Positron emission tomography with \([^{18}\text{F}]\text{fluorodeoxyglucose}\) to evaluate neutrophil kinetics during acute lung injury

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**Chen, Delphine L., and Daniel P. Schuster.** Positron emission tomography with \([^{18}\text{F}]\text{fluorodeoxyglucose}\) to evaluate neutrophil kinetics during acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 286: L834–L840, 2004. First published December 5, 2003; 10.1152/ajplung.00339.2003.—We measured neutrophil glucose uptake with positron emission tomographic imaging and \([^{18}\text{F}]\text{fluorodeoxyglucose}\) \(([^{18}\text{F}]\text{FDG-PET}) in anesthetized dogs after intravenous oleic acid—induced acute lung injury \((\text{ALI}; \text{OA} \text{group})\) or after low-dose intravenous endotoxin \((\text{known to activate neutrophils without causing lung injury})\) followed by OA \((\text{Etx} + \text{OA} \text{group})\). The following two groups were studied as controls: one that received no intervention \((n = 5)\) and a group treated with Etx only \((n = 6)\). PET imaging was performed \(-1.5 \text{h after initiating experimental interventions}\). The rate of \([^{3}\text{H}]\text{deoxyglucose}\) \(([^{3}\text{H}]\text{DG})\) uptake was also measured in vitro in cells recovered from bronchoalveolar lavage \((\text{BAL})\) after PET imaging. Circulating neutrophil counts fell significantly in animals treated with Etx but not in the other two groups. The rate of \([^{18}\text{F}]\text{FDG}\) uptake, measured by the influx constant \(K_i\), was significantly elevated \((P < 0.05)\) in both Etx-treated groups \((7.9 \pm 2.6 \times 10^{-3} \text{ ml blood-mL lung}^{-1}\text{min}^{-1} \text{in the Etx group,} 9.3 \pm 4.8 \times 10^{-3}\text{ ml blood-mL lung}^{-1}\text{min}^{-1} \text{in the Etx + OA group})\) but not in the group treated only with OA \((3.4 \pm 0.8 \times 10^{-3}\text{ ml blood-mL lung}^{-1}\text{min}^{-1})\) when compared with the normal control \((1.6 \pm 0.4 \times 10^{-3}\text{ ml blood-mL lung}^{-1}\text{min}^{-1})\). \([^{3}\text{H}]\text{DG uptake was increased (73 ± 7\%)} in BAL neutrophils recovered from the Etx + OA group \((P < 0.05)\) but not in the OA group. \(K_i\) and \([^{3}\text{H}]\text{DG uptake rates were linearly correlated (R}^2 = 0.65)\). We conclude that the rate of \([^{18}\text{F}]\text{FDG}\) uptake in the lungs during ALI reflects the state of neutrophil activation. \([^{18}\text{F}]\text{FDG-PET imaging can detect pulmonary sequestration of activated neutrophils, despite the absence of alveolar neutrophilia. Thus (18)FDG-PET imaging may be a useful tool to study neutrophil kinetics during ALI.}

**radionuclide imaging; acute respiratory distress syndrome; fluorodeoxyglucose; dogs**

**POSITRON EMISSION TOMOGRAPHY (PET) imaging after intravenous administration of \([^{18}\text{F}]\text{fluorodeoxyglucose}\) \(([^{18}\text{F}]\text{FDG})\) has become an established tool for diagnosis and treatment monitoring in oncology (28). However, inflammatory lesions are a common cause of false-positive results (4). Interestingly, a number of recent studies have shown that this “problem” can be turned to advantage and used to identify a variety of different inflammatory states and conditions (19, 23, 33, 34).

Evidence to date suggests that the ability of PET to detect an inflammatory focus relies on the extent to which \([^{18}\text{F}]\text{FDG}\) is taken up by cells responding to the inflammatory stimulus \((3, 7, 24, 30)\). During acute inflammation, these cells are primarily neutrophils \((12–14)\). When activated and recruited in a particu-
intravenous pentobarbital sodium and maintained by continuous infusion at 6 ml/min with adjustments as needed to ablale spontaneous breathing. After intubation with a cuffed endotracheal tube, mechanical ventilation was initiated with a Harvard pump respirator at a tidal volume of 15 ml/kg and an Fio2 of 1.0. The respiratory rate was maintained between 12 and 17 breaths/min.

