FDG-PET imaging of pulmonary inflammation in healthy volunteers after airway instillation of endotoxin

Delphine L. Chen¹, Daniel B. Rosenbluth², Mark A. Mintun¹, and Daniel P. Schuster¹⁺²

¹Departments of Radiology and ²Internal Medicine, Washington University School of Medicine, St. Louis, Missouri

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Chen, Delphine L., Daniel B. Rosenbluth, Mark A. Mintun, and Daniel P. Schuster. FDG-PET imaging of pulmonary inflammation in healthy volunteers after airway instillation of endotoxin. J Appl Physiol 100: 1602–1609, 2006.—Recent studies indicate that a focal, limited, inflammatory response can be safely elicited after direct bronchial instillation of small doses of endotoxin into a single lung segment. Because the radiotracer [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG) is taken up at accelerated rates within inflamed tissues, we hypothesized that we could detect and quantify this regional inflammatory response with positron emission tomography (PET). We imaged 18 normal volunteers in a dose-escalation study with 3 endotoxin dosing groups (n = 6 in each group): 1 ng/kg, 2 ng/kg, and 4 ng/kg. Endotoxin was instilled by bronchoscopy into a segment of the right middle lobe, with imaging performed ~24 h later, followed by bronchoalveolar lavage (BAL). A “subtraction imaging analysis” was performed in the highest dose cohort to identify the area of inflammation, using the preendotoxin scan as a baseline. BAL neutrophil counts were significantly higher in the highest dose group compared with the other two groups (1,413 ± 625 vs. 511 ± 396 and 395 ± 400 cells/mm³; P < 0.05). Autoradiography performed on cells harvested by BAL showed specific [¹³⁷I]deoxyglucose ([¹³⁷I]DG) uptake limited to neutrophils. In vitro [³⁵S]DG uptake in BAL neutrophils in the 4 ng/kg dose group (but not in the 2 ng/kg group) was statistically greater than in peripheral blood neutrophils obtained before endotoxin instillation. The rate of [¹⁸F]DG uptake was greatest in the 4 ng/kg group, with a consistent, statistically significant increase in the rate of uptake after endotoxin instillation compared with baseline. We conclude that the inflammatory response to low-dose endotoxin in a single lung segment can be visualized and quantified by imaging with FDG-PET. Neutrophils; cystic fibrosis; fluorodeoxyglucose; positron emission tomography

Persistent inflammation contributes to the progression of a number of pulmonary diseases. Cystic fibrosis (CF) and acute lung injury (ALI) in particular are characterized by inflammation that can cause respiratory failure and, eventually, death. Neutrophils are the predominant inflammatory cell type in the lungs of patients with CF and ALI (22, 23, 25). As neutrophils are recruited to the lungs, they cause or contribute to lung tissue injury via the increased production and extracellular release of oxidants and preformed proteases. In the case of CF, chronic inflammation, combined with bacterial infection and reduced mucous clearance, may be a root cause of progressive airway dysfunction. Although infection undoubtedly is one reason for persistent neutrophilic inflammation in the airways of CF patients, neutrophils appear early in the course of this disease, even before infection or pulmonary dysfunction is apparent. Accordingly, it has been suggested that chronic inflammation itself may be a valid target for therapy (20).

Unfortunately, few noninvasive methods are available to quantify the inflammatory response within the lungs of patients with CF, ALI, or other neutrophilic lung diseases. Studies in the past have relied primarily on measurements of pulmonary function, like the forced expiratory volume in 1 s (FEV₁) or invasive measurements of inflammation with bronchoalveolar lavage (BAL) (21); however, these measurements are highly variable and are unlikely to be sensitive to small to moderate changes in inflammation as a result of treatment.

Positron emission tomographic (PET) imaging with [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG), on the other hand, has already been used to image a number of inflammatory diseases (2, 3, 7, 11–19, 24, 33, 36, 37, 43). Recently, our laboratory showed that FDG-PET imaging can be used to quantify pulmonary inflammation in patients with CF (4). These results suggest that FDG-PET imaging might be a useful method for evaluating the anti-inflammatory effects of new drugs with potential utility for modulating CF-related inflammation.

