Molecular imaging of lung glucose uptake after endotoxin in mice

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Molecular imaging of lung glucose uptake after endotoxin in mice. Am J Physiol Lung Cell Mol Physiol 289: L760–L768, 2005. First published June 24, 2005; doi:10.1152/ajplung.00146.2005.—Positron emission tomographic imaging after administration of the glucose analog fluorine-18 fluorodeoxyglucose ([18F]FDG) may be useful to study neutrophilic inflammation of the lungs. In this study, we sought to determine the specificity of the increase in lung [18F]FDG uptake after intraperitoneal endotoxin (Etx) for neutrophil influx into mouse lungs and to determine the regulation of glucose uptake after Etx by Toll-like receptors (TLRs) and TNF-α. Lung tissue radioactivity measurements by imaging were validated against counts in a gamma well counter. Glucose uptake was quantified as the [18F]FDG tissue-to-blood radioactivity ratio (TBR) after validating this measure against the “gold standard” measure of glucose uptake, the “net influx rate constant.” TBR measurements were made in a control group (no intervention), a group administered Etx, and a group administered Etx plus an additional agent (e.g., vinblastine) or Etx administered to a mutant mouse strain. The glucose uptake measurements were compared with measurements of myeloperoxidase. Increases in TBR after Etx were significantly but not completely eliminated by neutrophil depletion with vinblastine. Increases in TBR after Etx were consistent with signaling via either TLR-4 or TLR-2 (the latter probably secondary to peptidoglycan contaminants in Etx preparation) and were decreased by drug inhibition of TLR-4 but not by inhibition of TNF-α. Thus molecular imaging can be used to noninvasively monitor biological effects of Etx on lungs in mice, and changes in lung glucose uptake can be used to monitor effects of anti-inflammatory agents. Such imaging capacity provides a powerful new paradigm for translational “mouse-to-human” pulmonary research.

respiratory distress syndrome (adult); positron emission tomography; fluorodeoxyglucose F18

IMAGING WITH POSITRON EMISSION TOMOGRAPHY (PET) after the intravenous administration of fluorine-18 fluorodeoxyglucose ([18F]FDG) has become a standard diagnostic and therapeutic management procedure in clinical oncology. In addition, several case series document that FDG-PET imaging can be used to identify inflammatory processes in various acute and chronic infectious and/or inflammatory conditions (4, 5, 10), including those involving the lungs (3, 7, 23–25, 27). Regardless of application, the underlying basis for FDG-PET imaging is that targets of interest (such as tumors) often have a high metabolic rate for glucose compared with surrounding tissues. However, although [18F]FDG is taken up by the same membrane transporters as glucose and is also similarly phosphorylated by hexokinase, it cannot, unlike glucose, be metabolized beyond this first phosphorylation step. In its phosphorylated form, [18F]FDG is trapped intracellularly and continues to accumulate until the nonphosphorylated form has been cleared from the blood via renal excretion. With sufficient accumulation of radioactivity, an imaging signal can be generated, detectable by PET.

Inflammatory cells, of course, also require energy to perform key functions, in this case chemotaxis, phagocytosis, and microbial killing (6, 11). They meet this energy need either by increasing glucose uptake or by increasing glycogenolysis of intracellular glycogen stores (6, 44). Indeed, the strong relationship between glucose metabolism and inflammatory cell function suggests that increased glucose uptake may be a sensitive marker of inflammatory cell activation. As with metabolically active tumors, any increase in glucose uptake in inflammatory cells will also lead to measurable increases in the uptake of [18F]FDG. In several acute models of pulmonary inflammation, the increase in [18F]FDG uptake appears to be confined to neutrophils (23–25). Even in tissues with a mixed inflammatory infiltrate, the accumulation of [18F]FDG has been reported to be greater in neutrophils than in lymphocytes (18).

Recently, in a canine model of acute lung injury, we reported that the rate of [18F]FDG uptake in the lungs depends on whether or not the animal had been exposed to small doses of endotoxin (9). Presumably, endotoxin activates neutrophils, leading to an increased rate of glucose uptake. In that study (9), FDG-PET imaging was able to detect the acute sequestration of activated neutrophils into the lungs, even in the absence of their penetration into the alveolar space. In the current report, we extend these observations to a mouse model of pulmonary inflammation, caused by the intraperitoneal injection of Escherichia coli endotoxin (Etx). We used this model to determine the specificity of the increase in lung [18F]FDG uptake after Etx for neutrophil influx into mouse lungs and to determine the regulation of glucose uptake after Etx by Toll-like receptors (TLRs) and TNF-α.

