

Real-time differential tracking of human neutrophil and eosinophil migration *in vivo*

Joanna J. Lukawska, MD,^{a,b*} Lefteris Livieratos, PhD,^{a,c*} Barbara M. Sawyer,^a Tak Lee, MD, ScD,^{b,†} Michael O'Doherty, MD, MD,^{a,c} Philip J. Blower, PhD,^{a,d} Martin Kofi,^c James R. Ballinger, PhD,^{a,c} Christopher J. Corrigan, MD, PhD,^b Gopinath Gnanasegaran, MD,^c Ehsan Sharif-Paghaleh, PhD,^a and Gregory E. D. Mullen, PhD^a London, United Kingdom

Background: Hitherto, *in vivo* studies of human granulocyte migration have been based on indiscriminate labeling of total granulocyte populations. We hypothesized that the kinetics of isolated human neutrophil and eosinophil migration through major organs *in vivo* are fundamentally different, with the corollary that studying unseparated populations distorts measurement of both.

Methods: Blood neutrophils and eosinophils were isolated on 2 separate occasions from human volunteers by using Current Good Manufacturing Practice CD16 CliniMACS isolation, labeled with technetium 99m-hexamethylpropyleneamine oxime, and then reinfused intravenously. The kinetics of cellular efflux were imaged over 4 hours.

Results: Neutrophils and eosinophils were isolated to a mean purity of greater than 97% and greater than 95%, respectively. Activation of neutrophils measured as an increase in their CD11b mean fluorescence intensity in whole blood and after isolation and radiolabeling was 25.98 ± 7.59 and 51.82 ± 17.44 , respectively, and was not significant ($P = .052$), but the mean fluorescence intensity of CD69 increased significantly on eosinophils. Analysis of the scintigraphic profile of lung efflux revealed exponential clearance of eosinophils, with a mean half-life of 4.16 ± 0.11 minutes. Neutrophil efflux was at a

significantly slower half-life of 13.72 ± 4.14 minutes ($P = .009$). The migration of neutrophils and eosinophils was significantly different in the spleen at all time points ($P = .014$), in the liver at 15 minutes ($P = .001$), and in the bone marrow at 4 hours ($P = .003$).

Conclusions: The kinetics of migration of neutrophils and eosinophils through the lung, spleen, and bone marrow of human volunteers are significantly different. Study of mixed populations might be misleading. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Granulocytes, eosinophils, neutrophils, kinetics, radio-labeled, lung, liver, spleen, bone marrow, technetium 99m-hexamethylpropyleneamine oxime

The biodistribution of granulocytes in animals and human subjects has been extensively investigated in both homeostasis and disease states. Most studies have used nuclear medicine methodologies and appear to have established that human granulocytes in homeostasis are distributed in approximately equal proportions in 2 dynamic pools: a marginating and a circulating pool.^{1,2} Granulocyte margination is interpreted as the consequence of transient adhesion of these cells to the microvasculature of major organs.^{3,4} Shortly after injection of radiolabeled granulocytes, the marginating pool was shown to be distributed predominantly between the spleen (35%), liver (25%), and lung (10%).⁵ In these studies the fact that granulocytes are not a pure population of cells has been set aside, and the tacit assumption has been that neutrophils, which are numerically the most common blood granulocytes, at least in healthy subjects, account for most of the signal when assessing biodistribution. This already somewhat dubious proposition has been further undermined by the observation that when a total granulocyte population was radiolabeled with technetium 99m-hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO), eosinophils were labeled with much higher efficiency than neutrophils, such that their mean cellular radioactivity was at least 10-fold higher.^{6,7} This implies that eosinophils *in vivo* could be contributing more than half of the overall signal, despite their significantly fewer numbers. Thus the assumption that granulocyte scans essentially reflect neutrophil distribution is not well founded and might be grossly misleading. This is of critical importance because neutrophils and eosinophils have fundamentally different roles in health and in particular disease processes. Whereas neutrophils act as professional phagocytes and home to sites of infection and some forms of inflammation, eosinophils are regarded as key effector cells in other inflammatory diseases, including asthma, eosinophilic pneumonia, eosinophilic colitis, and esophagitis. Furthermore, in patients with some diseases, such as asthma,

From ^aKings College London, Division of Imaging Sciences and Bioengineering, St Thomas' Hospital; ^bKing's College London, Department of Asthma, Allergy & Respiratory Science, Guy's Hospital; ^cthe Department of Nuclear Medicine, Guy's and St Thomas' Hospitals, London; and ^dthe Division of Chemistry, King's College London.

*These authors are joint first authors.

†Tak Lee, MD, ScD, is currently affiliated with the Allergy Centre, HK Sanatorium and Hospital, Hong Kong.

Supported by the Department of Health through the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and by the Lee Lu Cheung Fund.

Disclosure of potential conflict of interest: P. J. Blower has grants/grants pending with various funding agencies and has received travel/accommodations/meeting expenses for academic invited talks. C. J. Corrigan has consultant arrangements with Chiesi, Novartis, and Allergy Therapeutics; has received payment for lectures, including service on speakers' bureaus, from GlaxoSmithKline; has received payment for development of educational presentations from Henry Stewart Talks; and has received travel/accommodations/meeting expenses from Novartis. G. E. D. Mullen has received grants from Department of Health/Medical Research Council. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 7, 2012; revised June 18, 2013; accepted for publication June 28, 2013.

Corresponding author: Gregory E. D. Mullen, PhD, King's College London, Department of Imaging Chemistry and Biology, Division of Imaging Sciences and Bioengineering, Lambeth Wing, St Thomas' Hospital, London SE1 7EH, United Kingdom. E-mail: greg.mullen@kcl.ac.uk.