Even if FDG-PET imaging could be used for this purpose, however, it would still be highly desirable if the effects of a putative treatment on pulmonary airway inflammation could be predicted more reliably before embarking on a study in actual patients. Recently, investigators at the National Institutes of Health (NIH) (29) showed that focal, limited airway and surrounding parenchymal inflammation could be safely induced by the direct bronchial instillation of small amounts of endotoxin into the airway of a single lung segment in healthy human volunteers. Neutrophil concentrations in lung segments challenged in this way increased by fourfold (as assessed by BAL) 24 h after endotoxin instillation and returned to normal control values by 48 h. Importantly, this model of lung inflammation is directly relevant to CF and ALI given the ubiquitous presence of gram-negative bacteria in the airways of these patients. Furthermore, cytokine and other inflammatory responses to the instilled endotoxin are highly compartmentalized within the lungs, as they are in CF.

We surmised that this approach might eventually be used to screen anti-inflammatory drugs before initiating a more complicated functional trial in patients. To do so, it would first be necessary to show that FDG-PET imaging could quantify the focal inflammatory effect of endotoxin. Thus the purpose of the present study was to demonstrate the feasibility of imaging the inflammatory response to bronchially instilled endotoxin in healthy human research volunteers.
MATERIALS AND METHODS

This study was reviewed and approved by the Washington University School of Medicine’s Human Studies Committee (HSC), the school’s General Clinical Research Center (GCRC) Advisory Committee, and the school’s Radioactive Drug Research Committee, and the study was conducted under an Investigational New Drug authorization by the Food and Drug Administration (BB-IND no. 10420). An on-site, independent Data and Safety Monitoring Committee, approved by the HSC and the GCRC, was charged with reviewing the protocol and all adverse events and with making recommendations to the HSC concerning changes or discontinuance of the study.

The endotoxin (Escherichia coli O:113) was kindly provided by the NIH Clinical Center (Reference Endotoxin) and was reconstituted with sterile water (United States Pharmaceutical) to a final concentration of 2,000 endotoxin units/ml.

We screened 27 volunteers; 5 withdrew their consent before beginning the study, and 3 failed our screening procedures, which included normal chest radiographs, screening blood evaluations, pulmonary function tests (PFTs), and electrocardiograms. One individual withdrew from the study before instillation of endotoxin due to a vasovagal response before the procedure. Thus 18 individuals (8 men and 10 women) completed the protocol. We used a dose-escalation study design with three endotoxin dosing groups ($n = 6$ in each group): 1 ng/kg, 2 ng/kg, and 4 ng/kg.

Figure 1 illustrates the protocol sequence of events. Endotoxin was instilled into the lateral segment of the right middle lobe in 16 of 18 volunteers (the medial segment was used in 2 others). In the 4 ng/kg dose group, a baseline PET study was performed before instillation of the endotoxin. Some of the data from this baseline scan were used to define the rate of $^{18}$F-FDG uptake in normal lung in a previously reported study evaluating patients with CF (4). After endotoxin instillation, the PET study was repeated $\sim$24 h later, followed by repeat PFT measurements and blood withdrawal for a repeat complete blood count. Approximately 2 h later, a second bronchoscopy was performed, this time for BAL of the affected lung segment.

Endotoxin instillation and BAL. Participants were premedicated with 0.4 mg atropine via intravenous injection and 50 mg meperidine and 25 mg of hydroxyzine via intramuscular injection. Midazolam was given intravenously for sedation. Aerosolized lidocaine was administered before insertion of the bronchoscope, with lidocaine also administered bronchoscopically at the level of the carina to prevent coughing. The tip of the bronchoscope was wedged into a segment of the right middle lobe. A 5-Fr balloon-tipped monitoring catheter was then inserted through the bronchoscope to occlude the challenged segment (usually the lateral segment of the right middle lobe). Endotoxin (1, 2, or 4 ng/kg, in 1–2 ml water) was administered through the distal port of the balloon-tipped catheter. The catheter was flushed with 10 ml of normal saline followed by 10 ml air. The catheter was kept in place for 30 s, after which the balloon was deflated. The catheter and bronchoscope were then withdrawn, and the head of the bed was raised to 30$^\circ$. Postprocedure monitoring, including frequent vital signs and oxygen saturation monitoring, took place in the GCRC.