An indwelling arterial catheter was placed in the femoral artery for pressure monitoring. Another intravenous catheter was placed in the femoral vein for drug and radionuclide administration. Temperature, heart rate, oxygen saturation (SaO2), and arterial blood pressure (ABP) were monitored throughout the entire experiment.

Animals were divided into the following four groups: 1) a normal control group that received no experimental interventions (n = 5), 2) a group with lung injury induced by 0.08 ml/kg OA diluted in 1.5 ml of 70% ethanol (OA group, n = 6), 3) a group treated with 15 μg/kg Etx (Etx group, n = 6), and 4) a group with lung injury induced by OA preceded by treatment with the same dose of Etx (Etx/OA group, n = 7). The injury model was implemented by giving intravenously either Etx or placebo (3 ml normal saline) first, followed 30 min later by either OA or placebo (1.5 ml normal saline with 1.5 ml of 70% ethanol; Fig. 1).

PET Data Acquisition

The same scanning protocol was used in all animals. All data were acquired using an ECAT EXACT HR+ scanner, and all images were reconstructed using filtered backprojection. A 15-min attenuation scan was done initially, 1.5 h after experimental interventions were initiated, with placement of the animal such that the most caudal slice was approximately at the dome of the diaphragm. After completion of the transmission scan, the following scans were obtained: 1) lung water concentration (LWC) was determined using 15O-labeled water (H215O). A 5-min scan was performed 3 min after intravenous injection of ~1,665–1,850 MBq of H218O to allow equilibration of the radiolabeled water between the lung tissue and blood compartments and 2) 181 ± 19 MBq of [18F]FDG were injected intravenously at the start of a 58-min period of dynamic scan acquisition with the following protocol: 20 5-s, 6 30-s, 6 60-s, 4 120-s, and 8 300-s imaging frames. Blood samples (1 ml) were taken periodically throughout the scan and spun down at the end of the scan period. Activity in 200 μl of the plasma portion was measured in a gamma counter calibrated to the PET scanner. The data from these samples were used to determine the “input function” for calculating the rate of [18F]FDG uptake in the lungs (see below).

PET Data Analysis

Initial regions of interest (ROIs) were drawn on the lungs using the transmission and lung water scans to define the lung borders. ROIs were then adjusted using the last frame of the [18F]FDG scan to avoid including uptake in the heart. Five tomographic image slices (slice thickness = 0.24 cm, spaced 0.95 cm apart) were used, with the most caudal slice starting ~0.5 cm above the dome of the diaphragm. These ROIs were then used to determine LWC and the net rate of [18F]FDG uptake.

The net rate of [18F]FDG uptake, measured as the influx constant Ki, was calculated using a graphical analysis originally reported by Patlak et al. (26, 27). This form of quantitation, used by others (14) to quantify [18F]FDG uptake in the lungs, is appropriate when a radio-tracer is irreversibly trapped in target tissues, as is [18F]FDG upon phosphorylation by hexokinase. Patlak plots were constructed using an area-weighted average of the activity in the ROIs and the activity of [18F]FDG in plasma, measured from the blood samples. Linear regression was performed on all data points after 10 min of scanning with visual confirmation of linearity. All regressions had a correlation coefficient (R2) of 0.92 or greater. Ki was calculated from the slope of the equation generated by the regression.

Biological Markers

After PET data acquisition, BAL was performed by wedging a bronchoscope in a distal segment of the lung, instilling 30 ml of normal saline, and aspirating the fluid using low wall suction, sampling two to four different segments in each animal. BAL fluid retrieved from the different segments was pooled. A portion of every BAL sample was sent for total and differential cell counts, and the rest was used for in vitro determination of [3H]deoxyglucose ([3H]DG) uptake (see below). Sufficient numbers of cells for analysis were retrieved in three out of six dogs in each of the Etx and OA groups and four out of seven dogs in the Etx/OA group. Blood samples were also taken before the administration of Etx and at the end of the PET scans. These samples were used to determine complete blood counts with differentials and to serve as controls for the [3H]DG uptake assay.

