Dissociation of neutrophil emigration and metabolic activity in lobar pneumonia and bronchiectasis

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ABSTRACT: In animal models of pulmonary inflammation, neutrophils exhibit a dramatic influx of glucose in periods of high metabolic activity. This information was utilized to develop a technique, involving positron emission tomography (PET) of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG), which measures neutrophil activity *in situ*.

This technique was applied in a comparative study of neutrophil function in patients with acute lobar pneumonia or bronchiectasis. Neutrophil emigration was measured by γ-scintigraphy of intravenously injected ^{111}In-labelled granulocytes and neutrophil activity determined by PET of ^{18}FDG.

Neutrophil emigration was evident in 4 out of 5 bronchiectasis patients, whilst no emigration was apparent in the two pneumonia patients studied, consistent with animal studies showing maximum emigration soon after challenge. In contrast, ^{18}FDG uptake was markedly increased in 4 out of 5 pneumonia patients but not in the patients with bronchiectasis. Localization of radioactivity to neutrophils was confirmed by microautoradiography of lavage fluid in a patient with pneumonia.

These results suggest that the elevated uptake of glucose by neutrophils during the inflammatory response is a postmigratory event, most likely reflecting the respiratory burst, and that high levels of neutrophil emigration are not necessarily associated with significantly increased metabolic activity of these cells.
Eur Respir J 1997; 10: 795–803.

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The inflammatory process, and the neutrophil in particular, has been specifically implicated in the pathogenesis of a variety of lung diseases, including acute lung injury, a range of chronic inflammatory/scarring conditions in the lung, bronchial asthma and chronic obstructive pulmonary disease (COPD) [1]. The availability of a quantitative global index of "inflammation" or neutrophil activity in the human lung would be of considerable value in mechanistic studies and staging of lung diseases, and would greatly facilitate the design and testing of anti-inflammatory therapeutic agents. Markers of inflammation in the circulating blood and in bronchoalveolar lavage (BAL) fluid have been of considerable utility in a number of experimental and clinical situations, but they do not provide a quantitative index of inflammatory events in the lung interstitium. Transbronchial biopsy does not provide sufficient material for quantification of inflammatory cells. Even the "gold standard", open lung biopsy, may not provide quantitative information, especially in patchy disease, and it is rarely possible to perform this invasive investigation sequentially.

We and others have been investigating the use of noninvasive γ-camera scintigraphy following intravenous injection of ^{111}Indium-labelled granulocytes to monitor the trafficking of inflammatory cells in the lung. In experimental models, this approach has provided useful information on the sequestration of neutrophils [2] and monocytes [3] in the pulmonary vasculature, and on their emigration into lung tissue. These studies have been extended to human lung diseases, such as the adult respiratory distress syndrome (ARDS) [4], COPD [5], pneumonia [6] and bronchiectasis [7]. While these techniques can provide a dynamic quantitative index of global inflammatory cell trafficking, they give no information on the inflammatory activity of the cells *in situ*, which involves metabolic activation and is integral to downstream events in the inflammatory process.

Tomographic imaging of positron-labelled tracers has considerable potential for quantification of disease processes in the lungs [8]. Radioactive analogues of glucose with limited metabolic susceptibility, 2-deoxy-D-glucose (DG) and 2-fluoro-2-deoxy-D-glucose (FDG), have been used extensively to monitor the metabolic activity of cells *in vivo* [9]. These compounds are taken up by living cells by the same mechanism as glucose and are similarly phosphorylated by the enzyme hexokinase. However, unlike glucose, they can be metabolized no further and, hence, accumulate in cells during bouts of high activity. It was hypothesized, therefore, that FDG labelled with ^{18}F

(¹⁸FDG) could be used to measure and monitor the metabolic activity of the inflamed lung *in vivo* using positron emission tomography (PET).

There have been previous reports of increased pulmonary uptake of [18]FDG in sarcoidosis [10], cryptogenic fibrosing alveolitis (CFA) [11], and lung cancer [12], but the cellular processes responsible for the glucose uptake signal were unclear. We have demonstrated, in a rabbit model of lobar inflammation induced by *Streptococcus pneumoniae*, that [18]FDG uptake measured by PET is due to neutrophil activity *in situ* [13]. Six hours following challenge, when neutrophil emigration measured by ^{111}In-labelled neutrophil influx is at its peak, there was no significant [18]FDG signal, but at 15 h, when neutrophil emigration had largely ceased, [18]FDG uptake reached its maximum value. These observations suggested that [18]FDG uptake in neutrophils is a postmigration event, a hypothesis supported by autoradiography showing that labelled neutrophils were detected almost exclusively in the lung airspaces. When [18]FDG uptake in a rabbit model of bleomycin-induced chronic inflammation also appeared restricted to neutrophils, we speculated that, in lung inflammation, PET imaging of [18]FDG may provide a valuable noninvasive measure of the neutrophil metabolic burst *in situ* [13].

Neutrophil emigration has been examined in human pneumonia [6], where it has apparently ceased early in the course of the illness, and in chronic bronchiectasis [7] where it persists. However, the relationship of emigration to metabolic activity *in situ* as shown by [18]FDG accumulation is unknown. In this study, we examine the relationship of metabolic activity to neutrophil emigration in human pneumonia and chronic bronchiectasis.