0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2013.06.031>

Abbreviations used

FITC: Fluorescein-5-isothiocyanate

HSA: Human serum albumin

MFI: Mean fluorescence intensity

^{99m}Tc-HMPAO: Technetium 99m hexamethylpropyleneamine oxime

PE: Phycoerythrin

ROI: Region of interest

TAC: Time activity curve

heterogeneity has been proposed according to the relative contributions of neutrophils and eosinophils. Clearly, the ability to differentiate eosinophil and neutrophil migration is of fundamental importance.

In this study we used the Current Good Manufacturing Practice CD16 CliniMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany), which allows separation of blood neutrophil and eosinophil granulocytes according to expression or nonexpression of CD16 by using anti-CD16–conjugated magnetic beads. This allowed cellular separation, labeling, and reinfusion to be performed in a completely sterile environment in sufficient numbers and in a workable time frame with minimal stimulation of the cells that can modify their migration. We hypothesized that the time courses of efflux of neutrophils and eosinophils into the major organs of healthy adult subjects are fundamentally different.

METHODS**Participants and study design**

Four healthy volunteers were each scanned twice (2 male subjects aged 26 and 35 years and 2 female subjects aged 27 and 32 years). All volunteers had blood neutrophil and eosinophil counts within the laboratory's normal range and no clinical signs or symptoms whatsoever, particularly no signs or symptoms of upper or lower respiratory tract infection or inflammation, at the time of the study. The study was approved by an independent United Kingdom national research ethics committee and by the Administration of Radioactive Substances Advisory Committee of the United Kingdom. All subjects provided written informed consent to participate and were recruited at King's College London. On their first visit, volunteers were imaged after reinfusion of autologous radiolabeled neutrophils and returned within 11 weeks to be imaged again after reinfusion of autologous radiolabeled eosinophils.

Good Manufacturing Practice neutrophil and eosinophil isolation and radiolabeling

All open manipulations were carried out in a grade A environment in a pharmaceutical isolator located in a grade D background Good Manufacturing Practice facility in the Department of Nuclear Medicine at Guy's Hospital (London, United Kingdom). Each volunteer donated one 110-mL sample of venous blood on 2 separate occasions. At each visit, 105 mL of blood was collected in a heparinized syringe (sodium heparin at 1000 units per 105 mL of whole blood) of which 15 mL was centrifuged to retain a plasma sample and the remainder was diluted 1:1 with CliniMACS buffer (Miltenyi Biotec) and then layered over 95 mL of Ficoll Plus (GE Healthcare, Buckinghamshire, United Kingdom) and centrifuged at 450g for 25 minutes at room temperature. In addition, 4 mL of blood was collected into a Vacutainer EDTA tube (BD Biosciences, Oxford, United Kingdom) for cell counting, and 1 mL of blood was collected into 1 mL of 0.4% formaldehyde/PBS for flow cytometric analysis. After Ficoll separation, the diluted plasma was retained, whereas the mononuclear layer and Ficoll were discarded. The erythrocyte/granulocyte pellet was resuspended in autologous diluted plasma to a final volume of 95 mL, 20 mL of 6% Hespán (Grifols, Cambridge, United Kingdom) was added, and the mixture was centrifuged at 8g for 15 minutes at room temperature

(to separate the granulocytes from the red cells). The granulocyte layer was washed once with 100 mL of CliniMACS buffer containing 0.5% human serum albumin (HSA; Octapharma, Manchester, United Kingdom; CliniMACS-HSA buffer) and then resuspended in 10 mL of HSA buffer to which 0.8 mL of CD16 reagent (Miltenyi Biotec) had been added. After incubation at room temperature for 30 minutes on a slow cycle of a rotary mixer, the granulocytes were washed once with 50 mL of HSA buffer and then resuspended in the same buffer before being transferred to a sterile, non-pyrogenic blood collection bag (Miltenyi Biotec). The bag was then connected to the automated CliniMACS system, where the passage of the granulocytes in HSA buffer through the system and column eliminated CD16[−] cells (predominantly eosinophils), which were then collected in a sterile collection bag. After removal of the magnetic field by using the CliniMACS program, the CD16⁺ fraction (neutrophils) was eluted into another sterile bag attached to the CliniMACS tubing set. Eosinophils (approximately 1×10^7) or neutrophils (approximately 10×10^7) were incubated with up to 700 MBq of ^{99m}Tc-HMPAO (Ceretek, GE Healthcare) in accordance with the manufacturer's instructions. After incubation at ambient temperature for 15 minutes, the labeled cells were washed and resuspended in 2.5 mL of autologous plasma. Radiolabeling efficiency was measured by dividing the pellet activity (in MBq) by the total activity (pellet and supernatant) times 100.

