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Imaging Pulmonary Inflammation with Positron Emission Tomography: A Biomarker for Drug Development

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Abstract: Methods currently used to assess lung and airway inflammation are often poorly quantitative, invasive, nonspecific, or insensitive. Positron emission tomography (PET) with [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG), on the other hand, is a noninvasive, highly sensitive imaging technique that can be used to quantify pulmonary inflammation. [^{18}F]FDG, an analogue of glucose, is taken up by the same transporters that take up glucose into the cell; therefore, [^{18}F]-FDG uptake tracks cellular glucose transport, which is highly correlated to the rate of cellular glucose metabolism. Recent studies in animal models of neutrophilic lung inflammation, as well as in patients with inflammatory lung disease, indicate that increased [^{18}F]FDG uptake by the lungs correlates with the number of activated neutrophils recovered from the lungs. Therefore, the in vivo measurement of pulmonary glucose metabolism is a measure of neutrophil burden within the lungs. We propose that FDG-PET imaging can be used as a measurable biomarker in the development of drug therapies targeting lung inflammation.

Keywords: Lung inflammation; positron emission tomography; fluorodeoxyglucose; preclinical drug evaluation; drug evaluation

For drug development, biomarkers that identify toxicity or support efficacy can have a dramatic effect on go/no-go decisions for further development, on costs associated with development, and on the time to complete development. Such biomarkers are especially likely to be useful when therapeutic effects are difficult to measure, when long delays occur between exposure to the drug and the desired clinical effect, when a new therapy has a novel proposed therapeutic action, or when it would be useful to have estimates of the magnitude of potential therapeutic benefit before launching a large and expensive clinical trial.

Unfortunately, no consensus exists for an acceptable biomarker of the inflammatory response for lung diseases, despite the apparent importance of inflammation in the pathogenesis of such diverse conditions as acute pneumonia, acute respiratory distress syndrome, cystic fibrosis (CF), or chronic obstructive lung disease, among many others. Bronchoalveolar lavage (BAL) allows one to directly sample material from the lungs themselves, but because it is highly invasive, it is undesirable as an end point for most clinical trials of drug efficacy. Induced sputum[1,2] (a noninvasive alternative to BAL) is difficult to standardize, is often poorly tolerated by patients, and has never been shown to be sensitive to antiinflammatory drug effects. The forced expiratory volume in 1 s (FEV-1) is an important biomarker of pulmonary function but is certainly a nonspecific measure of airway inflammation, and in any case requires months (or longer) of observation to evaluate the effects of a new treatment. *Systemic* markers of inflammation are not an

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acceptable alternative, as they may not accurately reflect inflammatory responses within the lungs themselves, especially when such responses remain highly compartmentalized, as is often the case in pneumonia or CF.[3]

Accordingly, new tools are needed to measure lung inflammation efficiently, noninvasively, and quantitatively. The emergence of *molecular imaging* methods[4] offers an exciting new opportunity to develop and validate imaging biomarkers as adjuncts to diagnosis, tests of treatment efficacy, and treatment monitoring. Ideally, molecular imaging methods would be able to combine the attractiveness of noninvasive imaging with specificity for the inflammatory response afforded by analysis of material obtained from the lower airways.

PET as a Platform for Inflammation Imaging. While many cell types and mediators are required for a coordinated and effective inflammatory response, the vast majority of inflammation imaging methods[5] have focused so far on the neutrophil, especially the abnormal accumulation of neutrophils within an organ such as the lungs. For example, indium-111 labeling of blood leukocytes is one imaging method in which these cells are isolated from a blood sample obtained from a patient, incubated and labeled with indium-111, and then reinfused into the patient. A positive result relies on migration of the labeled cells to areas of active inflammation. Likewise, newer tracers utilizing murine antibodies or antibody fragments that bind specifically to cell-surface antigens on white blood cells may also be useful in identifying the presence of neutrophils.[6,7] These techniques employ either planar or single photon emission computed tomography (SPECT) imaging with standard γ camera systems. Qualitative image analysis may be useful for selected clinical applications but is not likely to be effective as a biomarker for drug development.