Patients

Lobar pneumonia

Five patients (4 males and 1 female, aged 24–71 yrs) admitted to hospital with acute symptoms and lobar consolidation were studied. None suffered from chronic lung disease. At the time of study, all patients were receiving antibiotics and only one patient (No. Pn2) was producing sputum. An aetiological diagnosis was achieved in only one patient (No. Pn2), who had a positive blood culture for *S. pneumoniae* on admission. However, *S. pneumoniae* was the likely pathogen in the other patients, as it is known to account for many cases of community-acquired acute lobar pneumonia, particularly that occurring in previously healthy, younger patients [14, 15].

Bronchiectasis

Five male patients, (aged 41–70 yrs) with longstanding bronchiectasis were studied, whilst in a chronic, stable state. Diagnosis was based on standard clinical, radiographic and computerized tomographic findings. All patients but one (No. Br4) were receiving antibiotic treatment. Sputum culture demonstrated the presence of *Enterobacter* sp. in patient No. Br1 and *Pseudomonas* sp. in patient No. Br3.

All patients gave informed consent to enter the study, which had been approved by the Research Ethics Committee of Hammersmith Hospital and the Administration of Radioactive Substances Advisory Committee.

Study design

Two noninvasive imaging techniques were used to determine two discrete components of neutrophil behaviour in the two groups of patients. Emigration of granulocytes from the blood into the lungs was monitored following intravenous injection of radiolabelled granulocytes, and metabolic activity was measured as the increase in the [18]FDG uptake by PET.

Granulocyte sequestration was measured by white cell scanning in two of the pneumonia patients, who were recovering well at 3–4 days, in close proximity to measurement of neutrophil metabolic activity by PET scanning. In the other patients with acute lobar pneumonia, whose slower recovery only permitted study at six or more days after onset, no white cell scan was obtained; previous evidence suggested that these would be negative [6], and we were anxious to avoid unnecessary radiation and too demanding a protocol in these patients.

[18]FDG uptake was measured in all pneumonia patients, with one patient (No. Pn4) being studied on two occasions. The interval between the onset of symptoms and the time of study ranged 3–22 days, but in all patients consolidation persisted at the time of the PET scan, subsequently resolving completely. The timing of PET scans was, in part, determined by the need for the patient to have recovered sufficiently to spend 2 h in the PET scanner.

The localization of [18]FDG was examined in cells obtained at BAL in two patients with acute lobar pneumonia, who required bronchoscopy to exclude airway obstruction: in patient No. Pn5, 21 days after admission; and an additional male patient (LH), aged 74 yrs, admitted with a right lower lobe pneumonia, who required bronchoscopy and BAL 4 days after admission, when lobar consolidation persisted. The latter patient was not sufficiently recovered for PET scanning. No obstructive lesions were found and both patients recovered over the next 2 weeks, with complete resolution of the consolidation.

Granulocyte migration and neutrophil metabolic activity were measured in all patients with bronchiectasis.

Methods

Measurement of granulocyte migration into the lung

Autologous granulocytes were isolated from the patients own blood and labelled in plasma with [111]Intropolonate [16]. Approximately 12 MBq of labelled cells were introduced by intravenous injection. Gamma-scintigraphy of the chest and abdomen was carried out at 4 and 24 h after injection to measure the distribution of labelled cells.

Analysis of γ-scintigraphy (¹¹¹In)

[111]In-granulocyte distribution was quantified from regions of interest (ROIs) over the lung fields. The right

and left lung fields were divided into equal thirds on the basis of vertical distance, measured from the dome of the diaphragm to the apex. Count rates were recorded in each ROI on images recorded at 24 h, when any remaining localization of radioactivity has been shown to represent emigrated cells [2], and were expressed as counts per second (cps) per MBq of injected dose per pixel. ROI were also placed over the iliac spine for measurement of bone marrow activity. The bone marrow signal from the chest wall was estimated from the iliac ROI, using a factor based on the relationship between iliac and chest wall bone marrow previously determined in a group of subjects with no evidence of inflammatory disease [17]. The cps per pixel from chest wall bone marrow was then subtracted from the total signal in the ROI to yield a signal, corrected for physical decay of the radionuclide, which represents intrapulmonary granulocyte migration. A value of 0 indicates that the count rate from the ROI over the lung is not significantly different from the estimated chest wall bone marrow signal in the same ROI. Values for migration determined in this way were then applied to all ROIs. An index of overall granulocyte migration was obtained by comparing the splenic counts, appropriately corrected for physical decay, between 4 and 24 h images. Since the early splenic signal predominantly reflects intrasplenic pooling of granulocytes, the decrease in splenic counts is more marked in the presence of extravascular granulocyte migration [18].

Measurement of inflammatory cell activity (^{18}FDG scans)

Patients were fasted for a minimum of 6 h before PET scanning. This reduces the uptake of glucose by normal tissue and increases the signal-to-noise ratio in areas of raised metabolic activity [19]. They were positioned supine within the PET scanner (Siemens 931, Bracknell, UK). A scan of regional thoracic density was performed by measuring the transmission of radioactivity through the field of view of the PET scanner from a ring source containing ^{68}Ge. An initial rectilinear scan was performed to select the appropriate area of thorax. The PET scanner acquires 15×0.7 cm transaxial planes of data, allowing a 10 cm section of the thorax to be studied. The selection of this section was based on the chest radiograph for the pneumonia patients and on the chest radiograph and white cell scan data for the patients with bronchiectasis. Once positioned, a 15 min transmission scan was performed to obtain transaxial data for estimation of thoracic density and for attenuation correction of the subsequently obtained emission data. The ring source was then retracted and approximately 120 MBq ^{18}FDG in normal saline infused intravenously over 2.5 min. The radioactive counts emitted were accumulated by the PET scanner for seven 3 min, followed by nine 5 min successive time-frames; a total of 66 min. These data were corrected for attenuation using the transmission scan data, and reconstructed to give 15 transaxial tomographic images of the distribution of radioactivity for each time-frame.

