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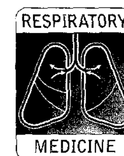
RESPIRATORY MEDICINE (1998) **92**, 1163–1166



Short Communication

Bimodal granulocyte transit time through the human lung demonstrated by deconvolution analysis

W. Y. USSOV*[†], A. M. PETERS[†], M. J. MYERS[†], D. M. GLASS[†] AND J. M. B. HUGHES*

*Departments of *Medicine and [†]Imaging, Hammersmith Hospital, London, U.K.*

The lungs are an important site of granulocyte pooling. The aim of the study is to quantify pulmonary vascular granulocyte transit time using deconvolution analysis, as has previously been performed to measure pulmonary red cell transit time. Granulocyte and red cell studies were performed in separate groups of patients. Both cell types were labelled with Tc-99m, which for granulocyte labelling was complexed with hexamethylpropyleneamine oxime (HMPAO). The red cell impulse response function (IRF) was monoexponential with a median transit time of 4·3 s. The granulocyte IRF was biexponential in 19 of 22 subjects, 18 of whom had systemic inflammation (inflammatory bowel disease, systemic vasculitis or graft-*vs*-host disease) and four were controls without inflammatory disease. The median transit time of the fast component ranged from 20 to 25 s and of the slow component 120–138 s in the four patient groups. The fraction of cells undergoing slow transit correlated significantly with (a) mean granulocyte transit time and (b) the fraction showing shape change *in vitro*. We conclude that granulocyte transit time through the pulmonary circulation is bimodal and that shape-changed (activated) cells transit more slowly that non-activated cells. The size of the fraction undergoing slow transit is closely related to mean granulocyte transit time and is an important determinant of the size of the pulmonary vascular granulocyte pool.

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Introduction

It is believed that a significant proportion of circulating granulocytes are present in the lungs (1,2). The size of this pool in relation to other sites of granulocyte pooling is controversial. Since granulocytes may have a role in lung damage in a range of inflammatory conditions (3–5), quantification of pulmonary vascular transit time is important and several techniques using labelled granulocytes have been described (5–9). Deconvolution analysis has been used in man to measure pulmonary vascular transit time of erythrocytes (10) but not granulocytes. Here we have quantified pulmonary vascular granulocyte transit time by deconvolution analysis. The impulse response function (IRF) so generated reproduces the time–activity curve that would be obtained following instantaneous deposition of cells in the lungs with no recirculation. Similarly, we also measured the pulmonary vascular transit time of erythrocytes.

Received 18 November 1997 and accepted in revised form 14 May 1998.
Correspondence should be addressed to: A. M. Peters, Department of Imaging, Hammersmith Hospital, Du Cane Road, London W12 0HS, U.K.

Patients and Methods

Granulocyte transit time was measured in 22 patients: six with active inflammatory bowel disease (IBD), five with graft-*vs*-host disease after bone marrow transplantation (BMT), seven with systemic vasculitis (SV) and four referred with suspected prosthetic joint infection but who had negative granulocyte scintigraphy and negative clinical follow-up (controls). Erythrocyte transit time was measured in nine more patients undergoing radionuclide ventriculography. No patient had radiological evidence of focal lung disease, none had heart failure and none were cigarette smokers. Differential white cell counts with respect to eosinophils were not significantly elevated.

Granulocytes were labelled with Tc-99m-hexamethylpropylenamine oxime (HMPAO) (Amersham International, U.K.) without isolation from plasma (11) and erythrocytes *in vitro* with Tc-99m using a standard technique. Although the granulocyte isolation technique eliminates mononuclear leukocytes, the population of cells labelled includes eosinophils, for which Tc-99m-HMPAO displays some selectivity (12), as well as neutrophils. Cells were injected as a bolus in a volume of less than 2·5 ml with the patient supine [~185 MBq (granulocytes); ~740 MBq (erythro-

0954-6111/98/091163+04 $12.00/0
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TABLE 1. Parameters of the pulmonary granulocyte impulse response function (IRF) and granulocyte shape change

	IBD	SV	BMT	Controls
Median transit time				
fast fraction (s)*	20	24	23	25
range	9–38	22–100	10–30	7–43
$P<$	n.s.	n.s.	n.s.	
Median transit time				
slow fraction (s)	120	128	138	130
range	69–310	66–408	99–193	62–266
$P<$	n.s.	n.s.	n.s.	
Median MTT (s)	37	66	52	36
range	20–247	39–196	39–69	11–87
$P<$	n.s.	n.s.	n.s.	
Median slowly-passing				
fraction*†	0·18	0·32	0·24	0·11
range	0·06–0·35	0·21–0·33	0·19–0·32	0·08–0·19
$P<$	n.s.	0·01	0·05	
Median fraction of cells showing shape				
change	0·22	0·32	0·41	0·11
range	0·07–0 34	0·19–0·58	0·33–0·82	0·09–0·15
$P<$	n.s.	0·005	0·01	

MTT in patients (two SV, one IBD) with monoexponential IRF were 66, 147 and 247 s, respectively.
*Excludes patients with monoexponential IRF.
P values are given in comparison to control group; n.s., not significant.
Fast fraction corresponds to first exponential with intercept *A*.
Slow fraction corresponds to second exponential with intercept *B*.
†Equal to $B/(A+B)$.
MTT, mean granulocyte transit time; IBD, inflammatory bowel disease; SV, systemic vasculitis; BMT, bone marrow transplant recipients.

cytes)]. Dynamic gamma camera scintigraphy was performed at a frame rate of $1\ s^{-1}$ for 2 min followed by $1\ 20\ s^{-1}$ up to 1 h (granulocytes) or $1\ s^{-1}$ for 30 s (erythrocytes).

