ceased.

8.5.7 *Inflammatory Bowel Disease*

IBD is a chronic inflammatory disease of the bowel and includes Crohn's disease, which may affect any part of the bowel, and ulcerative colitis (UC), which is usually confined to the rectum and colon.

In-111-labelled granulocyte scintigraphy in IBD has the ability to detect, anatomically locate, and define the severity of active inflammation and can image both the small and large bowel (Peters and Saverymuttu 1987). It has also been shown to correlate reliably with endoscopic findings and the histologically defined disease extent and activity (Saverymuttu et al. 1986). The intensity of uptake in the bowel in comparison to the uptake of the liver and spleen has been shown to correlate with clinical markers of severity (Peters and Saverymuttu 1987). The severity of IBD has also been shown to correlate with faecal granulocyte loss provided the stool collections are complete.

In the same way that granulocytes are lost in the sputum of patients with bronchiectasis and COPD, faecal loss of granulocytes occurs in IBD over the course of 4 days post injection and indicates the extent of migration of cells into the bowel lumen and subsequent loss from the body (Saverymuttu et al. 1983c). This may be demonstrated by the gradual reduction in image signal as the radiolabelled cells leave the body. Scanning with a WBC over 4 days also has the distinct advantage that it allows the administration of much lower levels of indium to quantify inflammation in IBD and the retention and loss of labelled cells (Cheow et al. 2005). It has been shown to be an accurate alternative to measuring the faecal excretion of these cells (Carpani de Kaski et al. 1992).

Tc-99m-HMPAO-labelled granulocytes are used more frequently in IBD due to the lower radiation dose of Tc-99m, greater image resolution, and capacity to detect small bowel pathology, which indium-scanning can miss (Fig. 8.8). Due to non-specific intestinal activity typical of Tc-99m-HMPAO-labelled granulocytes, false positives are possible when patients are scanned late particularly after 3 h; it is

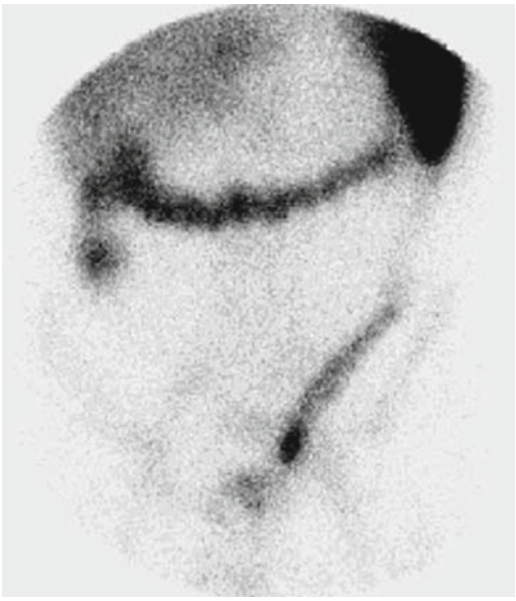

Fig. 8.8 Tc-99m-HMPAO scan in a patient with severe ulcerative colitis demonstrating severe colitis (Cheow et al. 2005)

important therefore that an early image is taken followed by sequential images. The sensitivity of Tc-99m-HMPAO for the detection of acute inflammation ranges from 91 to 95 % (Weldon 2003). Used in conjunction with SPECT, this approach has been shown to accurately quantify the extent of inflammation in IBD (Weldon et al. 1995a, b). Faecal counting with Tc-99m is not possible due to its short half-life (6 h) and the extent of non-specific gastrointestinal excretion (>24 h).

Tc-99m-labelled granulocytes can also be used to assess disease reactivation and to identify complications in Crohn's disease such as abscess formation and fistulae although imaging must be performed early in suspected colo-vesical fistulae due to the urinary activity caused by renal excretion (Peters 1994). The signal from a Crohn's abscess remains persistent at 24 h and can be distinguished from non-specific bowel activity, the signal from which diminishes with time. False positives may be seen in recent bowel anastomosis and gastrointestinal bleeding.

8.5.8 Nephrological Disease

Abnormal renal uptake has been demonstrated by In-111-leukocyte scintigraphy in patients with renal transplant rejection with a sensitivity of 73 % (Forstrom et al. 1981). However, many individuals with chronic renal disease referred for pyrexia of unknown origin (FUO) do not show renal uptake (Cousins et al. 2003b). Given the extent of renal uptake of secondary Tc-99m-HMPAO complexes in the kidney, imaging of the renal parenchyma should always be undertaken with In-111-labelled cells. Diffuse renal uptake of In-111 has also been seen in systemic vasculitis (Jonker et al. 1992).

8.5.9 Orthopaedic Infections

Osteomyelitis is an infection of the bone arising from haematological seeding, direct spread from adjacent soft tissue and joints, or direct infection through trauma and/or orthopaedic surgery. Without prompt diagnosis and treatment complications may occur including the progression to chronic osteomyelitis.

The use of labelled leukocytes in the detection of bone infection is made more difficult by the previously discussed physiological uptake of leukocytes by the bone marrow. Negative scans are also possible in cases of chronic osteomyelitis, where granulocyte migration can be impeded by poor blood flow to osteomyelitis and thus provide minimal signal (Peters and Saverymuttu 1987).