While SPECT can be used for quantitative imaging, several aspects make it less attractive than PET for this purpose. First, it is less sensitive than PET in detecting small changes in signal, a potentially significant problem when attempting to image processes such as pulmonary glucose uptake that inherently have low signal-to-noise ratios. Second, it has poorer spatial resolution, increasing the likelihood of partial volume artifacts that can degrade the accuracy of tissue activity estimates. Third, quantification with SPECT is more cumbersome because complicated attenuation correction procedures must be used. While newer hybrid SPECT and X-ray computed tomography (CT) systems may make such attenuation corrections easier to apply, these corrections will still be more complicated to apply when compared to PET.

Accordingly, in our opinion, positron emission tomography (PET) should still be considered the most accurate quantitative radionuclide imaging method currently available for imaging pulmonary inflammation. This accuracy derives primarily from coincidence detection of the high energy annihilation photons produced as a result of the positron decay process and the attenuation correction applied in PET image data processing. PET is also highly sensitive, requiring only nanomolar or femtomolar concentrations of tracer to generate a detectable signal without perturbing the metabolic processes being measured. While magnetic resonance imaging (MRI) can achieve much better spatial resolution, micromolar-level concentrations of contrast are required for an adequate signal to be detected and quantified. As a result of these features, inflammation imaging methods that use PET as a platform hold great promise as a noninvasive tool for quantifying the inflammatory response.

After compounds are labeled with positron-emitting isotopes, they are administered intravenously or inhalationally for lung imaging, and the tissue radioactivity concentration of the isotope is determined with the PET camera. Multiple two-dimensional image maps of the distribution of the radioactivity within the lungs are then reconstructed from the radioactivity data; in some cases, multiple images are obtained rapidly over a relatively brief period of time and quantified with appropriate mathematical compartmental models to represent a physiologic process (e.g., pulmonary perfusion or ventilation).

Recent advances in the scintillation materials used to make the radiation detectors in PET devices, and in the ability to transfer the scintillation light from the detectors to photomultiplier tubes,[8] now make it possible to manufacture devices with remarkable improvements in spatial resolution. These instrumentation advances have ushered in an era of "microimaging" that now make it possible to perform the same imaging studies in mice as well as humans. In addition, many modern devices combine CT capability with PET. Such multimodality imaging allows the information from high-resolution structural images to be merged with the molecular imaging of PET.

Increased Pulmonary Glucose Uptake as a Quantifiable Marker of Lung Inflammation. [^{18}F]Fluorodeoxyglucose ([^{18}F]FDG), the most widely used PET tracer in clinical practice, is a glucose analogue in which F-18 is substituted for the hydroxyl group at the second carbon position.[9,10] Entry into cells occurs via the same GLUT family of membrane transporters used by glucose, but unlike glucose, [^{18}F]FDG

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cannot be metabolized after it is phosphorylated by hexokinase. Thus, [^{18}F]FDG remains trapped after it is taken up by the cell. As [^{18}F]FDG accumulates in tissue cells, the concentration of radioactivity builds, eventually reaching a point at which it can be detected and quantified by an appropriately calibrated PET camera. Therefore, the uptake of [^{18}F]FDG reflects glucose uptake and is a useful proxy for cellular glucose metabolism.

[^{18}F]FDG has been shown to be useful in multiple studies to identify inflammatory processes.[9,11−15] However, to be optimally useful as a biomarker for drug development, quantitative measurements of [^{18}F]FDG uptake should be accurate, reproducible, and specific. In most organs (e.g., brain and heart), differences in the rate of [^{18}F]FDG uptake between different regions within the organ or among different individuals reflect differences in the rate of cellular glucose metabolism. The tissue density of the lungs, however, is highly variable, depending on the degree of regional aeration. Changes in tissue density occur as a result of both tissue destruction and infiltration by inflammatory cells. To date, most evidence currently indicates that differences in the rate of [^{18}F]FDG uptake over time or among individuals in the lungs are a function of both the number of inflammatory cells *and* their level of activation[16−18] (see below).