During the emission scan, blood was sampled continuously from a peripheral arm vein at 2 mL·min^{-1}, and passed over a detector for measurement of radioactivity. In addition, discrete samples were taken at intervals for measurement of whole blood/plasma radioactivity.

Immediately following the PET scan, a sputum sample was obtained from four of the patients with bronchiectasis to estimate the levels of radioactivity.

Analysis of PET data

Data from PET scanning were reconstructed to give images of regional density from the transmission scan and the distribution of ^{18}F following injection of ^{18}FDG for all 16 time-frames of the emission scan. ROIs were drawn on the relevant planes of the transmission scan, using image analysis software [20]. The area of inflammation in patients with pneumonia was identified as a region of increased density, a similar area in the opposite lung being used for comparison. In patients with bronchiectasis, the ROIs were drawn as peripheral and central lung areas on both lungs for all planes above the diaphragm, to differentiate between areas composed mainly of alveoli and those with a larger proportion of airways.

The ROIs were then transferred to the ^{18}F emission images and the mean radioactivity calculated for each of the 16 time-frames. Mean plasma radioactivity was calculated for each time-frame from the blood data. The rate of uptake of ^{18}FDG was determined from the slope of a Patlak plot, constructed from the tissue (ROI) and plasma radioactivity [21]. The ordinate intercepts of the plots at time = 0 give a measure of the tissue space (lung + blood) into which the ^{18}FDG was initially distributed before any uptake occurred. The greater the distribution volume for the ^{18}FDG the greater will be the slope, irrespective of the metabolic activity of the cells present. This relationship is directly proportional. The slopes were normalized for variations in distribution volume to enable us to measure any increase in metabolic activity of cells in the selected regions.

Cellular localization of ^{18}FDG

In patient No. Pn5 and the additional patient with acute lobar pneumonia (LH, studied 4 days after admission), BAL was carried out 80 min after *i.v.* injection of 120 MBq ^{18}FDG. Cytospins were prepared from the lavage fluid, fixed with 100% methanol and autoradiographs prepared by emulsion dipping. The preparations were coated in autoradiographic emulsion (Ilford K2 nuclear emulsion size A, Knutsford, UK). The sections were exposed overnight, developed (Kodak D19, Hemel Hempstead, UK), fixed (AMFIX, Ilford, Knutsford, UK), stained with haematoxylin and eosin and examined by light microscopy.

Results

Granulocyte emigration

Acute lobar pneumonia. Gamma-scintigraphy following intravenous injection of ^{111}In-labelled autologous granulocytes showed that in the two patients studied with acute lobar pneumonia, within 4 days of onset, there was no sequestration of labelled cells, indicating that active migration had effectively ceased, in confirmation of earlier studies [6]. The scan of patient No. Pn1, which
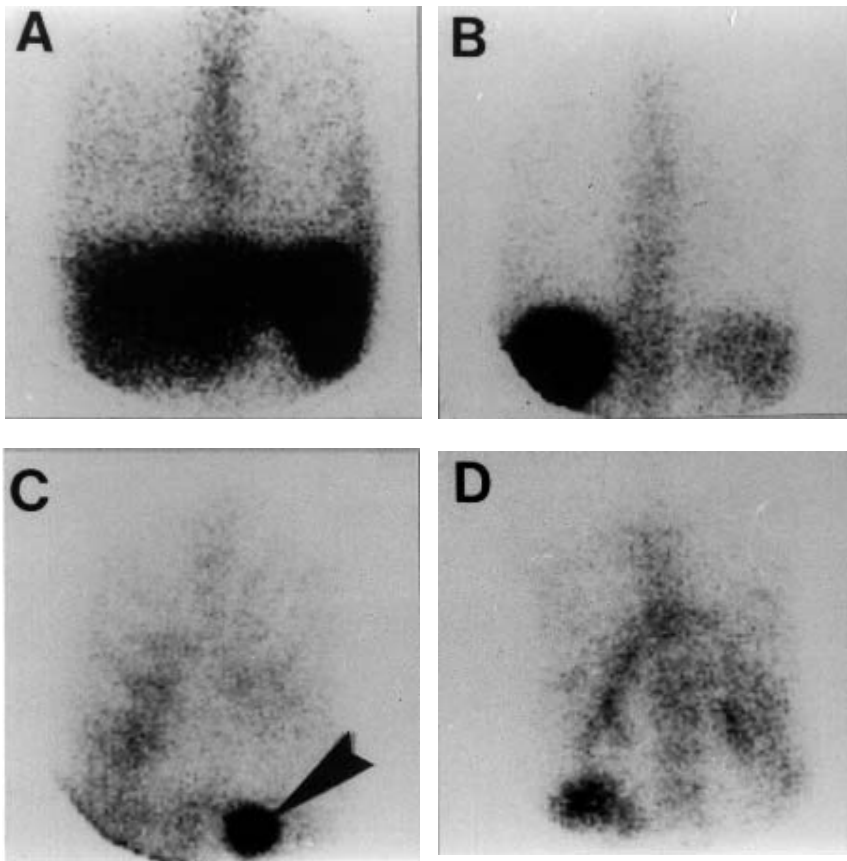
Fig. 1. – Distribution of ^{111}In-labelled granulocytes 24 h after intravenous administration. A) anterior view; and B) posterior view of patient No. Pn1, 3 days after onset of symptoms of acute lobar pneumonia. The radioactivity is located in the liver and spleen, whist the lung fields appear clear. Region of interest (ROI) analysis of the γ-scintigraphy revealed no signal in this patient and only a very small signal in patient No. Pn2 (table 2). C) anterior view; and D) posterior view of patient No. Br2 with bronchiectasis, showing patchy accumulation throughout the lung fields. The outline of the airways can be seen as radiolabelled cells are cleared from the lung and swallowed. The presence of radioactivity in the stomach (arrowed) confirms that the signal represents emigration into the lung and cannot be explained by margination in the capillary bed. ROI analysis shows variable migration between regions. The 10 cm section of the thorax with the highest uptake was selected for positron emission tomography (PET) scanning. A positive migration signal was apparent in three of the other bronchiectasis patients.