Viability, phenotype, and purity of purified radiolabeled neutrophils and eosinophils

Aliquots of 1×10^6 cells collected from whole blood after CD16 CliniMACS isolation and after radiolabeling were analyzed by means of flow cytometry. Cells were fixed with 0.4% formaldehyde in PBS at 37°C for 4 minutes and then washed in HSA buffer and resuspended in CliniMACS-HSA buffer. Cells were analyzed by using a FACSCalibur machine (BD Biosciences) with the following antibodies and fluorochromes, according to the manufacturer's instructions: CD16-phycoerythrin (CD16-PE) and CD15–fluorescein-5-isothiocyanate (CD15-FITC; Miltenyi Biotec), CD66-PE, CD11b-FITC, CD69-FITC, IgG-FITC/IgM-PE (isotype controls), and a mononuclear FITC cocktail of CD3, CD19, CD56, and CD80 (BD Biosciences). Staining of CD11b on neutrophils was performed on fixed cells at 4°C for no more than 10 minutes because it has been previously shown that staining for longer periods and at room temperature induces activation of CD11b on neutrophils.⁸ Possible activation of the CD66⁺CD16⁺ neutrophil population during *ex vivo* manipulation and radiolabeling was investigated by examining changes in the mean fluorescence intensity (MFI) of CD11b⁹ staining in whole blood and after isolation and radiolabeling and based on the percentage of cells coexpressing CD11b above a fluorescence threshold (CD11b^{High}) observed after incubating unfixed whole blood from healthy volunteers ($n = 10$) with GM-CSF (500 ng/mL, BD Biosciences) at 37°C for 2 hours.⁸ This was used to define a CD16⁺CD11b^{Low} and CD16⁺CD11b^{High} population (see Fig E1 in this article's Online Repository at www.jacionline.org). Quadrants were based on negatively stained cells and the isotype control. Possible activation of CD66⁺CD16[−] eosinophils was investigated by examining changes in CD69 MFI by staining cells from whole blood and after radiolabeling and based on the percentage of cells expressing CD69¹⁰ fluorescence compared with unstained cells and cells stained with isotype control with the stated reagents. Cellular viability was assessed with an automated cell counter (Countess; Invitrogen, Paisley, United Kingdom) based on Trypan blue exclusion.

Imaging protocol and data analysis

With the volunteer positioned in the dual-headed gamma camera (Symbia; Siemens Medical Solutions, Surrey, United Kingdom), 100 MBq (96 ± 27 MBq) of labeled autologous eosinophils or neutrophils suspended in 2.5 mL of autologous plasma was injected intravenously as a bolus. Dynamic anterior and posterior views were acquired with low-energy general purpose collimators as a sequence of 1-second frames for the first 2 minutes, followed by 30-second frames up to 32 minutes after injection. Further images (5 minutes per frame) were acquired at 1, 2, and 4 hours after injection. An outline/localization image with a ⁵⁷Co flood source positioned between the camera

and the subject was acquired for 30 seconds at the end of the dynamic scan for improved accuracy of lung region localization during data analysis. Images were transferred onto an offline workstation (Hermes Medical, Stockholm, Sweden) in which regions of interest (ROIs) were defined over the left and right lung, liver, spleen, and bone marrow. The ROIs were projected over the entire dynamic sequence to ensure that any influence by other organs was avoided. Bone marrow uptake was obtained by placing the ROI over the sternum at 4 hours after injection to allow for sufficient time for cell migration to the region. ROIs were applied onto both the anterior and posterior views to calculate geometric mean values of the acquired counts expressed as a percentage of counts in the whole image at each time point. Time activity curves (TACs) were extracted for each ROI, providing mean counts per second at each sampling time point. The left and right lung TACs, starting from the point they reached maximum activity, were fitted by using an exponential model, and the half-life was calculated. Statistical comparisons of neutrophil and eosinophil uptake through the lung, liver, spleen, and bone marrow at various time points after injection were performed by using a 1-tailed paired *t* test, and a *P* value of less than .05 was considered significant.

RESULTS

Isolation and radiolabeling of neutrophils and eosinophils

After the CliniMACS purification and radiolabeling procedure, the mean percentage of CD66⁺CD16⁺ cells in the neutrophil fraction was 97.7% ± 2.5%, whereas the mean percentage of CD66⁺CD16[−] cells in the eosinophil fraction was 95.1% ± 6.4% (Table I). Importantly, the neutrophil fraction contained less than 0.1% CD66⁺CD16[−] eosinophils and less than 0.1% mononuclear cells (CD3⁺, CD19⁺, CD56⁺, or CD80⁺), whereas the eosinophil fraction contained less than 0.1% neutrophils and less than 1% mononuclear cells. CD11b MFI in the neutrophil population, using identical antibodies and cytometer settings after analysis in whole blood compared with *ex vivo* manipulation and radiolabeling (ie, after isolation and radiolabeling), was not significantly higher at 25.98 ± 7.59 and 51.82 ± 17.44, respectively (*P* = .052), and the mean percentage of CD11b^{high} neutrophils was also not significantly different in whole blood (0.5% ± 0.1%) and after radiolabeling (1.1% ± 0.8%, *P* = .7027). CD69 MFI in the eosinophil fraction was significantly higher at 6.09 ± 2.16 and 20.27 ± 4.13, respectively (*P* = .005), but eosinophils expressing CD69 were not significantly different in whole blood (1.75% ± 1.3%) and after radiolabeling (4.8% ± 2.8%; *P* = .8956, Table I). Mean radiolabeling efficiencies of approximately 10 × 10⁷ neutrophils and approximately 1 × 10⁷ eosinophils were 26% and 72%, respectively. This resulted in mean injected activities of 95 ± 28 and 108 ± 6 MBq for neutrophils and eosinophils, respectively. Specific activities of injected cells were 1.3 Bq per cell (neutrophils) and 19.8 Bq per cell (eosinophils, Table I).

In vivo imaging of neutrophils and eosinophils

Representative images of biodistribution of ^{99m}Tc-HMPAO-labeled neutrophils and eosinophils in a healthy volunteer at 1, 5, 15, 25, and 120 minutes are shown in Fig 1 (also see Video E1 in this article's Online Repository at www.jacionline.org).