Approximately 24 h after bronchial instillation of endotoxin, an FDG-PET scan was obtained, as described below. Approximately 2 h after completion of the PET scan, bronchoscopy with BAL was performed. In this case, after wedging the bronchoscope into the same segmental airway used for endotoxin administration, three sequential 50-ml volumes of sterile normal saline ($37^\circ$C) were instilled through the suction channel of the bronchoscope. BAL fluid was recovered by gentle aspiration, and the samples were pooled (typically $\sim$100 ml total return volume), gauze filtered to remove excess mucin, and kept on ice for later processing. On completion of this second bronchoscopy, the research volunteer was returned to the GCRC for further monitoring until discharge. Criteria for discharge included normal vital signs, $<10\%$ decrease in the FEV$_1$, and $<3\%$ drop in oxygen saturation by pulse oximetry compared with baseline screening values.

The BAL fluid was analyzed for glucose levels, cell counts, and cell differential. $^{18}$F radioactivity levels in the BAL fluid were also determined by counting 1 ml of BAL fluid (with cells) and 0.5–0.8 ml of BAL fluid decanted from a sample (after centrifugation) in a well counter (2 and 4 ng/kg dose groups only). Cell pellets were reserved for an in vitro assay of $[^3H]$deoxyglucose ($[^3H]$DG) uptake and for autoradiography (see $[^3H]$DG assay and Autoradiography).

PET data acquisition. All participants fasted for at least 4 h before each PET scan. Imaging data were acquired with an ECAT EXACT HR+ scanner (CTI) and were reconstructed using filtered backprojection. A 15-min attenuation scan was done initially, with placement of the participant such that the most caudal slice was $\sim$0.5 cm below the dome of the diaphragm. After completion of the transmission scan, 361 $\pm$ 19 MBq (9.8 $\pm$ 0.5 mCi) of $^{18}$F-FDG were injected intravenously at the start of a 60-min dynamic scan acquisition with the following framing schedule: 24 ± 5, 6 ± 3 min and 8 ± 5 min frames. Blood samples were also collected during this time to determine the “input function” (see PET data analysis).

Because of the minimal inflammatory response in the lower dosing groups, $^{18}$F-FDG uptake in the right middle lobe could not be reliably differentiated from the normal variable uptake in the rest of the lungs. Therefore, the protocol was altered for the final dosing group to include a baseline scan, allowing the possibility of a paired, within-subject (“before-after”) analysis.

PET data analysis. To assist in locating the inflammatory response within the lungs, a “subtraction analysis” was performed on the scans obtained in the final dosing group, using a modification of previously reported methods for this type of analysis (8, 9, 26). The last four frames of the dynamic PET acquisition were summed for both the baseline and postendotoxin PET scans to increase the radioactivity count density within regions of interest. Then, the transmission scan from the baseline PET study was aligned to the postendotoxin PET study transmission scan to determine a transformation matrix for the FDG-PET data. A scaling factor was incorporated into the transformation matrix to account for differences in injected dose. The transformation matrix was then applied to the summed images and the dynamic image acquisition of the baseline PET study to coregister the two (“before” vs. “after”) images. The transformed summed images from before endotoxin instillation were subtracted from the postendotoxin summed images. The resulting subtraction image was displayed in Analyze version 6.1 using a color map scaled such that each color change represented a 5% change in activity. All negative values

![Fig. 1. Schematic of study design for fluorodeoxyglucose (FDG)-positron emission tomography (PET) imaging after bronchial instillation of endotoxin (Etx) in normal human volunteers. BAL, bronchoalveolar lavage.](http://jap.physiology.org/)
were set to zero. Regions of interest (ROIs) were drawn over the areas within the right lung demonstrating a twofold increase in activity over background on at least five consecutive transaxial slices. Control ROIs were created within the left lung on the same transaxial slices and at the same anterior-posterior level as the ROIs placed on the right. Based on the location of these ROIs from the subtraction analyses, ROIs were created on the postendotoxin PET scan images for the first two dosing groups in the expected location of the right middle lobe airway, approximately in the same location, with control ROIs similarly placed on the left. Tissue time-activity curves for each area of interest were then created for the next step in image analysis.