Most studies involving the use of [^{18}F]FDG suggest that increased uptake by the lungs is specific for neutrophilic infiltration. Animal studies evaluating [^{18}F]FDG uptake in models of pneumonia, pancreatitis-induced lung injury, and sepsis-induced lung injury,[19−21] as well as recent clinical studies,[22,23] not only show increased [^{18}F]FDG uptake by the lungs after the insult but also show by tissue autoradiography that [^{3}H]deoxyglucose ([^{3}H]DG) uptake was specifically limited to neutrophils. The autoradiography data from these animal studies also suggest that [^{3}H]DG uptake in neutrophils occurs prior to their entry into the alveolar space.[19,21] Additional in vitro data support the notion that increased deoxyglucose uptake is dependent on neutrophil priming and is not required for neutrophil functions such as the respiratory burst or degranulation.[24] Therefore, together, these studies suggest that increased deoxyglucose uptake occurs early in the process of neutrophil recruitment to the lungs, most likely as a result of neutrophil activation.

Data from our own studies support many of these conclusions. We first used canine models of oleic acid induced and low-dose intravenous endotoxin-modified acute lung injury to study the pulmonary uptake of [^{18}F]FDG.[17,25] Oleic acid causes disruption of the pulmonary vasculature resulting in pulmonary edema and protein leak, mimicking the pathophysiology of acute lung injury in humans.[26] Low-dose endotoxin alone causes temporary margination and retention of neutrophils in the pulmonary vasculature as a

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result of neutrophil activation without causing lung injury and can modulate the response to oleic acid, causing systemic hypotension and more severe hypoxemia.[27]

In our first set of studies, we hypothesized an increased rate of [^{18}F]FDG uptake by the lungs in the setting of acute lung injury. Indeed, we found that the rate of [^{18}F]FDG uptake, measured as the influx constant K_i using Patlak graphical analysis, by the lungs in animals exposed to endotoxin was 8 times higher than that seen in normal controls and 4 times higher than the uptake in animals exposed only to oleic acid.[17] The rate of [^{18}F]FDG uptake correlated with in vitro measurements of [^{3}H]DG uptake in neutrophils accessible by BAL. Interestingly, uptake was not significantly increased by oleic acid induced lung injury alone. Thus, these data, as proposed by others in the studies cited previously above, suggest that the increased rate of [^{18}F]-FDG uptake by the lungs in response to endotoxin was the result of neutrophil activation and sequestration even prior to migration out into the alveolar space.

Despite such data, these increases in lung [^{18}F]FDG uptake are not necessarily limited to neutrophils. In a recent study in mice, the increased [^{18}F]FDG uptake by the lungs in response to endotoxin was diminished only by ~50% in response to neutrophil depletion.[16] Furthermore, it is clearly established that significant increases in the lung uptake of [^{18}F]FDG can occur during inflammatory lung diseases not characterized by neutrophilic infiltration, such as sarcoidosis.[28,29] Nevertheless, the available evidence at this point strongly suggests that, in the appropriate experimental or clinical context (i.e., an acute inflammatory process), an increased rate of [^{18}F]FDG uptake is a highly reliable biomarker of the neutrophil burden within the lungs.

FDG-PET Imaging for Rapid Preclinical Assessment of Antiinflammatory Drugs. As further support for the proposition that FDG-PET imaging could be used to quantify antiinflammatory drug effects, we recently completed a study in mice using the anti-endotoxin antagonist E5564.[16] We demonstrated that the tissue-to-blood ratio (TBR) of [^{18}F]-FDG activity is highly correlated to the net rate of [^{18}F]FDG uptake as measured by the influx rate constant, K_i, obtained by Patlak graphical analysis.[30,31] Therefore, the TBR is a convenient ex vivo measurement of [^{18}F]FDG uptake that can be easily obtained in preclinical studies for rapid assessment of the efficacy of various antiinflammatory treatments and which can be correlated with imaging results. In our study, we found that E5564, a putative inhibitor of the TLR-4 receptor, effectively blocked endotoxin-induced increases in [^{18}F]FDG lung uptake as measured by the TBR (Figure 1). These data suggest that the TBR may be a useful adjunct to FDG-PET imaging in preclinical screening of new antiinflammatory drug therapies.