Mean lung TACs of labeled eosinophils and neutrophils in the 4 subjects are shown in Fig 2. Eosinophil activity decay was rapid and conformed to an exponential model. The mean ± SD half-life of clearance of eosinophils from the left and right lungs was 4.18 ± 0.36 and 4.13 ± 0.15 minutes, respectively (mean for

TABLE I. *In vitro* characterization of purified human neutrophils and eosinophils

	Neutrophils	Eosinophils
Phenotype*	CD66 ⁺ , CD15 ⁺ , CD16 ⁺	CD66 ⁺ , CD15 ⁺ , CD16 [−]
Purity (%)†	97.7 ± 2.5	95.1 ± 6.4
Activation‡: whole blood (%)	0.50 ± 0.1	1.75 ± 1.3
Activation§: after radiolabeling (%)	1.1 ± 0.8	4.8 ± 2.8
MFI : whole blood	25.98 ± 7.59	6.09 ± 2.16
MFI¶: after radiolabeling	51.82 ± 17.44	20.27 ± 4.13
Viability (%)	98.0 ± 2.1	97.0 ± 2.9
Radiolabeling efficiency (%)	26.1 ± 12.9	71.9 ± 12.4
Activity/cell (Bq)	1.3 ± 0.5	19.8 ± 5.3

*Phenotype after CD16 CliniMACS isolation.

†Contaminants were defined as CD3⁺, CD19⁺, CD56⁺, or CD80⁺ mononuclear cells.

‡Percentages (means ± SDs) of cells expressing CD11b (neutrophils) or CD69 (eosinophils) above an activation threshold of GM-CSF-activated cells *in vitro* (see the Methods section).

§Percentages (means ± SDs) of cells expressing CD11b (neutrophils) or CD69 (eosinophils) above the activation threshold after radiolabeling (see the Methods section).

||MFI (mean ± SD) for CD11b (neutrophils) and CD69 (eosinophils) in whole blood.

¶MFI (mean ± SD) for CD11b (neutrophils) and CD69 (eosinophils) after radiolabeling.

both lungs, 4.16 ± 0.11 minutes). Neutrophil lung TACs also conformed to an exponential model, but in comparison with eosinophils, the mean ± SD half-life of clearance of neutrophils from the lungs was considerably higher at 14.25 ± 3.97 minutes for the right lung, 13.18 ± 4.47 minutes for the left lung, and 13.72 ± 4.14 minutes for both lungs. The mean half-lives of clearance of neutrophils and eosinophils from the lung were significantly different (*P* = .009).

TACs for eosinophil and neutrophil mean counts over the liver and spleen are shown in Fig 3, A and B, respectively. In the liver there was sustained uptake from both neutrophils and eosinophils. Early uptake of neutrophils at the 15-minute time point was significantly lower than that of eosinophils (*P* = .001), although subsequent rates of change of the signals were not significantly different. Eosinophil and neutrophil uptake in the spleen was also sustained for the period of the experiment. Splenic migration of eosinophils was significantly greater than that of neutrophils after 1 hour (average plateau time in ROI, *P* = .014). The mean proportion of the eosinophil signal migrating to the bone marrow at 4 hours was 7.63% ± 1.9%, which is significantly higher than that of neutrophils at 2.19% ± 0.60% (*P* = .003).

Representative TACs for ^{99m}Tc-HMPAO-labeled neutrophils and eosinophils in the same healthy volunteer demonstrating different migration patterns through the lungs, liver and mediastinum are shown in Fig 4, A and B, respectively.

DISCUSSION

To our knowledge, this is the first report of a protocol that is able to discriminate between human eosinophil and neutrophil efflux into body organs in real time *in vivo*. We were able to isolate and radiolabel these cells without a significant increase in the number of activated cells or loss of viability, which has been reported to influence their migratory properties,¹¹ and perform dynamic scintigraphic imaging in human volunteers.

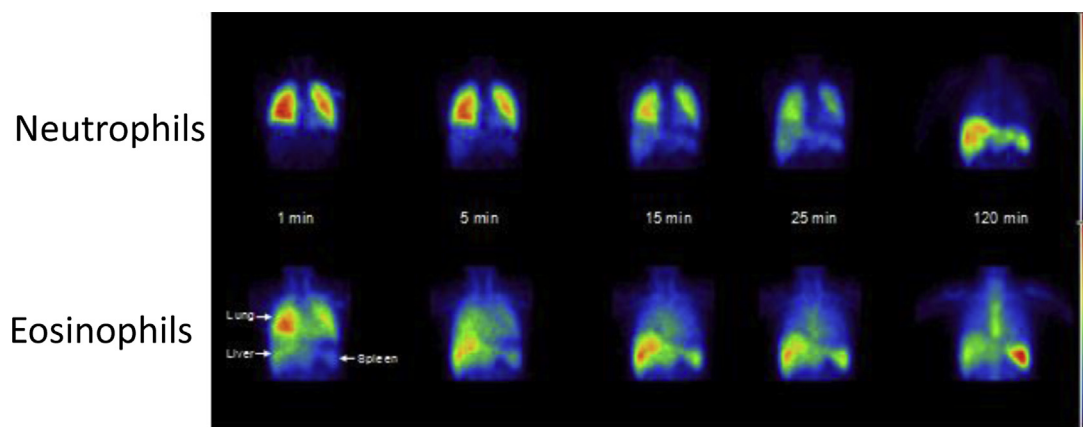


FIG 1. Example images (anterior view) of 1-minute frames from the acquired dynamic image sequence shown at 1, 5, 15, 25, and 120 minutes after injection of radiolabeled neutrophils (*top row*) and eosinophils (*bottom row*). The lung, liver, and spleen are indicated by arrows.