The net rate of $[^{18}F]FDG$ uptake, measured as the net influx rate constant $K_i$, was calculated using a graphical analysis originally reported by Patlak et al. (30, 31). This form of quantitation is appropriate for radiotracers that are irreversibly trapped in target tissues, as is $[^{18}F]FDG$ with phosphorylation by hexokinase. Patlak plots were constructed using the lung tissue activity in the right and left lungs as described above and the activity of $[^{18}F]FDG$ measured from the plasma obtained from the whole blood samples as the input function. Linear regression was performed on all data points after 5–10 min of scanning with visual confirmation of linearity.

By using the approach of Jones et al. (17), values for $K_i$ were also corrected for the initial volume of distribution by dividing by the intercept generated by the regression ("corrected $K_{i,0}$"). The metabolic rate for glucose uptake was calculated as $K_{i,0}$:plasma glucose concentration. Finally, the tissue-to-plasma ratio for lung tissue vs. blood radioactivity was calculated from data obtained at the end of the imaging period, as previously described (5).

$[^{3}H]DG$ assay. In vitro uptake of $[^{3}H]DG$ of peripheral blood neutrophils before and after endotoxin instillation and neutrophils from BAL obtained in the 2 and 4 ng/kg dose groups was assessed using previously published methods (6). Briefly, hypotonic cell lysis and Percoll gradients were used to purify peripheral blood neutrophils before assaying of $[^{3}H]DG$ uptake. Cells from BAL were simply washed with phosphate-buffered solution after collection. All cells were then divided into sample tubes; 37 MBq (1 μCi) of $[^{3}H]DG$ were added to each tube for 6 min; and then all samples were washed and spun down, and $[^{3}H]DG$ uptake was measured as previously described.

Autoradiography. Cells collected by BAL in the 4 ng/kg dose group were also incubated with $[^{3}H]DG$ using a previously described protocol for assaying deoxyglucose uptake in vitro. Briefly, cells were isolated from the BAL sample and washed with Krebs-Ringer-phosphate buffer three times. The cells were incubated with $[^{3}H]DG$ and then washed immediately with ice-cold Krebs-Ringer-phosphate buffer to stop the reaction. Cells were washed three times in total with additional cold buffer to remove excess $[^{3}H]DG$. Cytospin slides were created, and autoradiographic emulsion (LM-1, Amersham Biosciences) was placed using a thin-wire loop according to the manufacturer’s protocol. Slides were exposed for 5.5 wk at 4°C and then developed according to the manufacturer’s protocol.

Statistics. All group data are expressed as means (SD). One-way ANOVA tests were used to compare results among groups. In the highest dose group, a repeated-measures ANOVA, with time and side (right vs. left lung) as the main factors, was used to test for differences in $K_i$ before and after endotoxin. Post hoc comparisons were done using the Holm-Sidak method when data were normally distributed, and the Wilcoxon sum ranked test when they were not. Statistical significance was set at $P < 0.05$. Sigma-Stat v3.0 (SPSS, Chicago, IL) was used for statistical testing.

RESULTS

Table 1 shows vital signs, PFTs, and blood counts before and after endotoxin instillation as well as the most abnormal measurement acquired during the study of all 18 participants. No significant clinical events were noted after endotoxin instillation. There were no statistically significant dose-response differences observed for these variables. Three participants developed asymptomatic hypotension, which resolved in each case with intravenous administration of 250–500 ml normal saline. There were also clinically unimportant but statistically significant increases in body temperature and decreases in oxygen saturation by pulse oximetry when the most abnormal recorded value after endotoxin was compared with preendotoxin measurements. Similarly, spirometry, the hematocrit, and platelet counts decreased slightly but consistently when comparing pre- and postendotoxin values. None of these were symptomatic and none required any treatment. The differences shown in Table 1 were also nearly the same for just the six individuals given the highest dose of endotoxin (data not shown), except that the mean heart rate was not significantly