was obtained 3 days after the onset of symptoms, is shown in figure 1A and B. The radioactivity was located in the liver and spleen, while the lung fields appeared clear. ROI analysis of the γ-scintigraphy data from this patient revealed no signal at all. Radioactivity in the spleen fell only slightly between 4 and 24 h in these patients, confirming that by this time little granulocyte migration was taking place (table 1).

Bronchiectasis. γ-scintigraphy revealed active sequestration of ^{111}In-labelled cells in 4 of the 5 patients with bronchiectasis 24 h after injection. An example from patient No. Br2 (fig. 1C and D) shows patchy accumulation throughout the lung fields, and the outline of the airways can be seen as radiolabelled cells were cleared from the lungs and swallowed. The presence of radioactivity in the gastrointestinal tract (stomach arrowed) confirmed that the signal represents emigration into the lung and cannot be due to margination in the capillary bed. ROI analysis showed variable migration signals between regions. The region with the highest uptake was selected for PET scanning, the values for which are shown in table 1. Radioactivity in the spleen fell markedly between 4 and 24 h in all but one of the patients with bronchiectasis, patient No. Br5, who showed no evidence of neutrophil migration into the lungs.

Table 1. – Patient details and white cell migration

Pt No.	Age yrs	Sex	Radiographic dense region	Max [111]Indium signal at 24 h cps·MBq⁻¹·pixel	4–24 h Fall in splenic radioactivity
					%
Acute lobar pneumonia					
Pn1	48	M	Right middle lobe	0	12
Pn2	71	F	Right lower lobe	0.9	14
Pn3	45	M	Right middle lobe		
Pn4	24	M	Right upper lobe		
Pn5	28	M	Right lower lobe		
Bronchiectasis					
Br1	64	M	Whole right lung & both lower lobes	4.0	75
Br2	70	M	Right middle & both lower lobes	5.1	43
Br3	68	M	Left middle & right lower lobes	2.4	31
Br4	50	M	Left lower lobe	1.9	30
Br5	41	M	Both lower lobes	0.2	3

Pt: patient; cps: counts per second; Pn: pneumonia; Br: bronchiectasis; M: male; F: female.
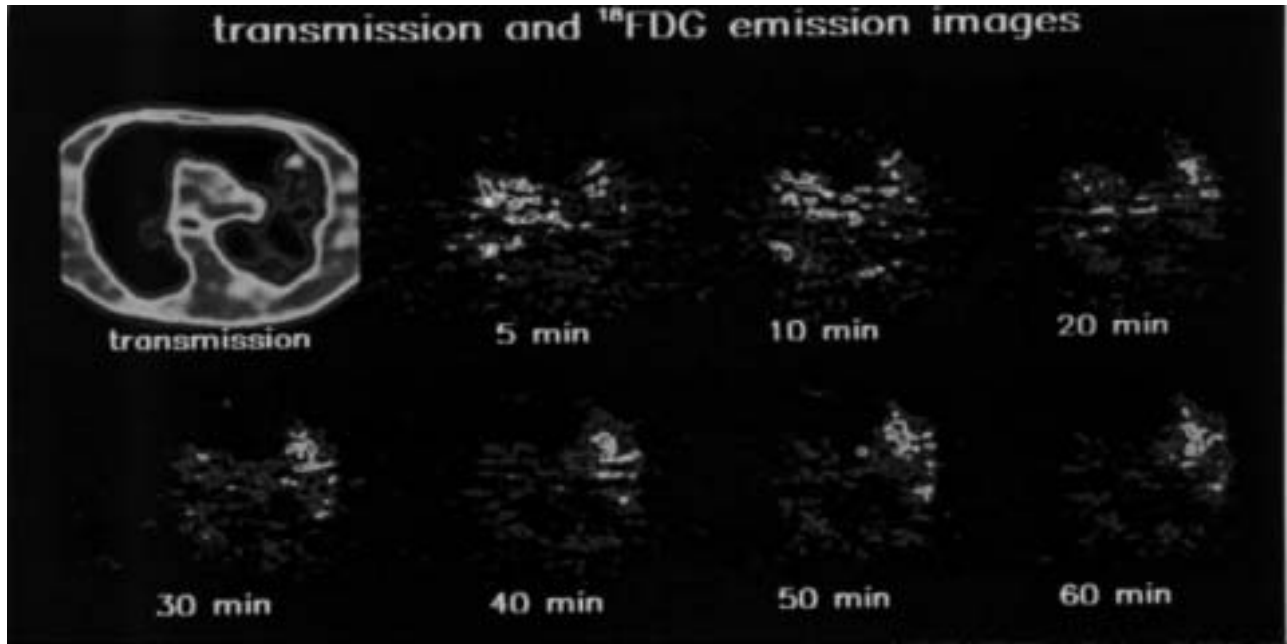
Fig. 2. – Positron emission tomography (PET) images obtained 3 days after the onset of symptoms in Patient No. Pn1 (pneumonia). The top left hand image shows regional density (high density areas are light, low density areas are dark). The lung fields show as areas of low density relative to the chest wall and heart. In this patient, the density of the inflamed right lung is raised, relative to the uninvolved left lung. This is in agreement with the appearance of the chest radiograph (not shown). The positron emission images obtained after intravenous injection of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG) show the distribution of radioactivity (high radioactivity areas are light, low radioactivity areas are dark), initially in the blood pool and then localizing to the affected area of lung over a period of 60 min. Similar images were obtained in all pneumonia patients.