[3H]DG Uptake Assay

Cell purification. Red blood cells were removed from whole blood samples using hypotonic cell lysis. After the blood samples were washed with PBS and spun down, the cell pellets were placed in 10 ml red blood cell lysis buffer and then placed on ice for 2–6 min with occasional gentle overhand mixing. The lysis buffer/cell solution was then neutralized with 1 ml of 10X PBS and spun down. Cell pellets were then resuspended in 45% Percoll solution and loaded on Percoll gradients using a modified version of a previously reported protocol (18). After the gradients were spun, neutrophils were collected from the 60–66% band of the gradient, washed in 10 ml Hank’s balanced salt solution + 0.1% BSA, and then spun down and resuspended in Krebs-Ringer-phosphate (KRP buffer solution).

Cells from BAL fluid were simply washed in PBS, spun down, and then resuspended in KRP.

[3H]DG uptake. We used a modification of a previously reported protocol (15). Cell pellets were washed three times with KRP buffer and then resuspended in KRP. Cells in each sample were counted using a hemocytometer and then divided into tubes containing 5 × 106 cells/tube. One tube from each sample group was treated with cytochalasin B (CB), an inhibitor of glucose uptake, to determine background uptake. After preincubation of all sample tubes, with or without CB, at 37°C for 6 min, 37 KBq [3H]DG solution was added to each sample. All samples were incubated again at 37°C with gentle shaking for 6 min and then spun down immediately at 4°C to halt further uptake. Cells were washed with cold KRP buffer three times and then lysed with PBS containing 1% Triton at 4°C. Cell debris was
Table 1. Select physiological variables

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
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<th>After Intervention</th>
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</thead>
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<td>38.2±1.0</td>
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<td>Heart rate, beats/min</td>
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<td>103±18</td>
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<td></td>
<td>Oxygen saturation, %</td>
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<td>OA</td>
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<td></td>
<td>Heart rate, beats/min</td>
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<td>115±23</td>
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<tr>
<td></td>
<td>Oxygen saturation, %</td>
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<tr>
<td>Etx</td>
<td>Arterial BP, mmHg</td>
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<td>114±31</td>
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<td>Heart rate, beats/min</td>
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<td>134±28*</td>
</tr>
<tr>
<td></td>
<td>Oxygen saturation, %</td>
<td>98±1</td>
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</tr>
<tr>
<td></td>
<td>Arterial BP, mmHg</td>
<td>107±11</td>
<td>101±16</td>
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</table>

Values are means ± SD. BP, blood pressure; Etx, endotoxin; OA, oleic acid. P < 0.05 compared with values before Etx and/or OA administration (*), compared with the OA group only (†), and compared with all other groups (‡).

spun down, and the supernatant was collected, with 350 μl used for quantification of radioactivity by scintillation counting and 50 μl for determination of protein content using the bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL), which was performed according to the manufacturer’s protocol. [3H]DG uptake was calculated after subtracting the background counts as measured in the CB controls. The data were expressed as picomoles per milligram protein per minute.

Statistical Tests

Group data are expressed as means ± SD. Standard one-way ANOVA tests were used to compare results among groups. Two-way repeated-measures ANOVA was used to compare results of measurements taken before and after the intervention among groups, using nonparametric tests when necessary for nonnormally distributed data. Post hoc comparisons were done using Tukey’s test. Statistical significance was set at P < 0.05. Sigma-Stat v2.0 (SPSS, Chicago, IL) was used for statistical testing.

RESULTS

Temperature, heart rate, SaO₂, and ABP at baseline and 3 h after lung injury are listed in Table 1. Temperature and heart rate increased significantly after Etx administration in both Etx-treated groups but not in the groups given Etx placebo. SaO₂ decreased after the experimental interventions only in the Etx + OA group (recall that all animals were ventilated with 100% oxygen). This change in oxygenation is consistent with a loss of perfusion redistribution away from edematous lung regions, thereby increasing the intrapulmonary shunt, as previously described (8). No group showed any significant change in ABP.

In both groups treated with Etx, peripheral white blood cell counts fell significantly (76 ± 13% decrease in the Etx group, 75 ± 8% decrease in the Etx + OA group, P < 0.05) when compared with those groups not treated with Etx (13 ± 30% increase in the normal group, 35 ± 36% increase in the OA group). A similar pattern was seen when examining specifically numbers of peripheral neutrophils across groups, as shown in Fig. 2A. Circulating neutrophils actually increased in the group treated only with OA.