<table>
<thead>
<tr>
<th>Table 1. Summary statistics of all volunteers</th>
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<td>---------------------------------------------</td>
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<tr>
<td>Vital signs</td>
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<tr>
<td>Temperature, ºC</td>
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<tr>
<td>Heart rate, beats/min</td>
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<tr>
<td>Respiratory rate, breaths/min</td>
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<tr>
<td>Blood pressure, mmHg</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
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<tr>
<td>Pulse oximetry, %</td>
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<tr>
<td>Pulmonary function tests</td>
</tr>
<tr>
<td>FEV1, ml</td>
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<tr>
<td>%Predicted FEV1</td>
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<tr>
<td>FVC, liters</td>
</tr>
<tr>
<td>%Predicted FVC</td>
</tr>
<tr>
<td>Blood cell counts</td>
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<tr>
<td>White blood cells, ×10^9/μl</td>
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<tr>
<td>%Neutrophils</td>
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<tr>
<td>Hemoglobin, g/dl</td>
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<td>Hematocrit, %</td>
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<td>Platelets, ×10^9/μl</td>
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Values are means (SD). Etx, endotoxin; PET, positron emission tomography; FEV1, forced expired volume in 1 s; FVC, forced vital capacity; NA, not applicable. *$P < 0.05$ compared with baseline. †$P < 0.025$ compared with baseline.
different at the three times, the diastolic blood pressure at the
time of the PET scan was not significantly different from the
baseline value, and the most abnormal pulse oximetry value
recorded after endotoxin was not significantly different from
baseline.

BAL cell counts and neutrophil counts were significantly
higher in the 4 ng/kg dose group (Table 2) than in the two
lower dosing groups. Glucose levels were undetectable in all
BAL fluid samples. Radioactivity in the cell pellet from the
BAL fluid was 3.6 times higher than that measured in BAL
fluid without cells (1.8 ± 1.1 vs. 0.5 ± 0.4 nCi/ml, respec-
tively) (i.e., ~80% of the activity was cell associated). Auto-
radiography performed on the BAL cells showed specific
uptake of [3H]DG limited to neutrophils (Fig. 2).

There was no difference in the in vitro rate of [3H]DG
uptake in neutrophils collected by BAL in the 2 ng/kg dose
group compared with rates measured in peripheral blood neu-
trophils obtained before or after endotoxin instillation. In the 4
ng/kg dose group, the rate of [3H]DG uptake was higher in
BAL neutrophils than in peripheral blood neutrophils (Fig. 3).

Figure 4 shows transformed single transaxial slices from the
attenuation and [18F]FDG emission scans before and after
endotoxin administration (after transformation of the baseline
study images). Also shown is an image of the subtraction
image at the same transaxial level. Figure 5 shows the Patlak
plots of the same subject shown in Fig. 4, clearly demonstrat-
ing an increased rate of [18F]FDG uptake after endotoxin in the
challenged segment ($P < 0.001$ for the slope) and no specific
uptake in the unchallenged segment as the slope was not
different from zero ($P = 0.41$).

[18F]FDG uptake, as measured by $K_i$, the slope of the Patlak
regression, in the 4 ng/kg dose group was significantly higher
than that measured in the lower dosing groups (Fig. 6A). There
was a weak correlation between the concentration of neutro-
phils present in BAL and $K_i$ ($R^2 = 0.21$). In the 4 ng/kg dose
group, there was a statistically significant increase in $K_i$ when
comparing data obtained after vs. before endotoxin adminis-
tration (Fig. 6B). None of the other methods of expressing
[18F]FDG uptake (corrected $K_i$, metabolic rate for glucose
uptake, or tissue-to-plasma ratio) were any better at distin-
guishing group differences than $K_i$ itself.

**DISCUSSION**

The main finding of this study is that FDG-PET imaging was
able to reliably detect and quantify the focal inflammatory
response to a small dose of endotoxin instilled directly into a
single segment of the right middle lobe. The potential impli-
cations of this capability are discussed below.