METHODS

The logic of our studies was as follows. First, we showed that the current generation of so-called “microPET” imaging devices (40) could accurately measure pulmonary radioactivity in the lungs of mice. These data allowed us to then carry out a second set of studies in which we performed dynamic imaging (i.e., the serial temporal acquisition of multiple PET images during a single imaging session) to measure the “net influx rate constant” (Ki) for glucose. This noninvasive measure of glucose uptake is equivalent to comparable measurements obtained by the classic Fick method (19, 20). Next, we used the information from these studies to demonstrate that pulmonary glucose uptake could be reliably estimated by a much simpler

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assay, the tissue-to-blood radioactivity ratio (TBR) obtained 1 h after \(^{18}\)F-FDG administration was 8. Compared with dynamic PET imaging, the measurement of TBR is both less time-consuming and much less expensive, allowing us to then proceed to measure TBR in multiple groups of mice, testing the relative importance of various interventions in mediating Etx-induced increases in pulmonary glucose uptake.

**Reagents.** Wild-type C57Bl/6 mice were used as controls (unless otherwise specified) and for intervention studies. In addition, some studies were performed in mice defective for TLR-4 (C3H/HeJ strain; C3H/OuJ mice were used as controls) or deficient in the TNF receptors 1 and 2 (B6; 129S-Tnfrsf1a^tm1ImxTnfrsf1b^tm1Imx; “TNF double knockout”). All of these mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Additional mice, defective in TLR-2 (41), were a generous gift of Dr. Marco Colonna (Washington Univ. School of Medicine, St. Louis, MO). All mice were 8–9 wk old, were kept on a 12-h light/dark cycle, and had free access to food and water (unless fasted before \(^{18}\)F-FDG administration). All experiments were approved by the Animal Studies Committee of Washington University.

The endotoxin (Etx) antagonist E5564 (33) was a generous gift of the Merck Research Institute (Andover, MA). Lipopolysaccharides from *E. coli* (055:B5) were obtained from Sigma-Aldrich (St. Louis, MO) and from List Biological Laboratories (Campbell, CA). The latter (Etxapo) is highly purified by phenol extraction and is free of peptidoglycans and other ligands that can bind to TLR-2 (21). The highly specific monoclonal antibody to TNF-α (41) [TN3–19,12 (TN3)] was obtained from Pharmingen (BD Biosciences, San Diego, CA). \(^{18}\)F-FDG was manufactured onsite, as previously described (16).

**Experimental preparation and experimental groups.** All studies were approved by the Washington University Animal Studies Committee. Mice were fasted for at least 4 h before the administration of \(^{18}\)F-FDG. Unless otherwise specified (usually in the figure legends), two to three groups of mice were studied at a time (*n* = 6–10/group). These group clusters included a control group, a group administered Etx, and in some cases, a group administered Etx plus an additional agent (e.g., vinblastine) or Etx administered to a mutant mouse strain. The Etx was administered intraperitoneally, 20 μg/g in 0.4–0.5 ml of normal saline, 30–45 min before \(^{18}\)F-FDG injection (the timing corresponds to that used in previously reported canine studies, e.g., see Ref. 9). Vinblastine was injected (4 μg/g) 5 days before \(^{18}\)F-FDG injection. TN3 (250 μg/g) in 0.5 ml of sterile injectable water was administered 20–24 h before \(^{18}\)F-FDG injection [the dose and timing were based on previously published data (41)]. E5564 [4 μg/g body weight (bw)] was administered 30 min before Etx [dose and timing were extrapolated from previously published data (33)].

**Instrumentation.** Venous and arterial catheters were placed for radiotracer administration and subsequent blood sampling. A microcatheter (Harvard Apparatus, Holliston, MA) was placed in the jugular vein for intravascular injection of \(^{18}\)F-FDG. Microurethane tubing (0.025 outer diameter × 0.012 inner diameter; Braintree Scientific, Braintree, MA) was placed into the left common carotid artery for blood sampling during dynamic PET scanning.