BAL data are shown in Fig. 2B. There were no statistically significant differences in total cell counts among the groups. However, neutrophils were present in significantly higher concentrations in the lung injury groups (64 ± 17% in the OA group, 72 ± 29% in the Etx + OA group) than in those groups without lung injury (2.6 ± 4.0% in the normal control group, 2.8 ± 2.0% in the Etx group).

LWC data are shown in Fig. 3. The increases in LWC in the OA and Etx + OA groups were consistent with lung injury and were statistically significant compared with groups without lung injury. Note that Etx alone did not cause an increase in LWC, nor did it increase LWC compared with OA alone. These results are consistent with previously reported data for both OA-induced injury and Etx effects in this model (8).

Examples of PET images obtained from one normal animal and one animal treated with Etx are shown in Fig. 4. The rate of 18F-FDG uptake is highest in the normal group, reflecting the presence of normal perfusion in the normal animal. This uptake is diminished in the OA group and further decreases in both the Etx and Etx + OA groups. These results are consistent with previously reported data for both OA-induced injury and Etx effects in this model (8).
of $^{18}$F FDG uptake was measured as the $K_i$ (calculated by Patlak analysis). Figure 5 shows examples of the Patlak plots from the same animals used in Fig. 4. $K_i$ was significantly elevated in both Etx-treated groups (Etx and Etx + OA) compared with the normal control group (Fig. 6). $K_i$ was also increased in the Etx + OA group compared with the group given OA alone. $K_i$ of the Etx group was also higher but not quite statistically significantly different compared with the OA group ($P = 0.065$). $K_i$ in the OA group was approximately double that of the normal control group, but the difference was not statistically significant.

Results of the $[^3]$H DG assay are shown in Fig. 7. Rates of $[^3]$H DG uptake in peripheral blood neutrophils before lung injury were used as the baseline against which uptake in the BAL cells was compared. The increase in the rate of $[^3]$H DG uptake in the BAL cells seen in the Etx + OA group ($73 \pm 6\%$ increase, $n = 4$) was statistically significant when compared with that seen in the OA group ($23 \pm 16\%$ increase, $n = 3$).

Cells collected from BAL samples in the Etx group, which consisted primarily of alveolar macrophages, were also assayed and showed a rate of $[^3]$H DG uptake that was only $\sim 2\%$ of the uptake in the Etx + OA group. This result indicates that the likely source of the signal in the two OA groups was the neutrophil, despite the fact that neutrophils were not specifically isolated from the BAL fluid.

There were no significant correlations between $[^18]$F FDG uptake and total cell or polymorphonuclear neutrophil concentrations from BAL samples. However, the rate of $[^18]$F FDG uptake was positively correlated with the rate of $[^3]$H DG uptake ($R^2 = 0.65$, Fig. 8).

**DISCUSSION**

One theory explaining the mechanism of lung injury in ALI/ARDS is the “neutrophil hypothesis,” which states that an initiating stimulus results in neutrophil activation and recruitment to the lungs, with subsequent oxidant production and protease release leading to tissue injury (17). The evidence that neutrophils can in fact cause or exacerbate ALI is very compelling (29), as follows: 1) neutropenia or interventions that block the migration of neutrophils in the air spaces (5) can significantly ameliorate lung injury in several animal models, 2) increased concentrations of neutrophils in the air spaces of patients with ALI/ARDS are observed universally (2, 6, 31), and 3) the magnitude of neutrophil infiltration correlates with the severity of lung dysfunction in ALI/ARDS (16, 36), whereas the persistence of neutrophils in the air spaces is associated with increased mortality (31).
In the current study, we found that the rate of \([^{18}\text{F}]\text{FDG}\) increased uptake of \([^{18}\text{F}]\text{FDG}\) either by lung cells or by cells in the tissue during nonspecific neutrophil infiltration in ALI/ARDS might be an important factor, with virtually no leakage in the lungs (22, 25). Such observations lead some to question whether neutrophil infiltration in ALI/ARDS might actually be an adaptive rather than a destructive response to injury (17). Therefore, the development and use of new tools such as PET imaging to study neutrophil kinetics could bring a new understanding to the mechanisms underlying lung injury in ALI.