Recently, our laboratory has shown in mouse and canine
models of lung injury, and in patients with CF, that changes in
pulmonary glucose consumption can be monitored and quan-
tified with FDG-PET imaging (4, 6, 44, 45). Indeed, in the CF
patients, we found that the FDG-PET imaging signal was
highest in patients with the most rapid decline in pulmonary
function; that the imaging signal correlated positively with the

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**Table 2. BAL cell counts and neutrophil counts**

<table>
<thead>
<tr>
<th>BAL Measures</th>
<th>1 ng/kg</th>
<th>2 ng/kg</th>
<th>4 ng/kg</th>
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<tbody>
<tr>
<td>Total cell count, cells/mm³</td>
<td>843 (794)</td>
<td>976 (769)</td>
<td>2,280 (652)*</td>
</tr>
<tr>
<td>%neutrophils</td>
<td>48 (21)</td>
<td>53 (15)</td>
<td>60 (13)</td>
</tr>
<tr>
<td>%lymphocytes</td>
<td>13 (9)</td>
<td>7 (6)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>%macrophages</td>
<td>37 (19)</td>
<td>35 (18)</td>
<td>22 (19)</td>
</tr>
<tr>
<td>%monocytes</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Neutrophil concentration, cells/mm³</td>
<td>395 (400)</td>
<td>511 (396)</td>
<td>1,413 (625)†</td>
</tr>
</tbody>
</table>

Values are means (SD). BAL, bronchoalveolar lavage. *$P < 0.05$ compared with 1 ng/kg group. †$P < 0.05$ compared with both 1 ng/kg and 2 ng/kg groups.
‡$P < 0.05$ compared with mean BAL value without cells.

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Fig. 2. Representative autoradiographic slide showing specific in vitro uptake of [3H]deoxyglucose in neutrophils from a subject in the 2 ng/kg dosing group. Inset: Magnified view of two PMNs. PMN, polymorphonuclear leukocyte; Mø, macrophage.

Fig. 3. Rate of [3H]deoxyglucose uptake in neutrophils (PMNs) isolated from the blood (before and after Etx instillation) and from BAL fluid (after Etx instillation). *$P < 0.05$ compared with blood PMNs before endotoxin.
number of neutrophils present in BAL fluid; and that, in cells isolated from BAL, the in vitro uptake (as detected by autoradiography) of another glucose analog, [3H]DG, was limited to neutrophils (4). In mice, neutrophil-depletion experiments show that these cells account for at least 40–50% of the increase in imaging signal after stimuli like endotoxin (44).

The accelerated rate of [18F]FDG uptake in the lungs of patients with a variety of different acute and chronic inflammatory diseases (such as CF, acute respiratory distress syndrome, and pneumonia) suggest that this measurement might be a useful new biomarker to improve the efficiency of evaluating novel anti-inflammatory drugs. For instance, using our data in CF patients, only 10–15 patients with “rapid” rates of decline in lung function would be required to show that an anti-inflammatory agent could produce a 35–50% decrease in the PET imaging signal at the $P < 0.05$ level, which would represent a considerable improvement in efficiency over current methods of evaluating new interventions in this disease.

Even so, small studies of CF patients could still take a year or more to complete at a single institution because CF is a relatively rare disease, and the study would target only the subset of patients with more active disease. To justify the cost and difficulties of implementing such a trial, and indeed trials of anti-inflammatory therapy more generally, it would be very useful to be able to predict more reliably the effects of a putative treatment on pulmonary airway inflammation before embarking upon a trial in patients. Recently, investigators at the NIH (29) showed that focal, limited airway inflammation could be safely induced by the direct bronchial instillation of small amounts of endotoxin into the airway of a single lung segment in normal human volunteers. The use of this model is directly relevant to CF given the ubiquitous presence of gram-negative bacteria in the airways of these patients and the fact that the inflammatory response in this model remains largely compartmentalized to the lungs, as is true in the stable CF patient.

However, to evaluate the effects of endotoxin instillation, it was necessary to perform a second bronchoscopy, this time to recover fluid for biochemical analysis by bronchoalveolar lavage. Given the ability of tomographic imaging methods like PET to measure regional variation within organs, we surmised that it should be possible to eliminate the need for this second invasive procedure in certain settings by using FDG-PET imaging to quantify the inflammatory response.