**PET imaging and analysis.** Gas anesthesia (1–2% isoflurane) was administered continuously during imaging. Micro-X-ray computed tomography (microCT) images were obtained using a MicroCAT II scanner (Imetek, Knoxville, TN). \(^{18}\)F-FDG (15–20 μCi/g bw), in ~50 μl of saline, was administered via the jugular vein over 60 s. Mice were scanned for 75 min on a microPET-R4 scanner (CTI Concorde Microsystems, Knoxville, TN). The microPET-R4 camera consists of four rings of 24 lutetium orthosilicate (2 cm × 2 cm × 1 cm) scintillators optically coupled to position-sensitive photomultiplier tubes with fiberscopes. Each detector is subdivided into an 8 × 8 array, resulting in 6,144 individual crystals. The field of view is 12 cm transaxially by 8 cm axially. The camera is capable of a spatial resolution of 2 mm and has an absolute sensitivity of 900 cps/μCi. Data are acquired in list mode and can be histogrammed in frames as short as 1 s. Images are reconstructed by Fourier Rebinning followed by 2D-Filtered Back Projection (ramp filter at Nyquist frequency) using all tilt angles. Images were collected serially in the following order (number and duration of image “frames”): 11 × 8 s (s), 1 × 12 s, 1 × 20 s, 2 × 60 s, 1 × 180 and 10 × 400 s. Mean activity per milliliter of tissue was determined by drawing a lung region on a single slice, located just above the diaphragm. During scanning, multiple arterial blood samples (~5 μl each) were obtained: 12 at 5-s intervals, then 4 at 10-s intervals, then 1 each at 30-, 60-, and 180-s intervals, and finally 10 samples were obtained approximately every 400 s. Samples were drawn into a disposable micropipette, pre- and postweighed, and counted in a gamma counter.

The kinetics of \(^{18}\)F-FDG uptake in the lungs were analyzed with a standard three-compartment model using nonlinear regression (8, 22), where *K*₁ is the forward rate constant between the blood and tissue compartment, *K*₂ is the reverse rate constant between the same two compartments, and *K*₆ is the rate constant representing trapping of \(^{18}\)F-FDG intracellularly after phosphorylation by hexokinase. Model parameters were obtained by fitting the experimental time-activity data to the following equation

\[
C_t(t) = \frac{K_1}{\alpha_1 - \alpha_2} \left[ (K_4 - \alpha_2)e^{-\alpha_2 t} + (\alpha_2 - K_4)e^{-\alpha_1 t} \right] \times C_B(t) + BV \times C_H(t)
\]

where

\[
\alpha_{2,1} = \frac{1}{2}(K_2 + K_3 + K_4 \pm \sqrt{(K_2 + K_3 + K_4)^2 - 4K_2K_3})
\]

\(\otimes\) is the convolution operator, BV is the estimated blood volume component, *t* is time, and *K*₁, *K*₂, *K*₃, and *K*₄ represent the individual rate constants.

It was assumed that dephosphorylation in the lungs is negligible over the 1-h scanning period, so *K*₄ was set to 0 and dropped from the equation. *K*₁, *K*₂, *K*₃, and BV were then estimated by nonlinear regression using 0.04, 0.04, and 0.03 as initial estimates for the rate constants.

The net influx constant *K*ᵢ was then calculated from the compartmental model rate constants as follows (32, 34)

\[
K_i(\text{ml blood · ml lung}^{-1} · \text{min}^{-1}) = K_i \frac{K_1}{(K_1 + K_3)}
\]

For illustration of differences between experimental groups, the *K*ᵢ was also calculated from the tissue and blood time-activity data using the graphical method of Patlak et al. (34, 35). The plot was constructed from an area-weighted average of activity in the image regions of interest and the activity of \(^{18}\)F-FDG in blood. Linear regression was performed on all data points after 10 min of scanning with visual confirmation of linearity. *K*ᵢ was calculated from the slope of the equation generated by the regression. The following equation was used to construct the Patlak plots and to generate the linear regressions

\[
C_H(t) = K_1 \frac{C_B(t)}{C_H(t)} + \text{Int}
\]

where *C_H(t)* and *C_B(t)* are, respectively, tissue and blood radioactivity at each sample time point, *τ* is the integration variable, and Int is a parameter representing the initial volume of distribution of the tracer in both the tissue and blood (the intercept when plotted graphically).