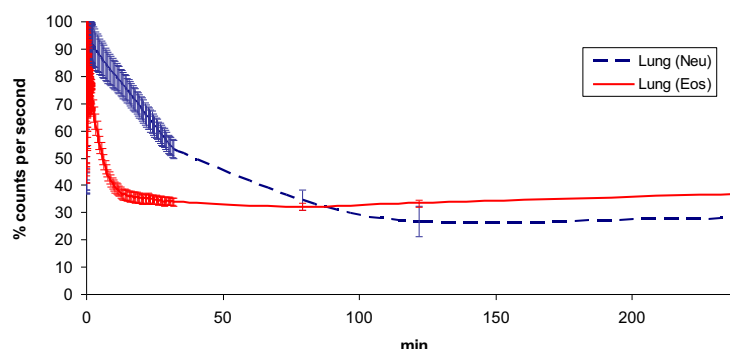


FIG 2. Percentage mean lung TACs of labeled neutrophils and eosinophils in 4 volunteers with a fitted curve. Error bars represent ± 1 SD, with a fitted curve. Rapid clearance of eosinophils from the left and right lungs was exhibited (mean half-life, 4.16 ± 0.11 minutes). Neutrophils had significantly slower lung clearance (mean half-life, 13.72 ± 4.14 minutes).

Each volunteer was imaged on 2 separate occasions to minimize between-subject physiologic, anatomic, or hemodynamic variability. The TACs demonstrated substantial differences in migratory kinetics between neutrophils and eosinophils, most obviously in the lungs but also in the spleen and bone marrow.

Previous studies have suggested that the degree to which leukocytes are retained in the lung capillaries depends principally on their size and deformability.¹² This does not accord with what we have observed here: neutrophils, which are similar in diameter to eosinophils and appear to have similar rheology (deformability),¹³ migrated more slowly through the lung vasculature. The question also arises of whether these phenomena might be influenced by activation of the cells during the isolation and radiolabeling procedure. We detected no significant increase in MFI CD11b on neutrophils or in the number of cells expressing CD11b. In contrast, in the eosinophil fraction, although the isolation and radiolabeling procedure did not significantly change the small percentage of cells expressing CD69, it did significantly influence the MFI of CD69 staining on CD69⁺ cells. Previous studies¹⁴ would suggest that increased expression of such markers would prolong cellular transit time, yet in spite of this, eosinophils in our study showed faster lung egress than neutrophils, which showed less clear evidence of activation. It might be possible that other subtle changes in cellular physiology caused by the

process of isolation and radiolabeling might have influenced cellular migration *in vivo*. We believe a significant effect of the magnetic beads is unlikely: although the use of macrobeads (1–4.5 μm) has been observed to result in cellular activation during isolation,¹⁵ this has not been observed with nanobeads (<100 nm).¹⁶

In summary, there will be ongoing scrutiny in this and every similar study as to whether cells isolated from whole blood and manipulated *in vitro* behave like unlabeled cells. This has existed for as long as these techniques have been in use, and this study does not resolve the issue. Nevertheless, within the specialty of nuclear medicine, our work is analogous to other studies on leukocyte kinetics, in which the pattern of migration through the lungs and liver can be seen as a sensitive index of activation or damage of leukocytes during radiolabeling. In the present study we observed rapid efflux of both eosinophils and neutrophils through the lungs and minimal sequestration in the liver, which is again consistent with our contention that this technique does not fully activate either cell population. Finally, the kinetics of migration of neutrophils in the pulmonary vasculature in our study were very similar to those reported in other studies with Tc-99m labeling in which density gradient and magnetic bead separation were not used,^{17,18} although as previously emphasized, such studies involve assumptions about and corrections for the

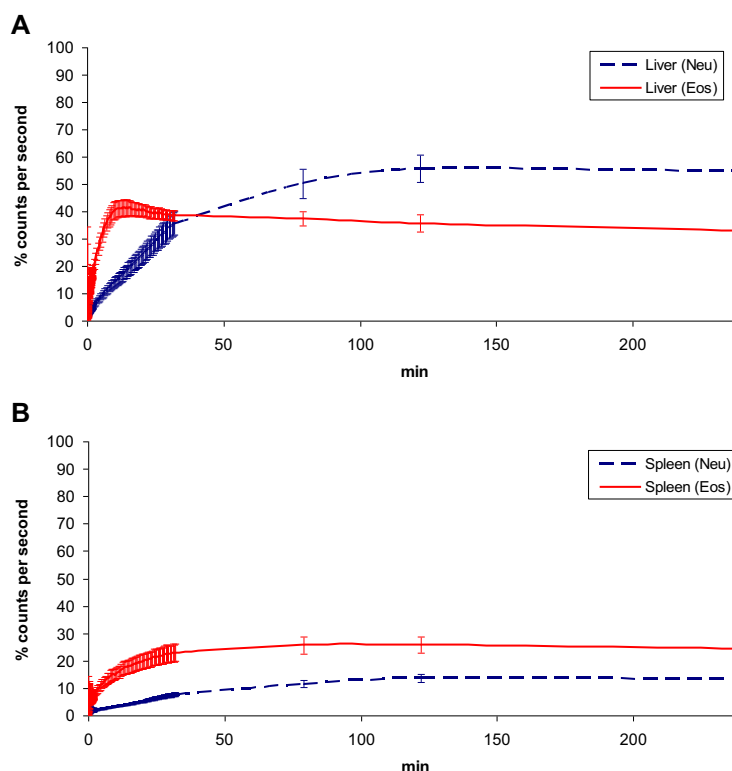


FIG 3. Mean TACs for neutrophils and eosinophils of the liver (A) and spleen (B). Error bars represent ± 1 SD. In the liver eosinophils showed initial faster uptake, a reflection of faster lung migration. There were no significant differences noted after the first 15 minutes. In the spleen eosinophils demonstrated significantly higher uptake than neutrophils ($P = .014$).