Pulmonary ^{18}FDG uptake as a marker of inflammatory cell activity

Acute lobar pneumonia. Representative images for a single transaxial tomographic slice in the thoracic region of patient No. Pn1 are shown in figure 2. The top left hand image shows the distribution of density. The lung fields appear as areas of low density relative to the chest wall and heart. In this patient, the density of the inflamed right lung was slightly raised, relative to the uninvolved left lung. This is in agreement with the appearance of the chest radiograph (not shown). The positron emission images obtained after intravenous injection of ^{18}FDG show the distribution of radioactivity, initially in the blood pool and then localizing to the affected area of lung over a period of 60 min.

Patlak plots constructed from ROI data in the affected area and radioactivity measured simultaneously in the blood showed an increased slope (fig. 3 and table 2, column A). This was occurring at a time when no emigration signal could be detected by γ-scintigraphy (fig. 1A and B and table 1). Increased uptake of ^{18}FDG was apparent in the affected lung regions of all patients with acute lobar pneumonia (table 2, column A), whilst accumulation of ^{18}FDG in the uninvolved lung was in the range obtained in normal subjects (table 2, column B). The ordinate intercepts of the plots for the affected regions were also raised, indicating an increase in the tissue space (lung + blood) into which the ^{18}FDG was distributed (table 2, columns C and D). The magnitude of the signal was greatest in those patients studied at an early stage of the disease. When corrected for an increase in the ^{18}FDG distribution volume (table 2, columns E and F) the Patlak slopes of ^{18}FDG uptake in patients Nos.

Pn1–4, studied up to 2 weeks after the onset of their symptoms, were well above the normal range of values. However, the raised slope in patient No. Pn5 at 3 weeks (table 2, column E) can be explained entirely by increased
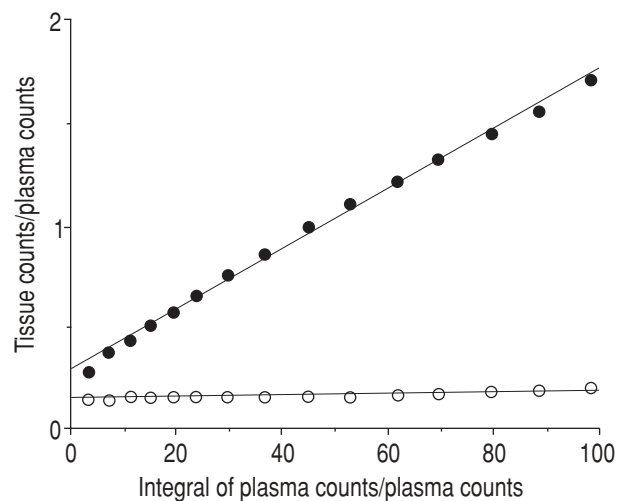

Fig. 3. – Example of Patlak plots constructed from region of interest (ROI) from positron emission tomography (PET) scan (fig. 2, acute lobar pneumonia, patient No. Pn1) and the radioactivity measured simultaneously in the blood. Slopes represent the clearance of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG) from the plasma into the metabolically active cells in the different regions of interest. Accumulation of ^{18}FDG in the uninvolved lung is in the range obtained in normal subjects. Uptake in the right middle lobe is dramatically increased, indicating raised metabolic activity in this area, at a time when no cell emigration signal could be detected (*c.f.* fig. 1A). The ordinate intercept of the plot was also raised, indicating an increase in the tissue space (lung + blood) into which the ^{18}FDG initially distributed. ——●——: right middle lobe, slope = 148×10^{-4} (min^{-1}); ——○——: left lung, slope = 4.9×10^{-4} (min^{-1}).