The pulmonary IRF was derived by deconvolution analysis (13) using an input curve from a region of interest (ROI) over the right ventricle and an output curve from an ROI over the periphery of the mid-zone of the right lung. Mean transit time was calculated as the initial height of the IRF divided by the enclosed area. Most granulocyte IRFs were bi-exponential; the fraction of cells undergoing slow transit was accordingly calculated as the ratio $B/(A+B)$ where A is the zero-time intercept of the fast exponential and B of the slow exponential. $B/(A+B)$ was taken as unity for monoexponential IRFs. Activation of labelled granulocytes was assessed using an *in vitro* shape-change assay which assesses, on an all-or-nothing basis for individual cells, the effect of stimuli upon polarization of cytoskeletal elements (14). Cell labelling itself has no activating effect by this assay (15).

Statistical analysis was non-parametric: Wilcoxon ranked sum test and Spearman rank correlation coefficient (r).

Results

Erythrocytes gave monoexponential IRFs with a median transit time of 4·3 s (range 1·7–14·7), compared with about 40 s for granulocytes (Table 1). The lung IRF was bi-exponential in 19 patients and monoexponential in three. The rapidly-passing fraction had a transit time of about 20 s. No significant differences, compared with controls, could be identified in patients with inflammation with respect to the exponential rate constants or to mean granulocyte transit time. However, even after exclusion of patients with monoexponential IRFs, the fraction of slowly-passing granulocytes [i.e. ratio $B/(A+B)$] was significantly higher in patients with SV ($P<0·01$) and transplant recipients ($P<0·05$) compared with controls. $B/(A+B)$ correlated strongly with mean transit time ($r=0·8$, $P<0·001$). There was a weak but insignificant association between the eosinophil differential and transit times of both fractions.

The fraction of granulocytes showing shape-change was significantly higher in patients with SV ($P<0·005$) and transplant recipients ($P<0·01$) compared with controls. The shape-changed fraction was similar to $B/(A+B)$ [with an

average ratio of the two of 0·95 ($n=19$, SD 0·55)] and correlated significantly with $B/(A+B)$ ($r=0{\cdot}58$, $n=22$, $P<0{\cdot}01$), but not with mean granulocyte transit time. There was no correlation between the eosinophil differential and either $B/(A+B)$ or the fraction of granulocytes showing shape change.

Discussion

Although similar in size to red cells, and larger than pulmonary capillary diameter, granulocytes transit the lungs more slowly than red cells because they are less deformable (16–21). Reducing deformability by exposure to cytokines or glutaraldehyde results in a prolongation of transit time which correlates with cell stiffness as measured by a cell 'poker' (19–21).

Using videomicroscopy in the dog, Lien *et al.* (17,18) observed cells either passing through lung capillaries at the same speed as erythrocytes, without being arrested at any point, or to be completely immobilized for periods up to several minutes. Using the indicator dilution technique, Hogg's group (9,22,23) measured a discrete fraction of granulocytes extracted in the lung on a single pass. Extraction of granulocytes measured by outflow detection in the isolated perfused lung is also consistent with bimodal transit (24), in which the fast fraction has the same transit time as erythrocytes.

The current findings fit well with the concept of bimodal transit in several respects. Thus, we found a fraction of cells with rapid transit which could be separately identified in the IRF from a fraction with more prolonged transit. However, the mean transit time of the rapidly-passing fraction was not the same as mean red cell transit time, but four to five times longer. This may be the result of labelling as we have previously shown that the transit time of labelled granulocytes decreases over about 30 min after injection by a factor of about 3 (8). In the controls, the rapidly-passing granulocytes represented about 85% of the total; this is in contrast to 50% with videomicroscopy [dog (17,18)], 10–40% with indicator dilution [dog (22), rabbit (23), man (9)] and 12% with outflow detection [dog (24)]. It is not known whether bimodal granulocyte transit could be a reflection of differing transit times of granulocyte sub-populations. This is of relevance in the present study because Tc-99m-HMPAO has an element of selectivity for eosinophils (12). Although the eosinophil count did not correlate with the fraction of granulocytes showing slow transit, weak associations were observed with transit times of the rapidly and slowly passing fractions. In any event, it is unlikely that differential labelling of eosinophils and neutrophils with Tc-99m-HMPAO could explain the current findings because first oesinophil counts were not elevated and second the kinetics of granulocytes respectively labelled with Tc-99m-HMPAO and In-111-tropolonate (which is not known to be selective for eosinophils) are indistinguishable (25).

The fraction of slowly-passing cells and mean transit time were higher in SV and transplant recipients compared with controls, but the separate transit times of the two fractions did not differ between patient groups. The fraction of slowly-passing cells was similar to and correlated with the fraction showing shape change, suggesting that activated cells transit slowly. Because shape change is considered all-or-nothing, this *in vitro* assay is of direct relevance to the transit time of granulocytes through the pulmonary capillaries in relation to reduced deformability (20). We do not know, however, whether neutrophils and eosinophils behave differently in the assay.

We conclude that granulocyte transit time through the pulmonary circulation s bimodal and that shape-changed (activated) cells transit more slowly than non-activated cells. The size of the fraction undergoing slow transit is closely related to mean granulocyte transit time and is an important determinant of the size of the pulmonary vascular granulocyte pool. The relationships between granulocyte sub-populations and pulmonary vascular transit time deserves further study.

Acknowledgements

W. Y. Ussov was supported by The Wellcome Trust.

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