Finally, the pulmonary uptake of \(^{18}\)F-FDG was also measured as the TBR. For this measurement, mice were killed via cervical dislocation 1 h post-\(^{18}\)F-FDG injection. The chest was opened, and a 5-
10-μl blood sample was collected in a preweighed capillary tube via heart puncture. The lungs and heart were removed en bloc, and excess tissue and the heart were separated from the lungs. The lungs were not flushed but were then placed in a preweighed centrifuge tube. Blood and lung weights were determined, and radioactivity was measured in a gamma counter. After decay corrections, TBR was calculated as the ratio of lung tissue radioactivity to blood radioactivity. After counting, the lung tissue was frozen for later assay of myeloperoxidase (MPO) activity. After showing that Ks and TBR were highly correlated (see Results), we measured TBR alone in subsequent groups of mice, by the procedure just described, after injecting [18F]FDG (4–8 μCi/g bw) via tail vein while the mice were under anesthesia.

MPO activity assay. The previously frozen lung tissue was homogenized for 30 s in 5 ml of 50 mM potassium phosphate buffer, pH 6, containing 0.5 g/dl hexadecyltrimethyl ammonium bromide, incubated in the water bath at 55°C for 2 h, and centrifuged for 20 min at 13,000 rpm at 4°C. Ten microliters of the supernatant were added to 250 μl of reaction solution (25 ml of 50 mM potassium phosphate buffer, pH 6, 4.175 mg of O-dianisidine, and 416.5 μl of 0.003% hydrogen peroxide) in a well of a microplate and read by a spectrophotometer microplate reader. Each sample was assayed in triplicate. Absorbance of 460 nm of visible light was measured for 6 min. MPO activity per microgram of protein of supernatant (measured by the standard bicinchoninic acid method) was calculated as follows: MPO activity = ΔOD·min⁻¹·μg⁻¹ protein, where ΔOD equals the rate of change in absorbance at 460 nm between 1 and 6 min.

Statistics. Group data were expressed as means ± SD. Standard one-way ANOVA tests were used to compare results among groups. In some cases (specified in the figure legends), data were log transformed to achieve normal data distributions required for ANOVA. Post hoc comparisons were done using the Holm-Sidak multiple comparison test. Statistical significance was set at P < 0.05. The SigmaStat 3.1 program (Systat Software) was used for these calculations.

RESULTS

Examples of microCT and microPET images from a normal control mouse and a mouse treated with Etx are shown in Fig. 1. Note that in mice treated with Etx, there is a generalized increase in lung tissue radioactivity compared with control normal mice (indicating increased uptake of [18F]FDG) as well as increased uptake in the heart and over the spine (presumably representing increased uptake of [18F]FDG by marrow inflammatory cells).

A prerequisite for using the images as a measure of inflammatory response is that the radioactivity measurements from regions of interest on the images are in fact accurate. Figure 2 demonstrates this to be the case by showing a strong correlation between radioactivity data obtained from the final image at the end of the imaging session with lung tissue radioactivity measured ex vivo in lungs placed in a gamma well counter (Fig. 2). [Note that the slope of 0.35 is consistent with previous results (38) and with the known density of lung tissue (~0.3 g/ml) in vivo (29). The PET data are expressed in units per

Fig. 1. Examples of micro-X-ray computed tomography (microCT) (left) and microPET (middle) images from a normal control mouse (top) and from a mouse 90 min after intraperitoneal endotoxin (Etx). The positron emission tomography (PET) images were obtained 1 h after tail vein injection of fluorine-18 fluorodeoxyglucose ([18F]FDG). Images at right are merged microCT and microPET images after coregistration, using fiducial markers on both the CT and PET images. The color bar along the bottom indicates increasing tissue radioactivity on the PET images, from the “cool” colors on the left to the “warm” colors on the right (each color change represents a 5% increment). Note that after Etx, there is a generalized increase in lung tissue radioactivity (indicating increased uptake of [18F]FDG) as well as an increase in lung tissue radioactivity compared with control mouse and a mouse treated with Etx are shown in Fig. 1. 