A theoretical concern about using PET imaging in ALI, however, is that, because of the damaged endothelial barrier, \([^{18}\text{F}]\text{FDG}\) might leak in the alveolar spaces, becoming a major, nonspecific determinant of the \([^{18}\text{F}]\text{FDG}\)-PET signal during ALI. In the current study, we found that the rate of \([^{18}\text{F}]\text{FDG}\) uptake in animals treated with OA alone was approximately twice that in the uninjured group (Fig. 6). Although this difference might have been statistically significant if the study groups had been larger in number, it was still small relative to the differences measured in either of the groups administered Etx (Fig. 6). Thus it seems reasonable to conclude that pulmonary vascular leak, by itself, does not contribute significantly to the PET imaging signal in the lungs during ALI.

In contrast, \(K_i\) increased significantly after animals were exposed to Etx (Fig. 6). Because vascular leak itself is probably not an important factor, the increase in \(K_i\) likely represents increased uptake of \([^{18}\text{F}]\text{FDG}\) either by lung cells or by cells recruited to the lungs. [Note that, despite the increase in \(K_i\), it is difficult to appreciate the increase in uptake visually on the PET images (Fig. 4), primarily because the baseline uptake of \([^{18}\text{F}]\text{FDG}\) in the lungs is low relative to the uptake of \([^{18}\text{F}]\text{FDG}\) in the heart, emphasizing the importance of appropriate quantitation to assess \([^{18}\text{F}]\text{FDG}\) uptake in the lungs and not just a visual assessment.]

A number of studies suggest that the neutrophil is the primary cell type responsible for increased \([^{18}\text{F}]\text{FDG}\) uptake in acute inflammatory lesions. For instance, Jones et al. (12) have shown, with autoradiography, in a rabbit model of \textit{Streptococcus pneumoniae}-induced pneumonia, that only neutrophils (obtained by BAL) were specifically labeled with \([^{3}\text{H}]\text{DG}\). Hartwig et al. (9) obtained similar autoradiographic results with neutrophils sequestered in the lungs in a pancreatitis model. Our data support these previous studies in that significant increases in both \([^{18}\text{F}]\text{FDG}\) and \([^{3}\text{H}]\text{DG}\) uptake were seen only when neutrophils (albeit activated by Etx) could be recovered from BAL (Fig. 7).

Intravenous Etx is known to result in neutrophil sequestration within the lungs (17), and the declines in peripheral neutrophil counts after treatment with Etx in the current study are consistent with such a phenomenon (Fig. 2A). Pulmonary \([^{18}\text{F}]\text{FDG}\) uptake was increased in the Etx group (Fig. 6), even though cells harvested from BAL samples from this group (primarily alveolar macrophages) showed no significant increase in \([^{3}\text{H}]\text{DG}\) uptake. Likewise, in BAL samples from the OA group, relatively little increase in \([^{3}\text{H}]\text{DG}\) uptake was measured (Fig. 7), despite the presence of increased neutrophil concentrations (Fig. 2B). Only in samples from animals given both Etx and OA did both \([^{18}\text{F}]\text{FDG}\) (Fig. 6) and \([^{3}\text{H}]\text{DG}\) uptake in cells recovered from BAL samples increase (Fig. 7). Overall, we interpret these observations to indicate that Etx caused activation and sequestration of neutrophils in the lungs along with increased glucose and hence \([^{18}\text{F}]\text{FDG}\) uptake, and OA-induced injury permitted penetration of these cells in the air spaces, allowing them to be recovered by BAL.

It should be noted that Hartwig et al. (9) reported in their study of ALI in a pancreatitis model that increased lung tissue \([^{18}\text{F}]\text{FDG}\) uptake did not detect neutrophil sequestration within the lungs. However, they quantified \([^{18}\text{F}]\text{FDG}\) uptake simply as the percent injected dose. This approach is relatively insensitive when the absolute rate of uptake is low and background radioactivity in blood is proportionally high. In contrast, the Patlak graphical approach used in the current study is a more sensitive method for quantifying uptake rates, since it takes the contribution of blood activity into account.