differential labeling and relative abundance of various leukocytes, particularly neutrophils and eosinophils, in the mixed cell populations. In conclusion, although we cannot completely exclude the possibility that the cells might have been “primed” or “partially activated” after *in vitro* manipulation and radiolabeling, this does not appear to have had a marked effect on their overall migration kinetics.

It is difficult to place our data in the context of other studies because they are largely without precedent. Granulocytes are known to be retained in the liver, spleen, and bone marrow in the setting of normal homeostasis^{5,14,19,20} and might be released by various natural stimuli, such as adrenaline or exercise,²¹ or in response to autonomic nervous signaling.²² It seems reasonable to hypothesize that migration of neutrophils and eosinophils to the liver portends apoptosis. Preferential migration of eosinophils compared with neutrophils to the spleen and bone marrow might reflect physiologic roles for these cells in the lymphatic organs, such as in B-cell²³⁻²⁵ or T-cell²⁶ priming.

Finally, our data and those of others indicated that eosinophils radiolabeled with ^{99m}Tc-HMPAO had a far higher specific activity (10-25 times higher) than neutrophils and thus are likely to contribute more than 50% of the signal in unseparated granulocyte populations *in vivo*.^{6,7} Considering that eosinophil labeling is a function of time and total dose of activity,⁷ the discrepancy in labeling efficiency might be even higher when total granulocytes, rather than pure populations, are used. Therefore it is likely that white cell and granulocyte scans grossly overestimate neutrophil uptake and underestimate eosinophil uptake and hence are representative of neither population.

The results of a recent publication²⁷ also addressing kinetics of eosinophil efflux from the lungs of healthy human subjects are largely congruent with our own data. Although these authors do not quote the half-life of the eosinophil signal for the lungs, it appears to be somewhat more prolonged compared with our results. The reasons for this are not clear but could relate to the influence of different radioisotopes on the behavior of the cells in terms of their activity, viability, or both. Clearly, it is important to take into account these issues, as we have done in the present study.

In terms of human physiology, our study begins to explore the immense complexity of normal leukocyte migration and underlines the fact that virtually nothing is known about it. Although it is a challenge to track the migration of individual leukocyte populations in human subjects in real time, we have already been able to show that eosinophils and neutrophils, despite sharing many similar physical and biological characteristics, show clearly distinct kinetics. We suspect that temporary arrest of neutrophils in the alveolar circulation might contribute to immunosurveillance in a site in which these cells are separated from the environment by a barrier less than 1 μ m thick (blood in pulmonary capillaries–alveolar air).

We trust that our technique will provide the opportunity and impetus to characterize further what controls differential human neutrophil and eosinophil migration in health and disease. It offers an opportunity to subphenotype diseases, such as asthma, in which neutrophils and eosinophils are thought to play varying roles in different patients. It also offers the opportunity for minimally invasive, high-throughput screening of potential therapeutic agents designed to target the influx of any defined population of

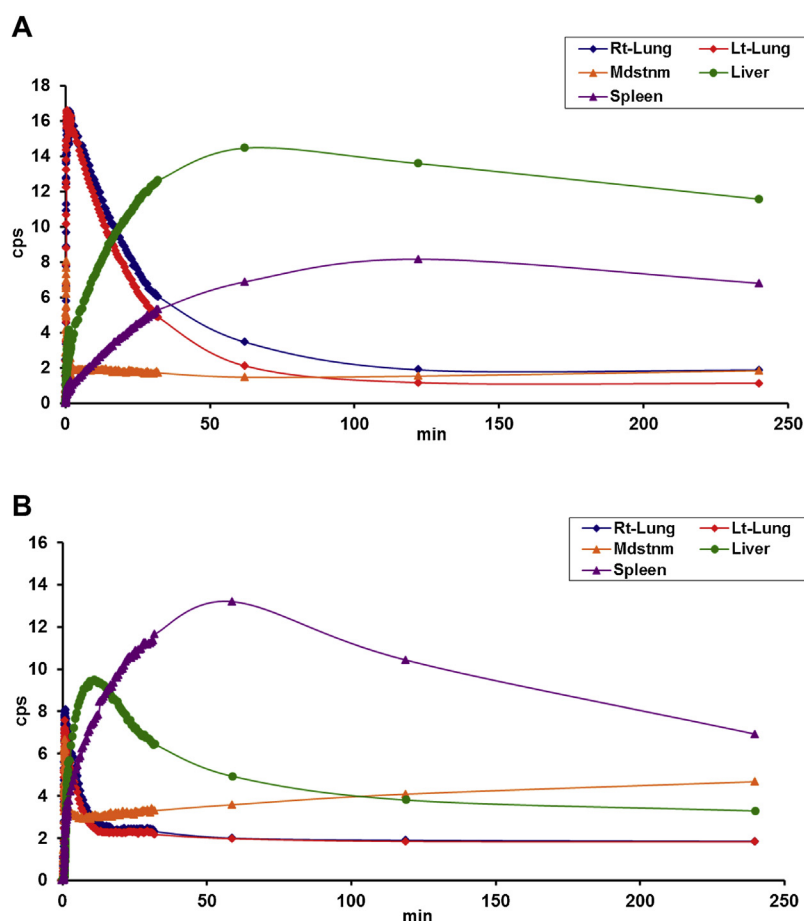


FIG 4. Example of the distribution of radioactivity over 250 minutes for the lungs, liver, spleen, and mediastinum in a single representative healthy volunteer for purified autologous neutrophils (A) and purified autologous eosinophils (B). cps, Counts per second.

cells into disease sites in any clinical arena. In short, this work can be seen as a starting point for the study of spatiotemporal regulation of immune responses in human subjects.