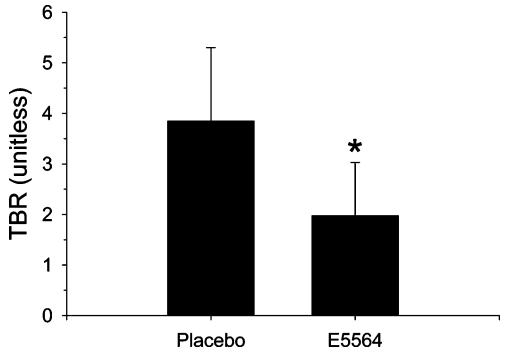

Figure 1. Effect of E5564 on lung uptake of [^{18}F]FDG in mice (measured as the tissue-to-plasma radioactivity ratio) after ip endotoxin administration compared with mice administered ip endotoxin alone (placebo) ($n = 10$ per group). * = $p < 0.05$ compared with the placebo group. Reprinted with permission from ref 16. Copyright 2005 American Physiological Society.

Clinical Studies Using FDG-PET Imaging To Quantify Pulmonary Inflammation. Jones et al. were the first to suggest that FDG-PET imaging could be used to study pulmonary inflammation.[18,32,33] In a study of patients with acute pneumonia, they found that the rate of [^{18}F]FDG uptake remained elevated even after neutrophils were no longer migrating into the alveolar space. While they interpreted this finding to indicate that the uptake of [^{18}F]FDG was a postmigratory event, other possibilities (such as cells other than neutrophils contributing to the imaging signal) could account for the same finding.[32]

Given the presumed important role of the neutrophil in the pathogenesis of cystic fibrosis (CF), and given the need for a suitable biomarker to quantify lung inflammation in this disease, we recently evaluated FDG-PET imaging as a biomarker of pulmonary inflammation in CF patients.[22] We studied 20 adult patients with stable CF with FDG-PET imaging; we also correlated the rate of [^{18}F]FDG uptake with neutrophil counts in the BAL in a subset of 7 patients.[22] The

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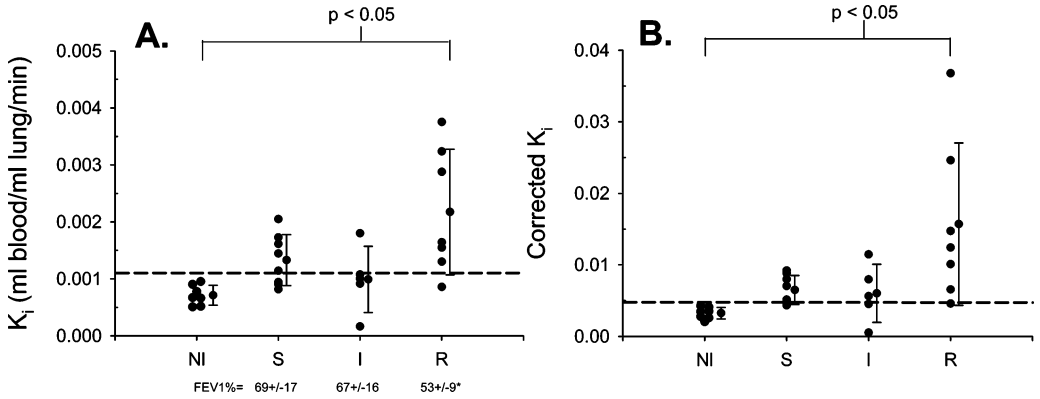

Figure 2. (A) Rate of [^{18}F]FDG uptake by the lungs, K_i, in healthy volunteers (NI) and in patients with cystic fibrosis, divided into stable (S), intermediate (I), and rapidly declining (R) groups based on changes in FEV-1 during the previous 4 years. Also shown are the mean and SD for each group, and the average % predicted FEV-1 in each group. The dashed line indicates 2 SD above the mean in the healthy volunteers. (B) Rate of [^{18}F]FDG uptake corrected for the volume of distribution, demonstrating similar results for all three groups when compared to K_i without the correction. Reprinted with permission from ref 22. Copyright 2006 American Thoracic Society.