Fig. 2. Correlation of lung tissue radioactivity as measured by PET imaging in intact mice with lung tissue radioactivity measured ex vivo in lungs placed in a gamma well counter. Data were obtained in 5 normal control mice and 7 mice treated with intraperitoneal Etx. Also shown are the coefficient of determination (R²), the regression line, and the regression equation describing the relationship between the 2 radioactivity measurements. The slope of 0.35 is consistent with the density of lung tissue in vivo (note the PET data are expressed in units per ml of lung tissue and the gamma counter data are expressed in units per g of lung tissue).
milliliter of lung tissue, and the gamma counter data are expressed in units per gram of lung tissue.

As the net uptake rate constant, $K_i$ is analogous to measuring the tissue uptake of any tracer by the classic Fick method. Measuring $K_i$ by noninvasive imaging requires serial measurements of tissue radioactivity (in this case, in the lungs) simultaneously with measurements of radioactivity in blood. Figure 3, A and B, show examples of the resultant tissue and blood time-activity curves, respectively, from both a normal control mouse and from a mouse administered intraperitoneal Etx. Data such as these were used to then calculate $K_i$ by compartmental modeling and by Patlak graphical analysis (see Methods). The low noise present in the time-activity curves yielded excellent modeled fits to the experimental data, as evidenced by $R^2 > 0.9$ in all cases.

While the differences between the curves in Fig. 3, A and B, may appear to be small, they are cumulative over time, as clearly seen when displayed graphically by the method of Patlak (34, 35) (Fig. 3C). The linear regression analysis of the Patlak plots to compute $K_i$ was similarly robust, with $R^2 > 0.9$ in all cases. For these two groups, the mean $K_i$ in the untreated control mice was $5.9 \times 10^{-3} \pm 1.0 \times 10^{-3}$ vs. $11 \times 10^{-3} \pm 3.5 \times 10^{-3}$ ml blood·ml lung$^{-1}$·min$^{-1}$ in the Etx-treated mice ($P < 0.05$). In contrast, the mean intercept (related to the initial volume of distribution for the radiotracer) was not significantly different (0.51 ± 0.14 in controls vs. 0.47 ± 0.20 ml blood/ml lung in the Etx group).

The measurement of $K_i$ with a combination of both serial imaging and blood drawing can be technically challenging, especially in mice. However, in dogs, $K_i$ correlates strongly with the much simpler TBR measurement, which is the tissue radioactivity measurement obtained ~1 h after $[^{18}\text{F}]$FDG administration divided by a simultaneous measurement of radioactivity in blood. Figure 3D shows that $K_i$ by Patlak analysis and TBR measurements were also strongly correlated to one another in mice. As a consequence, we used TBR as the index of lung tissue $[^{18}\text{F}]$FDG uptake in the remaining experiments.

Mean values for TBR in groups of normal control mice and in mice treated with Etx are shown in Fig. 4A. In normal mice, mean TBR is $>1$, indicating net uptake of $[^{18}\text{F}]$FDG into lung tissue (if all the radioactivity was limited to blood, the ratio would be $<1$). Figure 4A also shows that MPO activity levels are very low in normal lung tissue, indicating the presence of relatively low numbers of neutrophils in the control condition. Thus lung parenchymal cells are likely responsible for the net uptake of $[^{18}\text{F}]$FDG into the lungs of normal mice.

![Figure 3](image-url)

**Fig. 3.** A: lung tissue time-activity curves obtained by serial PET imaging from 1 normal control mouse and 1 mouse administered intraperitoneal (i.p.) Etx. B: blood time-activity curves obtained from the same mice as in A, by serial microcatheter blood sampling. C: Patlak graphical analysis of the data obtained in A and B. Note the increased slope of the relationship in the mouse administered Etx, consistent with an increased rate of lung glucose ($[^{18}\text{F}]$FDG) uptake. The intercepts of the 2 regression lines are nearly identical, suggesting similar volumes of distribution for the radiotracer in this example. D: correlation of the tissue-to-blood radioactivity ratios (TBR; obtained at the end of each experiment by placing the lungs and a blood sample into the gamma well counter) with $K_i$ (the net influx rate constant for $[^{18}\text{F}]$FDG, obtained with dynamic PET imaging, as explained in Methods). Data were obtained in the same set of mice as in Fig. 2. Also shown are the coefficient of determination ($R^2$), the regression line, and the regression equation describing the relationship between the 2 measurements of glucose uptake.
In mice treated with Etx, both lung MPO activity levels and $[^{18}F]$FDG uptake increase. Figure 4A shows a moderately strong correlation between these two variables (using the individual data in Fig. 4A to calculate group means).