Table 2. – ^{18}FDG uptake and distribution

Pt No.	Time post onset of symptoms days	Slope* min^{-1}×10^{-4}		Intercept*		Corrected slope† min^{-1}×10^{-4}	
		A Affected region	B Control region	C Affected region	D Control region	E Affected region	F Control region
Acute lobar pneumonia							
Pn1	3	148	5	0.27	0.12	82	6
Pn2	3–4	194	4	0.46	0.14	63	4
Pn3	6	124	7	0.30	0.21	52	5
Pn4	13	78	5	0.43	0.22	27	3
	22	28	6	0.31	0.21	13	4
Pn5	21	35	8	0.64	0.17	8	7
Normal range		3–8		0.10–0.20		3–8	
Bronchiectasis							
Br1		13	7	0.19	0.12	10	9
Br2		9	8	0.17	0.17	7	9
Br3		15	10	0.17	0.11	13	14
Br4		7	5	0.17	0.10	6	8
Br5		5	6	0.18	0.11	4	8
Normal range		3–8		0.10–0.20		3–8	

*: of the Patlak plot; †: corrected for tissue volume using M × (c normal)/c; M: slope of Patlak plot; c: intercept of Patlak plot; c normal: average of range of values of c obtained from normal subjects (*i.e.* 0.15);. ^{18}FDG: 2-[^{18}F]-fluoro-2-deoxy-D-glucose; Pt: patient; Pn: pneumonia; Br: bronchiectasis.

volume of ^{18}FDG distribution within the tissue, due possibly to increased fluid, but also to the presence of large numbers of other cell types. Cytospins prepared from lavage samples taken from this patient (No. Pn5), 80 min after *i.v.* injection of 120 MBq ^{18}FDG showed a large number of macrophages with less than 1% neutrophils. ^{18}F microautoradiography showed no cell associated radioactivity. In contrast, microautoradiography of ^{18}F in lavage fluid from patient LH, 4 days after admission with pneumonia, showed radioactivity localized to >90% of the large number of neutrophils present.

Bronchiectasis. In the patients with bronchiectasis, PET scanning (*e.g.* fig. 4) showed patchy increases in density throughout the lung, but the positron emission images following ^{18}FDG injection showed no significant accumulation of radioactivity in the lung. The Patlak plots
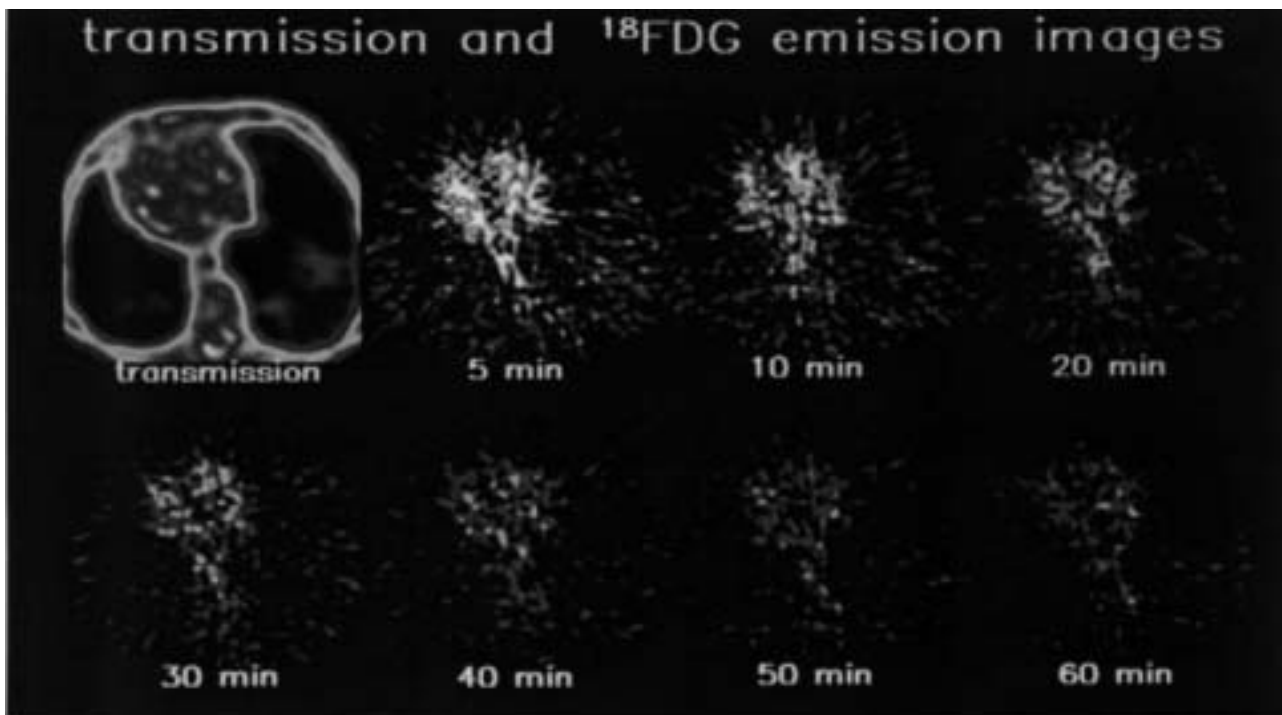