In their original report, O’Grady et al. (29) reported statistically significant changes in body temperature, heart rate, mean arterial pressure, and gas exchange after endotoxin in-

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Fig. 4. Transmission (top) and FDG-PET (bottom) images from 1 healthy volunteer, before and 24 h after direct bronchial instillation of 4 ng/kg of Etx into the right middle lobe. An area of increased density (presumably due to inflammation) and [18F]fluorodeoxyglucose ([18F]FDG) uptake are clearly visible (arrows). Also shown is a subtraction [18F]FDG image, in which activity of the before and after images are normalized for injected dose, and the “before” image is subtracted from the “after” image.

Fig. 5. Patlak plots for the same subject shown in Fig. 4 demonstrating differences in uptake as measured by the influx constant $K_i$ (the slope of the linear regressions of the Patlak plots).
stillation in 28 research volunteers. More recently, Nick et al. (28) reported no severe or unexpected adverse events in 14 persons. Collectively, these findings are similar to our own observations in the present study. Statistically significant changes after endotoxin occurred for body temperature, heart rate, blood pressure, oxygen saturation, pulmonary function, blood hematocrit, and platelet count. However, all were transient and asymptomatic, and except for the administration of small amounts of normal saline for mild hypotension, none required treatment.

O’Grady et al. (29) reported that neutrophil concentrations in BAL fluid peaked 24 h after 4 ng/kg endotoxin were instilled into a segment of the right middle lobe, producing an average of $0.5 \times 10^5$ neutrophils/mm$^3$, which returned to normal by 48 h. Nick et al. (28) found that this dose of endotoxin produced a slightly higher average neutrophil response of $1.3 \times 10^5$ cells/mm$^3$, which is virtually identical to our own results ($1.4 \times 10^5$ cells/mm$^3$, Table 2).

In the present study, we found only a weak correlation between the rate of uptake of $[18F]$FDG and neutrophil counts in BAL fluid. This relatively poor correlation may have several explanations. First, not only the number of neutrophils, but their degree of activation (as indicated by the rate of glucose uptake per cell) will influence the total amount of $[18F]$FDG uptake by the lungs. In a previous study of the canine oleic acid model of acute lung injury performed with and without prior administration of endotoxin (6), we found that neutrophil concentrations in the BAL were comparable during the two conditions, but only neutrophils (harvested from BAL) from animals exposed to endotoxin showed an increased uptake of $[3H]$DG in vitro. In the present study, cells in BAL from the lung segment challenged with endotoxin (mostly neutrophils) showed an increased rate of uptake of $[3H]$DG in vitro compared with neutrophils simultaneously obtained from the blood (Fig. 3). An analogous phenomenon was noted by Jones et al. (17) when they found that patients with bronchiectasis (a disease characterized by high numbers of neutrophils in the airways) had little to no increase in pulmonary $[18F]$FDG uptake as measured by PET imaging in contrast to patients with acute pneumonia (presumably a condition more likely to be associated with exposure to endotoxin or other similar inflammatory activators).

Another factor is the extent to which neutrophils in the BAL fluid reflect the total neutrophil burden within the lungs. In our previous dog study (6), the uptake of $[18F]$FDG by the lungs increased nearly to the same extent in animals administered endotoxin alone as it did in animals given both endotoxin and oleic acid. We interpreted this observation to indicate that the uptake of $[18F]$FDG by the lungs was influenced by the total neutrophil burden within the lungs, including cells sequestered in the vasculature as well as those that had penetrated into the alveolar spaces. This speculation was supported by a recent study in mice (44).

Given these considerations, it is possible that the uptake of $[18F]$FDG in the segment of lung challenged by endotoxin might have been greater at an earlier time than we chose for study. On the basis of the temporal data of O’Grady et al. (29), which showed that the peak neutrophil counts after endotoxin occurred 24 h after instillation, we decided to image volunteers with PET at approximately this same time. However, most of the peak changes in physiological variables occurred within the first 8 h, when neutrophil counts were nearly as high as they were at 24 h. Further study will determine whether the uptake of $[18F]$FDG in this model peaks at an earlier time, and whether or not this correlates with differences in the in vitro rate of uptake of $[3H]$DG (a measure of individual cell metabolism).