The use of In-111-oxine-labelled leukocytes has however been used to detect osteomyelitis with a sensitivity of 0.91 and specificity of 0.62 (Palestro et al. 2002). These values are improved further if white cell scanning is combined with Tc-99m sulphur colloid. Limitations again include negative scans in chronic infections, and the local uptake of labelled leukocytes in lymph nodes (e.g. in the groin), which can confuse the interpretation of joint infection (Palestro et al. 2006).

Within the axial skeleton, the detection of abnormal uptake in the bone depends on the demonstration of leukocyte activity in the absence of colloid uptake (bone scintigraphy) (Peters 1994). Leukocyte scintigraphy in cases of vertebral body osteomyelitis will paradoxically demonstrate reduced uptake in affected areas due to comparatively greater physiological uptake in the bone marrow of adjacent healthy vertebral bodies (Seabold and Nepola 2003). Although the bone marrow in the peripheral skeleton is not usually a problem for the detection of osteomyelitis, it can sometimes be difficult to differentiate bone infection from adjacent soft tissue infection. CT or MRI anatomical discrimination may be of assistance together with the localised leukocyte uptake. Leukocyte scintigraphy may also be used to assess the response to treatment with antibiotics and to detect infection of and loosening of joint prostheses resulting in non-specific inflammation.

8.5.10 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a symmetrical, inflammatory polyarthropathy characterised by the accumulation of inflammatory cells in the synovial tissue of joints leading to chronic inflammation and destruction of joints.

Imaging of Tc-99m-HMPAO-labelled monocytes in patients with active RA has demonstrated similar initial kinetics to those seen with labelled mixed leukocytes with initial margination to the lungs followed by margination in the liver and spleen (Bennink et al. 2008). Within 2 h however, the labelled monocytes accumulate in the large and small joints of the hands and feet. Tc-99m-HMPAO-labelled lymphocytes have also been shown to migrate to joints and a positive joint scintigram is predictive of active synovitis (Jorgensen et al. 1995).

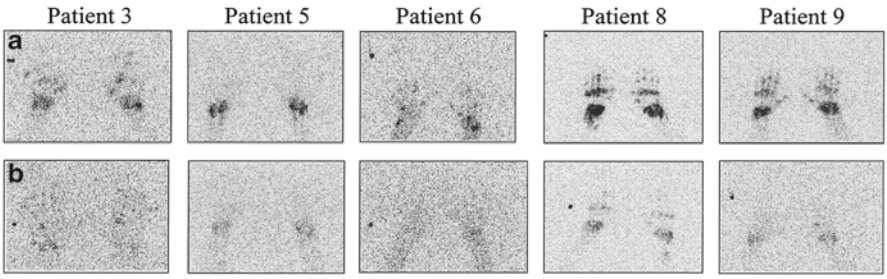

Fig. 8.9 In-111 scans of the hands and wrists of five patients taken 22 h after injection (**a**), before and (**b**) after a single intravenous bolus of anti-tumour necrosis factor α antibody. A reduction in the signal detected is demonstrated after treatment in all five patients (Taylor et al. 2000)

Leukocyte scintigraphy has also been used to assess the response to treatment in RA. In-111-granulocyte scintigraphy has been carried out in RA patients before and after treatment with agents targeting the tumour necrosis factor-α (TNFα) pathway. Following such treatment, granulocyte counts within the synovial fluid of patient samples are lower and this is accompanied by a significant reduction of the In-111-granulocyte signal in the knees, hand, and wrist joints (Taylor et al. 2000) (Fig. 8.9).

8.5.11 Systemic Vasculitis

Systemic vasculitis is associated with marked changes in granulocyte kinetics involving both the lung and spleen (Jonker et al. 1992). Patients with granulomatosis with polyangitis (GPA) and microscopic polyarteritis (MP) studied with autologous Tc-99m-HMPAO- or In-111-troponolate-labelled leukocytes, demonstrate diffuse lung uptake which is most marked in GPA patients. Activity can also be detected in the nose of GPA patients, and one such patient with rapidly progressive glomerulonephritis had diffuse uptake of indium-labelled cells in the kidneys. There was also abnormal splenic uptake in patients with systemic vasculitis.

8.6 Summary

Modern imaging modalities with ever-impressive improvements in spatial resolution and methods to interpret cell kinetics, have led to a revolution in leukocyte imaging via scintigraphy, and such methods have allowed for the quantitative interpretation of cell trafficking and migration of leukocytes under both physiological and pathological conditions.

Published studies have clearly demonstrated the utility of radioscintigraphy to assess the response of disease conditions (and normal physiology) to pharmacological intervention. Careful leukocyte preparation and radiolabelling, coupled to modern imaging modalities such as SPECT/CT, now provides an exciting platform to test the efficacy of new drugs and biologics, as well as novel cell therapies, in altering the migration characteristics and kinetics of inflammatory cells. The ability to image the anti-inflammatory responses to treatment with existing and novel drugs and biologics will continue to be innovative and a valuable asset in the understanding of how we can best control or treat infection and inflammation.

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