The increase in MPO after Etx is due to the influx of neutrophils into the lungs. However, the increase in $[^{18}F]$FDG uptake by the lungs after Etx could be due to the influx of activated neutrophils and/or to increased glucose uptake by lung parenchymal cells. To determine the extent to which neutrophils are responsible for the increased uptake of $[^{18}F]$FDG after Etx, groups of mice were treated with the neutrophil-depleting chemotherapeutic agent vinblastine and then 5 days later were treated with Etx or placebo.

Figure 5A shows the effect of 5-day treatment with vinblastine on circulating peripheral neutrophil counts in mice treated with or without Etx. Vinblastine alone caused a decrease in circulating neutrophil counts, consistent with tissue sequestration. Vinblastine also caused a decrease in circulating neutrophil counts, consistent with its expected cytotoxic effects.

Figure 5B shows the effect of vinblastine on MPO and TBR. As a result of neutrophil depletion, vinblastine decreased tissue MPO almost to control levels, even in the presence of Etx. Vinblastine also decreased TBR in mice given Etx (compared with those not treated with vinblastine, $P < 0.05$) but not to control levels. These results suggest that the Etx-induced increases in lung $[^{18}F]$FDG uptake occurred in both parenchymal cells (still present after vinblastine) as well as neutrophils (diminished or eliminated by vinblastine).

Recent data suggest that TNF-$\alpha$ can mediate increases in neutrophil uptake of $[^{18}F]$FDG (26). Therefore, we investigated the effect of blocking TNF-$\alpha$. Figure 6 shows that neither $[^{18}F]$FDG uptake nor MPO activity levels were altered by pretreatment with the highly specific anti-TNF-$\alpha$ antibody TN3 (41). This lack of effect was confirmed in mice genetically deficient for TNF receptors 1 and 2 (Fig. 6).

We tested the effect of using the TLR-4 antagonist E5564 (33). The results of these experiments (Fig. 7) show that E5564...
reduced TBR compared with mice treated with Etx alone ($P < 0.05$). MPO was also reduced but not significantly.

The effects on TLR-4 antagonism with E5564 were confirmed in studies in mice defective for the TLR-4 receptor (Fig. 8). As previously shown in Fig. 4B, Etx caused a significant increase in TBR compared with control mice (no Etx). Mice administered Etx in these studies actually included two subgroups, namely mice administered Etx purified by phenol extraction (Etxnph) and mice administered Etx that was not phenol extracted (Etxnp). The former is free of peptidoglycans that contaminate Etx preparations that are not phenol extracted.

Figure 8A shows comparable increases in TBR in wild-type mice regardless of type of Etx used. In mice defective for the TLR-2 receptor, TBR still increased significantly after exposure to Etxnph (consistent with TLR-4 mediated effects). In contrast, when the highly purified Etxnph was used in C3H/HeJ mice lacking functional TLR-4, TBR did not increase. [The increase in TBR in control C3HeB/FrJ mice after Etx (2.05 ± 1.34), of similar genetic background to the C3H/HeJ mice, was not significantly different from the increase after Etx administration to C57Bl/6 mice (2.69 ± 1.32, $P = $ not significant)]. TBR did increase, however, if Etxnph was used in C3H/HeJ mice (consistent with TLR-2 mediated effects by peptidoglycans known to be present in Etx not purified by phenol extraction). Overall, the MPO results in these mice (Fig. 8B) mirrored the TBR data.

**DISCUSSION**

The main findings of this study are 1) the increase in lung $[^{18}F]$FDG after intraperitoneal Etx in mice is significantly but not solely due to neutrophil influx into the lungs, 2) the increase in lung $[^{18}F]$FDG after Etx is not dependent on TNF-$\alpha$, and 3) the increase in lung $[^{18}F]$FDG is consistent with either TLR-2 or TLR-4 mediated signaling. Furthermore, the results demonstrate that molecular imaging of the biological effects of Etx on the lungs can be monitored noninvasively in mice with the current generation of new microPET scanning instruments and that changes in lung $[^{18}F]$FDG uptake can be used to monitor the effects of anti-inflammatory agents. Because such imaging can be performed in both rodents and humans, this capacity provides a powerful new paradigm for translational “mouse-to-human” research.