Fig. 4. – Positron emission tomography (PET) scan in patient No. Br2 (bronchiectasis). The density image (upper left) shows patchy increases in density throughout this tomographic slice. Positron emission images following 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG) injection show no accumulation in the lung regions over the period of the scan in this or any other bronchiectasis patients.
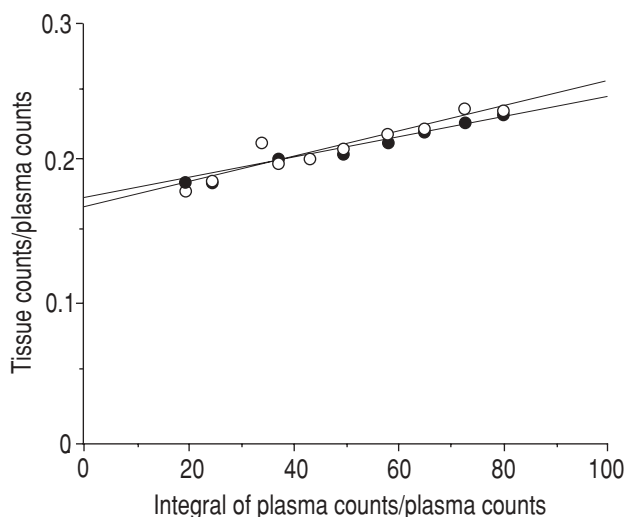
Fig. 5. – Example of Patlak plots reconstructed from the region of interest (ROI) data from positron emission tomography (PET) scan (fig. 4, bronchiectasis, patients No. Br2). there was no increase in 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG) accumulation in either the central or peripheral regions of lung, nor was there an increase in the intercept. ——●——: central regions slope = 9×10^{-4} (min^{-1}); ——○——: peripheral regions slopes = 8×10^{-4} (min^{-1}).

constructed from the R01 data (fig. 5) demonstrated little increase in ^{18}FDG accumulation (compare values for control lung in patients with pneumonia) either in the central or peripheral regions of the lung, and no increase in intercept (fig. 5 and table 2). Although ^{18}F radioactivity was present in sputum samples obtained at the end of PET scanning, confirming that the tracer had diffused through to the airways, no cell-associated ^{18}F radioactivity was apparent.

Discussion

The present studies show a clear dissociation between the migration of neutrophils and the demonstration of increased metabolic activity both in acute lobar pneumonia and in chronic bronchiectasis.

Lobar pneumonia

There are obvious ethical and logistic difficulties in subjecting patients with acute pneumonia to extensive investigations. The present studies aimed to complement existing information that we have derived previously from an animal model of localized pneumococcal pneumonia [13], and from studies of the migration of ^{111}In-labelled neutrophils in human pneumonia [6]. In the rabbit model, in which a right upper lobe pneumonia was induced by bronchoscopic instillation of *S. pneumoniae*, neutrophil emigration measured by ^{111}In-labelled neutrophil influx was at its peak at 6 h and had largely ceased 15 h after instillation. In contrast ^{18}FDG uptake is absent at 6 h but reached its peak at 15 h after instillation of pneumococci. Furthermore, ^{18}FDG uptake has been shown to be due to neutrophil activity *in situ* [13]. Analogous studies of ^{111}In-labelled neutrophils in human pneumococcal pneumonia have shown no influx to the inflamed lung at the time of study (1–2 days after admission to hospital [6]); and such studies involve the

administration of considerable amounts of radioactivity (9 mSv) and require repeated scanning.

The objectives of the present study in human pneumonia were essentially based on our findings in rabbits, namely to examine whether enhanced metabolic activity could be demonstrated in the inflamed lung after significant neutrophil emigration had ceased and to determine the cells responsible for any enhanced metabolic activity. These investigations had to be achieved within the practical and ethical constraints of being unable to obtain complete scanning and lavage data in individual patients at the most acute phase of their illness.

No significant sequestration of the ^{111}In-labelled neutrophils could be detected in the two patients studied within 4 days after the onset of pneumonia, confirming the previous study [6]. Despite the lack of detectable neutrophil emigration to the lung, the metabolic activity of the inflammatory cells measured by PET was very high in these two patients, indicating postmigratory activity of the accumulated cells.

A significantly increased ^{18}FDG signal was still present at 13 days in patient No. Pn4, whereas in rabbits the ^{18}FDG signal returned to baseline within a few days. Nearly normal values were found in two patients, when clinical symptoms were considerably improved, in areas of residual consolidation 3 weeks after admission. ^{18}FDG uptake measured by PET depends on the density as well as the metabolic activity of tissue in the lungs. The raised intercepts of the Patlak plots in the affected lung regions of the pneumonia patients (table 2) indicate an increased amount of ^{18}FDG distributed through the tissue, which is unrelated to the metabolic activity in these areas. *In vitro* the survival of neutrophils is modestly extended by the presence of inflammatory mediators [22], but this is unlikely to explain the present findings. It is more likely that there is a dramatic initial wave of neutrophil migration, which declines rapidly to a level which is too low to detect by external γ-scintigraphy by the time the patients are admitted to in hospital, but which persists for some time.

It was possible to obtain lavage fluid for microautoradiography from the pneumonic area in two patients in whom bronchoscopy was performed to exclude obstructive lesions (patient No. Pn5, and additional patient LH). In patient LH, studied 4 days after onset, neutrophils were labelled; in patient No. Pn5, very large numbers of macrophages were present in lavage fluid but none were radiolabelled, nor was there any radioactivity localized to the few neutrophils present. Although the right lower lobe in this patient still had significantly raised density, metabolic activity detected by PET was that of normal lung, suggesting almost complete resolution of the neutrophil-related component of inflammation.