As reported previously by our laboratory and others (10, 14, 15, 17), autoradiography of cells in the BAL showed specificity for uptake of $[3H]$DG in neutrophils (Fig. 2), and analysis of $^{18}$F radioactivity in the BAL showed that 80% of the activity was cell associated. Additionally, the $[3H]$DG uptake assay showed increased uptake in the BAL neutrophils compared with quiescent peripheral blood neutrophils, consistent with our previously published findings that neutrophil activation by endotoxin leads to an increase in glucose metabolism and hence $[18F]$FDG uptake (6). Nevertheless, in our laboratory’s recent neutrophil-depletion studies in mice, the uptake of $[18F]$FDG after intraperitoneal endotoxin was decreased only by $\sim 50\%$, indicating that other cells within the lungs (macrophages, epithelium, endothelium) likely also contribute to the total PET imaging signal (44).

It may well be then that the signal from FDG-PET imaging represents an integral of the total inflammatory response. If true, this does not negate its potential utility; indeed, it is just as possible that correlations with relevant clinical outcomes may be better with an index of the total response rather than one limited to a single cell type. Further studies are needed to clarify the components of the imaging signal under different
relevant experimental and clinical conditions, and how they may or may not be affected by therapy.

The best method for quantifying \( ^{18}\text{F} \)FDG uptake in the setting of inflammation still remains to be determined. Jones et al. (17) introduced the concept of “correcting” \( K_i \) for the initial volume of distribution (represented by the intercept of the Patlak linear regression) to normalize for differences in the degree of inflation of the lungs, which can alter the apparent rate of \( ^{18}\text{F} \)FDG uptake. In contrast, our laboratory has reported that the intercept correction was not useful in the setting of experimental ALI (5), a finding replicated in the present study as well as in our laboratory’s study of CF patients (4). Similarly, in both the present study and in the study of CF patients, other methods of quantifying the rate of \( ^{18}\text{F} \)FDG uptake by the lungs were either equivalent or inferior to the measurement of \( K_i \) itself. Thus, at present, we favor \( K_i \) as the preferred method of quantitation, but this conclusion will need to be tested under different experimental and clinical conditions.

Additionally, the threshold for detecting lung inflammation remains to be determined. Because we did not perform baseline scans in the lower dosing groups, we cannot say for certain whether we would have detected a change in the PET signal in these lower dosing groups. The advantage of PET imaging over other modalities is its sensitivity in detecting such low rates of uptake in a given region of interest and the ability to quantify that signal.

Although PET imaging is noninvasive, it does carry a potential clinical risk due to radiation exposure. The radiation exposure for an FDG-PET study with 370 MBq (10 mCi) of \( ^{18}\text{F} \)FDG is 7 mSv (38) for an adult, which is similar to the national average effective dose of 7.8 mSv for chest X-ray computed tomography (39). In addition, current federal regulations severely limit the amount of radiation that can be administered to persons under the age of 18 yr for research purposes (i.e., there is no federal regulation limiting radiation exposures when needed for clinical purposes). This limitation may be especially significant if studies in patients under the age of 18 (such as in patients with CF) were to be contemplated. Given the relatively low rate of uptake of \( ^{18}\text{F} \)FDG by the lungs, it is likely that limiting the amount of radioactivity administered so that persons younger than 18 yr of age could be studied would result in PET scans of unacceptable image quality. The results of the present study, together with our previous studies in mice, dogs, and patients (5, 6, 32, 44, 45), suggest the possibility of a new paradigm for evaluating novel anti-inflammatory therapies in which the drugs would first be tested in normal human volunteers in the bronchial endotoxin instillation model to determine whether the drug decreased the inflammatory response as measured by FDG-PET imaging, followed by a test of the drug in a small group of patients. Such data would help drive a decision for further testing in a large phase III study to demonstrate improvements in functional outcomes or clinical outcomes. Such a paradigm would be analogous to the current practice of first testing potentially useful immunomodulatory drugs for sepsis in normal volunteers before and after the intravenous infusion of endotoxin before embarking on multicenter phase II and phase III studies in septic patients (1, 27, 34, 35, 40–42).

In summary, FDG-PET imaging can reliably quantify the inflammatory response to a small dose of endotoxin directly instilled into a single lung segment in normal human volunteers. As such, it represents a new tool available to investigators interested in studying such responses in humans.

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