We thank the Department of Nuclear Medicine at Guys and St Thomas' Hospital for providing radiopharmacy, medical physics, and technical/radiographer support for the study.

Key message

- In the setting of homeostasis, eosinophils and neutrophils display significantly different patterns of lung, spleen, and bone marrow migration.

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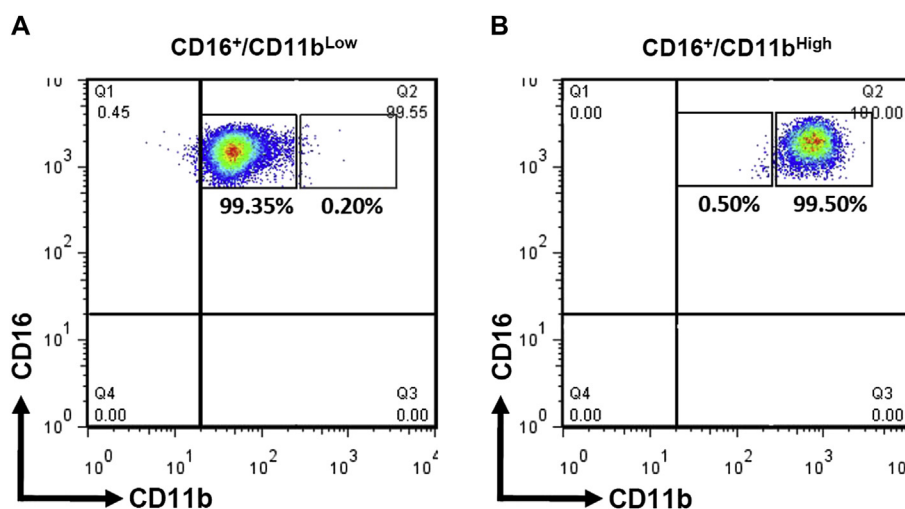
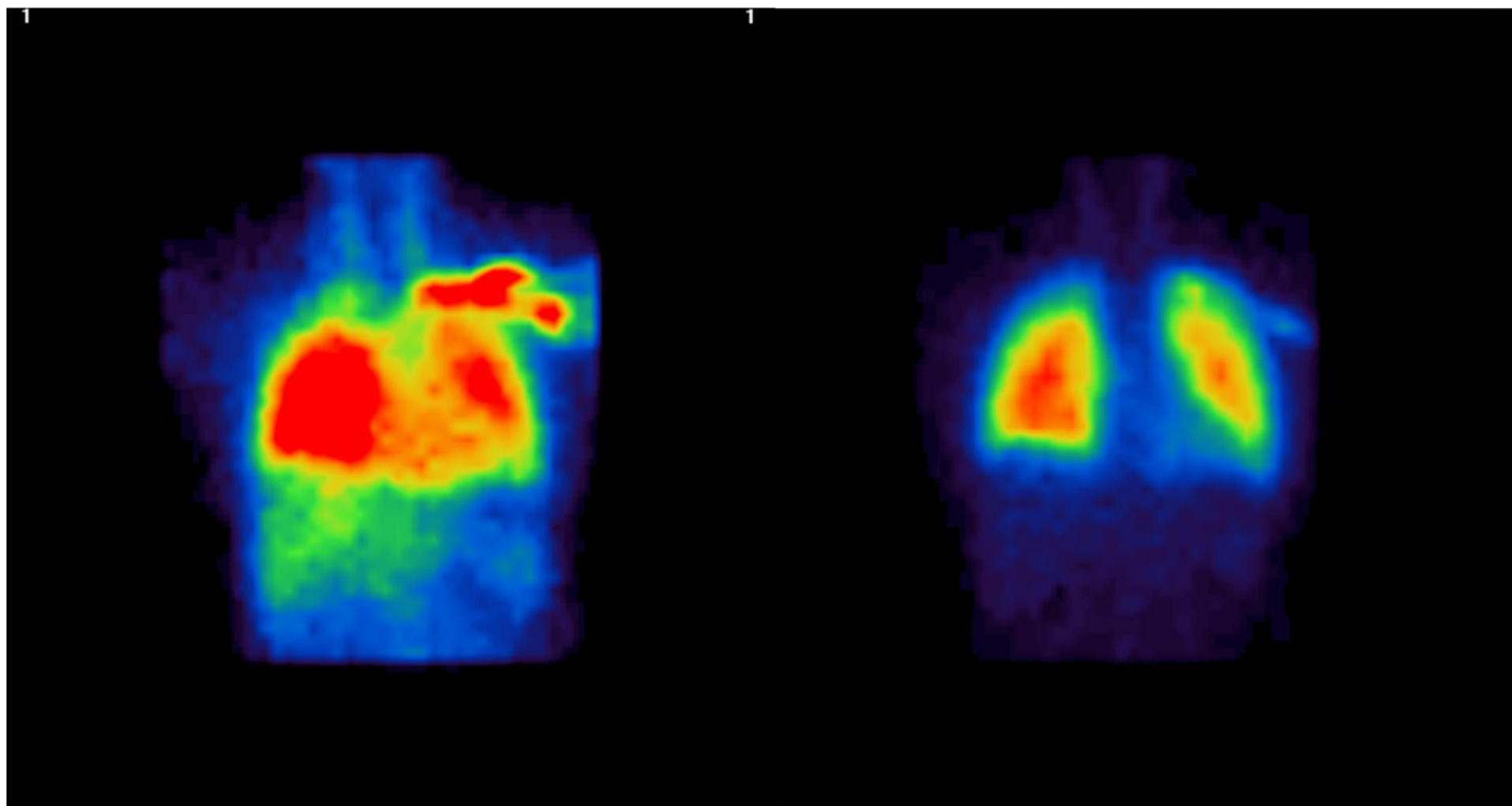