These results, therefore, confirm the findings in experimental pneumonia, although the time course of enhanced ^{18}FDG activity (and persistence of consolidation) is more extended. Clearly, there is a need for further studies identifying the cell type responsible, particularly in the resolution stage of pneumonia. We have previously identified the neutrophil as the cell type responsible for the increased ^{18}FDG signal in experimental pneumonia by microautoradiography of tritiated deoxyglucose in lung tissue sections [13]. In these sections, the neutrophil was the only cell which showed autoradiographic

labelling. This does not mean that activated neutrophils are the only cells to increase their glucose uptake but that the magnitude of this increase must far outweigh that of other cells. It is likely that the contribution to the [18]FDG signal due to cells other than neutrophils is minimal, especially in view of the fact that when large numbers of macrophages were present (patient No. Pn 5) the [18]FDG signal was not elevated.

Because bronchoscopy was not clinically indicated in most of these patients, it was disappointing that only one of the initial five patients (and additional patient LH) had sputum at the time of scanning and that microbial aetiology was not identified in four of the patients. However, this is a common finding in hospital practice, when the first cultures are made after antibiotics have already been started before admission, and it is widely accepted that most of the healthy patients with acute lobar consolidation with negative microbial cultures have infection with *S. pneumoniae* [14, 15]. Much more extensive studies would be required to establish the time of persistence of enhanced [18]FDG activity and whether this varies with the infecting organism.

Bronchiectasis

Previous studies have shown a high level of on-going emigration into the lung in human patients with bronchiectasis [7], but there have been no previous studies of [18]FDG PET scans to assess metabolic activity at these sites of active neutrophil emigration. In the present study, four of the five patients with bronchiectasis showed a high level of ongoing emigration of neutrophils into the lungs. Localization of ^{111}In-labelled granulocytes does not distinguish between entrapment of neutrophils and their emigration. At 4 h, increased radioactivity may represent margination of the labelled cells; however, a signal 24 h after injection is most likely to represent emigration into the lungs [2]. The appearance of radioactivity in the gastrointestinal tract in patient No. Br2 confirms the clearance of the radioactivity through the lungs. In addition, the fall in splenic counts between the 4 h and 24 h scans is indicative of emigration of labelled cells from the blood [18]. Despite this ongoing emigration of neutrophils, very little increase in metabolic activity could be detected by PET scanning.

We know that the [18]FDG was reaching the sites of interest, as radioactivity was detectable in the sputum immediately following an [18]FDG scan. The detection by PET of an increase in [18]FDG uptake by inflammatory cells depends not only on their metabolic activity but also on there being sufficiently large numbers of such cells present. Although cells are continually migrating to the site, mucociliary clearance and cough are responsible for their removal from the lungs. The number of cells present per cm^3 of diseased lung at any one time is estimated as 10^7 (see Appendix 1). Histological studies in our animal model of pneumonia show that 30×10^6 active granulocytes·cm^3 provide a strong [18]FDG PET signal, and as few as 1×10^6 granulocytes·cm^3 are detectable by PET, provided that these cells are activated.

The implication of the present finding is that in bronchiectasis the cells are not as highly activated when they reach the disease site as they are in acute lobar pneumonia. The behaviour of neutrophils in lavage fluid has

been shown to be compromised in some disease states, such as ARDS [23], and it is possible that this also occurs in bronchiectasis. However, preliminary studies *in vitro* (data not shown) showed that in bronchiectasis the metabolic activity of neutrophils in sputum samples can be increased by phorbol myristate acetate (PMA), which acts *via* a non-receptor-mediated mechanism [24]. It is possible that colonizing bacteria present in the lungs of bronchiectatic patients produce factors which suppress the respiratory burst of neutrophils by affecting cell surface receptors. Another possibility is that the mucus secreted into the airways, itself, contains substances which are capable of affecting neutrophil activity.

The present study supports the conclusion that in human pneumonia, as in animals, maximum metabolic activity of neutrophils occurs after peak emigration of the cells to the site of inflammation. Further, high levels of neutrophil emigration are not necessarily associated with significantly increased metabolic activity of these cells. The implication that this has in the pathophysiology of bronchiectasis has yet to be determined.

Appendix 1

Estimation of granulocyte density in bronchiectasis

Assuming circulating granulocyte count = $10,000 \times 10^6 \cdot L^{-1}$ and blood volume = 5 L.

If the circulating granulocyte pool is equal to the marginated pool, the effective blood volume is 10 L [25, 26] and the total granulocyte count = 1×10^{11}. Mean ^{111}In granulocyte residence time in the blood is 10 h [25, 17]; therefore, granulocyte turnover = 10^{10} granulocytes·h^{-1}.

Extensive bronchiectasis is associated with a granulocyte migration into the lungs of approximately 50% of an injected dose [7]. Lower lung regions were selected on the basis of the white cell scan images for PET scanning in all cases. The volumes of each of these lower ROIs constitute at least one fifth of the totalling volume. Thus, approximately 10% of the injected dose will migrate to each lung ROI; therefore, granulocyte migration into bronchiectatic region = 10^9 granulocytes·h^{-1}.

Assume mobilization rate, k, for the bronchiectatic region (*i.e.* bronchi to sputum) of 10%·h^{-1} [7]. Let n be number of granulocytes in ROI;

thus,
$$dn/dt = 10^9 - kn$$
$$10^9 - n/10$$

at equilibrium:
$$dn/dt = 0$$
thus, $n/10 = 10^9$
and, $n = 10^{10}$

Assuming the volume of the lung ROI to be about 1 L (maximum estimate), then the granulocyte count is at least 10^7 granulocytes·cm^{-3}.

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