**Lung uptake of $[^{18}F]$FDG as a marker of neutrophilic inflammation.** Martin (30) has noted that “direct studies (about neutrophil trafficking in the acute respiratory distress syndrome) in humans are very limited,” and the reasons why are obvious. The only way to harvest neutrophils from the lungs of living animals is via bronchoalveolar lavage (BAL), and the mere presence of these cells in BAL does not necessarily correlate with their functional state at the time of harvesting because the cells may no longer be activated or they may be in an altered state of activation from the harvesting procedure itself. Likewise, the interpretation of assays of soluble mediators of inflammation in BAL is difficult unless one takes into account production and clearance rates, as well as the presence of counterregulatory molecules. Given the dependency of neutrophil function on glucose metabolism, others have proposed that an acute increase in tissue glucose uptake is a marker of tissue neutrophilic inflammation (23, 24). Accordingly, PET imaging could be a particularly powerful research tool because
the measurements can be obtained noninvasively in “real time” (i.e., they reflect function at the time of the actual measurement). Before these imaging methods are implemented to monitor neutrophil kinetics in the lungs, however, it is important to verify the specificity of the imaging signal for neutrophil activation and to better understand the factors regulating the accumulation of $^{18}$F-FDG uptake (and, therefore, of the imaging signal).


Recently, in a canine model of acute lung injury, we reported that FDG-PET imaging could be used to detect early stages of neutrophilic inflammation associated with vascular sequestration of neutrophils after intravenous Etx (9). The increased lung uptake of $[^18]$F-FDG appeared to reflect the state of neutrophil activation after Etx and was not the result of nonspecific leak of the tracer through an injured alveolocapillary endothelial membrane.

The current study, limited to the effects of Etx alone, extends these observations. First, we validated the use of microPET imaging in mice as a means of measuring tissue radioactivity. Despite limitations in spatial resolution (~2 mm in plane) and “spillover” effects of increased radioactivity from the heart and/or bone marrow into the lung region of interest (Fig. 1) (39), there was a strong correlation between image-derived lung radioactivity measurements and those obtained in a gamma well counter (Fig. 2). Furthermore, the time-activity data with serial imaging provided high-quality data for compartmental modeling of the $K_i$ [the noninvasive imaging equivalent of measuring tracer uptake by the classic Fick method (19, 20)] (Fig. 3) and for calculation of $K_i$ by Patlak graphical analysis.

As in dogs (9), we showed that the much simpler TBR measurement correlated strongly to measurements of $K_i$ (Fig. 3), despite [and in contrast to reports by others (23–25)] no correction for differences in the volume of distribution of the radiotracer (estimated by the intercept calculation from Patlak graphical analyses). In the current study, the mean intercept value for a control group of mice was not different from a group treated with Etx, so the value of such a correction may be moot. The lack of a difference in the intercept after Etx is consistent with a mild state of inflammation, and it is certainly possible if not likely that with more severe inflammation, the intercept group means would have been different. However, in the canine study, we found that TBR and $K_i$ were strongly correlated despite significant differences in the intercept value among the experimental groups. Even so, these strong correlations may be related to the specific experimental setting, and the potential value of such a correction in other settings requires additional study.

In dogs, we reported that the average value for TBR in normals was $0.33 \pm 0.06$. In the current study, TBR averaged $2.32 \pm 0.53$ in mice (Fig. 4). Values >1 mean that there must be significant uptake of the radiotracer into lung tissue since values would have to be <1 if all of the radiotracer was confined to the blood pool in lung tissue. This apparent species difference (which suggests increased glucose metabolism of lung parenchymal cells compared with dogs) may be important in explaining the results of neutrophil depletion experiments (Fig. 5) since vinblastine failed to completely prevent the increase in TBR after Etx, suggesting continued stimulation of glucose uptake by Etx-sensitive cells in the lung parenchyma, even in the absence of significant neutrophil influx into lung tissue. The significance of these findings for the use of FDG-
PET imaging in clinical settings is at present uncertain since comparable measurements have yet to be reported in humans.