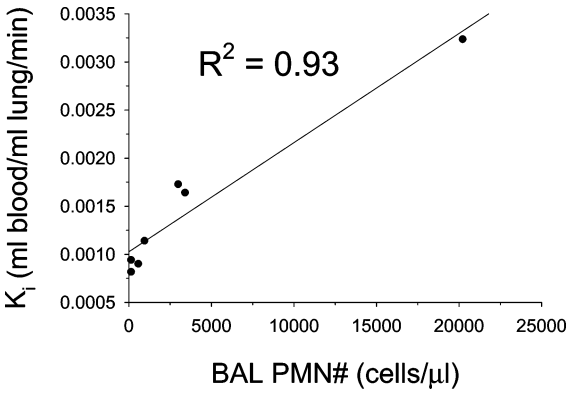

Figure 3. Correlation between neutrophil numbers in BAL fluid and K_i, as a measure of in vivo uptake of [^{18}F]FDG by PET. The R^2 without the "outlier" point with the highest cell count was still high ($R^2 = 0.95$). Reprinted with permission from ref 22. Copyright 2006 American Thoracic Society.

imaging results were grouped according to the patients' rate of decline in pulmonary function during the previous 4 years.[34] We found that the rate of [^{18}F]FDG uptake was higher in CF patients than in normal controls, and that the highest rates of [^{18}F]FDG uptake occurred in those patients with the most rapid decline in lung function (Figure 2). Additionally, we found that the imaging signal correlated strongly with neutrophil numbers in BAL (Figure 3). These results imply not only that the rate of [^{18}F]FDG uptake may be useful as a biomarker of active lung inflammation but also that it may predict the onset of more rapid deterioration in pulmonary function in CF patients.

Our findings in CF patients are different from results previously reported by Labiris et al.[35] in a smaller group of CF patients in whom no correlation to pulmonary function (FEV-1) was observed. However, the *rate* of decline in pulmonary function may be a better correlate than the absolute level of pulmonary function at any one time.[34] Thus, like Labiris et al., we failed to find a significant correlation between the pulmonary uptake of [^{18}F]FDG and the *absolute* value for FEV-1 or % predicted FEV-1, but we *did* find that significant differences in subgroups could be identified by FDG-PET imaging if segregated on the basis of their *rate of decline* in FEV-1.[22] Indeed, in retrospect, Dr. Labiris found a similar association between the FDG-PET imaging signal and the rate of decline in FEV-1 (personal communication).

Even if FDG-PET imaging could be used, however, to more efficiently study antiinflammatory drugs in patients with a variety of lung diseases, the cost and difficulties of implementing appropriate trials in these patient populations warrant a method that would allow investigators to reliably predict the effects of a putative treatment on pulmonary airway inflammation before undertaking a patient study. Previously, Taylor et al. suggested that FDG-PET imaging could be used to quantify focal inflammatory responses in the airways of the lungs induced by bronchoscopic deposition of grass pollen or other allergens.[36] More recently, investigators at the NIH[37] 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

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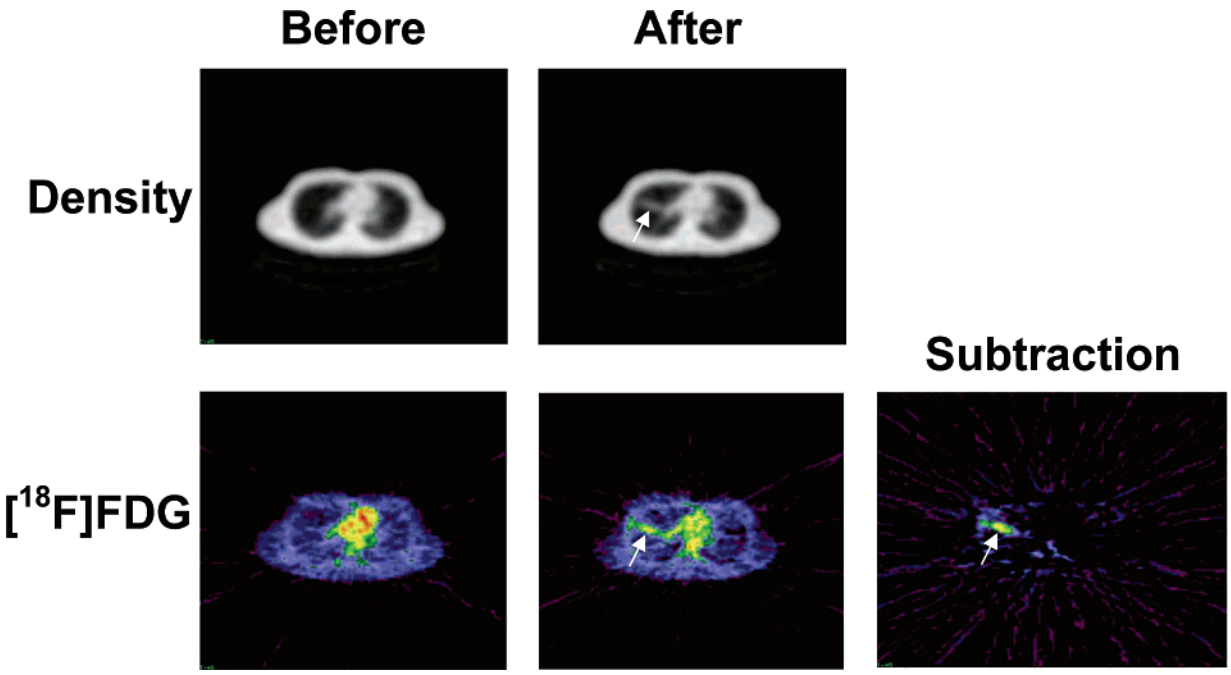