FIG E1. CD16/CD11b^{high} neutrophil population established by means of flow cytometry. **A**, Unfixed healthy volunteer whole blood was stained with CD16 and CD11b and analyzed by means of flow cytometry. Quadrants are based on negatively stained cells and isotype controls. Cells were gated on granulocytes and analyzed for the CD16/CD11b^{low} population (*left square gate*). **B**, The CD16/CD11b^{high} neutrophil population was determined by means of incubation of unfixed healthy volunteer whole blood with GM-CSF and then analyzed by means of flow cytometry. Quadrants are based on negatively stained cells and isotype controls. Cells were gated on granulocytes and analyzed for the CD16/CD11b^{high} population (*right square gate*).

Subject #1 / Eosinophils

Subject #1/ Neutrophils



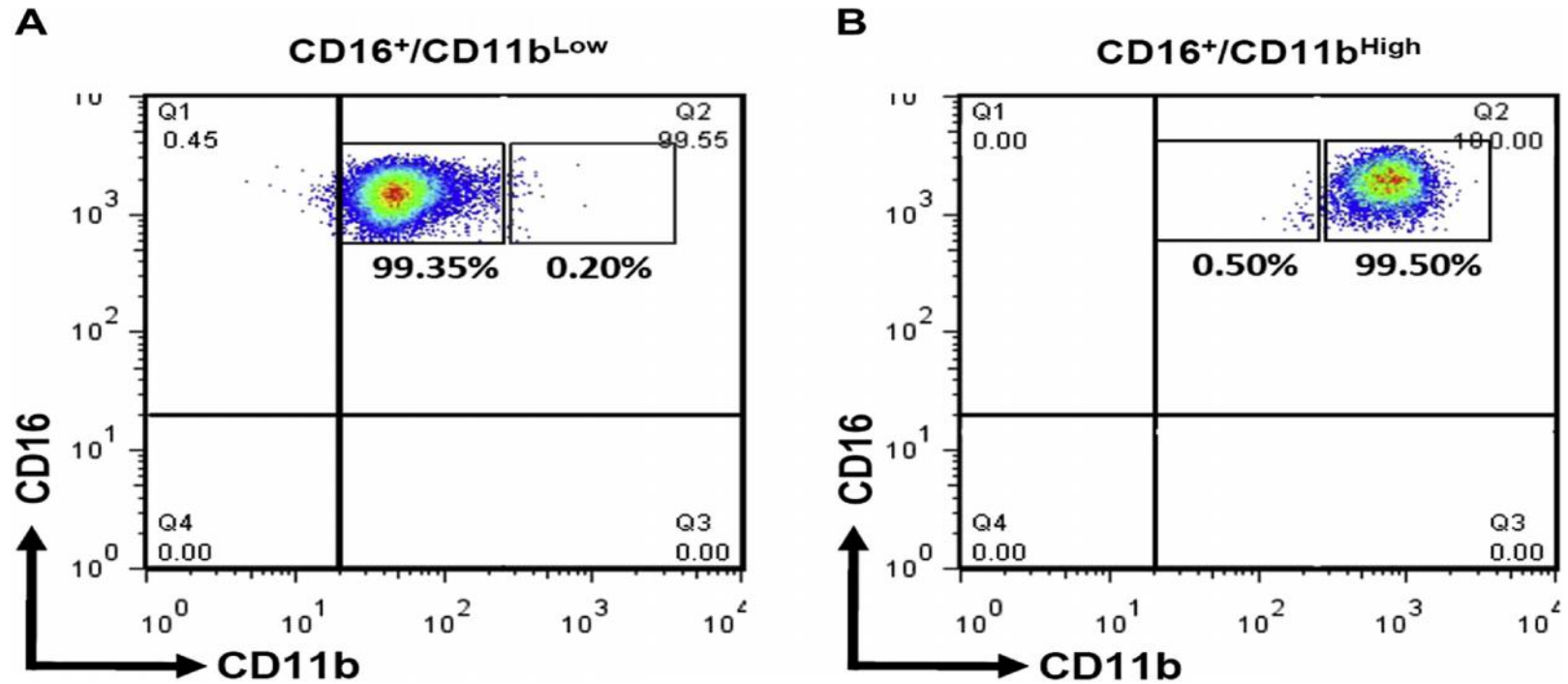


Fig E1 CD16/CD11b high neutrophil population established by means of flow cytometry. A, Unfixed healthy volunteer whole blood was stained with CD16 and CD11b and analyzed by means of flow cytometry. Quadrants are based on negatively stained cells and is...

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Journal of Allergy and Clinical Immunology null 2013 null

<http://dx.doi.org/10.1016/j.jaci.2013.06.031>