**Regulation of lung \(^{18}F\)FDG uptake.** If FDG-PET imaging is to be used in studies of acute lung injury (or other settings characterized by acute neutrophil influx into the lungs), then understanding how glucose uptake is regulated upon neutrophil activation might suggest new targets for ameliorating neutrophil-dependent tissue injury. These same mechanisms would also affect the magnitude of the imaging signal during FDG-PET imaging.

Our understanding of how glucose is transported into inflammatory cells or into the lung parenchyma is incomplete, however. It is usually presumed that glucose (and therefore \(^{18}F\)FDG) enters neutrophils via the constitutively expressed glucose transporter-1 (GLUT-1) protein (36). However, neutrophils have been reported to express several different glucose transporters (12, 28, 31), and specifically which one facilitates increased glucose uptake after Etx has not been identified. GLUT-1 expression is increased in alveolar epithelial cells in response to hypoxia, but changes in response to inflammatory stimuli like Etx also have not been studied.

Likewise, what regulates \(^{18}F\)FDG (or glucose) accumulation in activated neutrophils or stimulated lung parenchymal cells is uncertain. Inflammatory activators such as PMA, the tripeptide formyl-methionyl-leucyl-phenylalanine, and granulocyte/monocyte colony-stimulating growth factor each in a drug that interferes with Etx binding to TLR-4 (25, 26) has been specifically implicated as a major factor affecting neutrophil glucose uptake, we first tested the effect of inhibiting the effects of TNF-α (either with a specific monoclonal antibody or in mice defective for the TNF-α receptors) on lung \(^{18}F\)FDG uptake after Etx. We found that TNF-α was not a necessary factor regulating increases in lung glucose uptake after Etx in mice (Fig. 6).

In contrast, we were able to show definitively but not unexpectedly that lung glucose uptake after Etx was completely inhibited when studies were performed in mice deficient for TLR-4 (Fig. 8) and could be substantially reduced by a drug that interferes with Etx binding to TLR-4 (Fig. 7) (33). Furthermore, the fact that an impure Etx preparation, shown by others to contain peptidoglycans and other ligands that can bind TLR-2 (21), was able to increase lung glucose uptake in TLR-4-deficient mice (Fig. 8) suggests that TLR-2 signaling may also mediate changes in glucose metabolism.

Although neutrophils, which express both TLR-2 and TLR-4, are a likely cause of the increased PET imaging signal and increased lung \(^{18}F\)FDG uptake after Etx, lung epithelial cells and tissue macrophages also express both receptors (1, 2, 14, 15); therefore, they too could contribute to the measured increases in lung \(^{18}F\)FDG uptake. The molecular mechanisms that link TLR-2 or TLR-4 signaling with increased glucose uptake via changes in glucose transporter expression or activity, regardless of cell type, however, are unknown at this time.

**Use of \(^{18}F\)FDG-PET imaging to monitor anti-inflammatory therapies.** A major advantage of PET imaging is the opportunity to readily translate insights gained in experimental animals into clinical studies. FDG-PET imaging is already widely used for cancer detection and treatment planning. Others, however, have suggested that FDG-PET imaging might also be useful as a clinical diagnostic test in the management of infection and inflammation (37, 45). Despite the apparent nonspecificity of the imaging signal for neutrophils, at least in mice, a strong correlation between the imaging signal (as a measurement of overall inflammatory response) and neutrophil activation may still make these measurements useful and valuable. Such a correlation remains to be documented but is not unreasonable. If and when present, FDG-PET imaging could prove useful in studies of patients with acute respiratory distress syndrome, again, with the assumption that glucose uptake by infiltrating neutrophils and by resident lung cells would be closely coupled in this setting. Such studies might help resolve uncertainties about the “kinetics” (onset, magnitude, and/or duration) of the inflammatory response as a whole. In this and other disease states, particularly those characterized by neutrophil influx into the lungs (e.g., pneumonia, cystic fibrosis), noninvasive FDG-PET imaging might also be useful as a means of testing anti-inflammatory therapies. The effect of the anti-Etx drug E5564 on lung \(^{18}F\)FDG uptake (Fig. 7) provides the first proof-of-principle evidence to support this potential application. Overall, the current study supports the further study of FDG-PET imaging as an indicator of pulmonary inflammatory disease severity, acuity, or response to anti-inflammatory treatment.

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**REFERENCES**


Molecular Imaging of Lung Glucose Uptake