Additionally, our data point to a potentially important limitation of BAL, one of the most commonly used procedures to sample lung tissue in ALI. Obviously, BAL is effective in detecting the presence of neutrophils only if they penetrate into the air spaces. Our data, however, indicate that significant sequestration of activated neutrophils can occur without significant alveolar neutrophilia. Even more importantly, our data also indicate that neutrophils in the air spaces during lung injury are not necessarily activated (at least, when using increased glucose uptake as the criterion for activation). Thus not only may BAL underestimate the influx of neutrophils in the lungs but it is also inaccurate in estimating the current state of cell activation if the number of cells present is used as the indicator of activation. \([^{18}\text{F}]\text{FDG}\)-PET imaging, on the other hand, should be a useful tool to study the early phases of neutrophil trafficking in the lungs as well as the state of neutrophil activation at the actual time of measurement. Combining these data with biochemical and other information derived from BAL could be especially powerful.

Our data, then, also suggest a different interpretation of a previous study by Jones et al. (14). These authors reported that an increased \([^{18}\text{F}]\text{FDG}\)-PET imaging signal could be measured in patients with acute bacterial pneumonia despite no evidence...
of neutrophil emigration (by 111In-labeled neutrophil scintigraphy). On the other hand, patients with chronic bronchiectasis had evidence for neutrophil emigration without an increase in the uptake of [18F]FDG. They interpreted their findings to indicate that increased neutrophil glucose metabolism (consistent with “activation”) was a postmigratory event.

The present study, however, indicates that an increased [18F]FDG-PET imaging signal can be detected in neutrophils that are sequestered within the lungs, before migration out of the vascular space. We believe these results indicate that the key determinant of the [18F]FDG-PET imaging signal is the state of neutrophil activation (Fig. 8), induced by Etx in the current study or by bacterial pneumonia in the study by Jones et al., and not whether or not the cells have migrated out of the pulmonary vasculature. Indeed, our study (Fig. 7) and the study by Jones et al. indicate that large numbers of neutrophils can penetrate in the air spaces without being activated (or at least maintaining that state of activation). Interestingly, Martin et al. (20) observed some time ago that bronchial deposition of the chemoattractant leukotriene B4 in the air spaces of normal volunteers could result in significant alveolar neutrophilia without evidence of injury. We speculate that the neutrophils in that case also, despite emigration out of the vasculature, were not significantly activated and would not have had an increase in glucose uptake.

Overall, the magnitude of the [18F]FDG-PET imaging signal probably represents a combination of the absolute number of neutrophils that infiltrate the lungs as well as their state of activation. It is possible that a threshold concentration of cells is required before a signal can be detected, which would of course affect the sensitivity of PET to assess neutrophilic inflammation during ALI. Our studies were not designed, however, to determine this threshold level.

As already noted, our data indicate that the state of neutrophil activation is a likely determinant of the imaging signal (Fig. 8), since a similar concentration of neutrophils was found in the BAL of both groups given OA (Fig. 2B), but only those animals to which Etx was also administered showed an increase in the PET imaging signal or in [1H]DG uptake. The latter observation is important because these data were normalized to the protein content of each cell, indicating that there was an increase in [1H]DG uptake per cell.

If the state of neutrophil activation is a significant contributor to the increase in [18F]FDG uptake during ALI, then it will be important to understand the regulatory mechanisms underlying glucose metabolism in neutrophils. Glucose and [18F]FDG enter cells via one or more of the GLUT family of membrane transporter proteins (3, 7). After phosphorylation by hexokinase, further metabolism of [18F]FDG is not possible, effectively “trapping” it within the cytoplasm. However, whether changes in the activity of hexokinase or the expression of the GLUT transporters is primarily responsible for the increases in [18F]FDG uptake associated with Etx stimulation is not known. Likewise, the regulatory control of such changes is also unknown.

We have shown that it is possible to detect early stages of neutrophilic inflammation associated with vascular sequestration of neutrophils in an animal model of ALI using PET imaging with [18F]FDG. The rate of [18F]FDG uptake appears to reflect the state of neutrophil activation. Importantly, the imaging signal is not primarily the result of nonspecific pulmonary vascular leak associated with an injured alveolocapillary endothelial membrane. Thus [18F]FDG-PET imaging appears to be a promising new tool to study neutrophil trafficking and kinetics during ALI.

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