Figure 4. Transmission (top row) and [^{18}F]FDG (bottom row) images from one normal subject, before and 24 h after direct bronchial instillation of endotoxin (Etx) into the right middle lobe. An area of increased density (presumably due to inflammation) and [18F]FDG uptake are clearly visible (arrows). Also shown is a subtraction [18F]FDG image, in which activity of the "before" and "after" images is normalized to the same peak activity and the "before" image is subtracted from the "after" image. Reprinted with permission from ref 23. Copyright 2006 American Physiological Society.

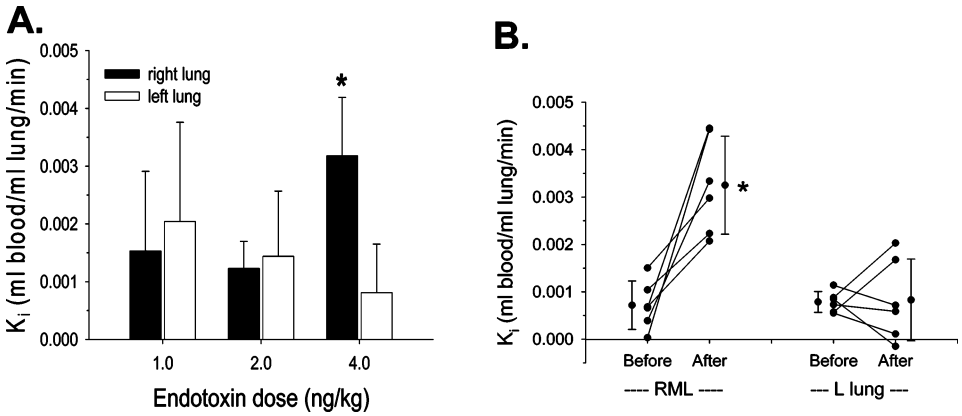

Figure 5. (A) Rate of [^{18}F]FDG uptake (expressed as the net uptake rate constant, K_i, in the lung segment of the right middle lobe (RML) affected by bronchial instillation of endotoxin (Etx) in each of the dosage groups ($n = 6$/group). The highest dose of Etx produced a significant increase in K_i compared with both lower doses of Etx. (B) Uptake of FDG by the lungs both before and after direct bronchial instillation of Etx into the RML of 6 normal volunteers. Note that, in every case, FDG uptake in the RML increases after Etx, while, on average, there is no significant change in a comparable region of interest on the contralateral, unaffected, left side. Reprinted with permission from ref 23. Copyright 2006 American Physiological Society.

normal humans. Neutrophil concentrations in the BAL fluid from lung segments challenged this way increased by 4-fold 24 h after endotoxin instillation, but were back to control values by 48 h. We surmised that this method could be used to screen antiinflammatory drugs for lung diseases characterized by neutrophilic inflammation, using FDG-PET imaging to quantify the antiinflammatory effect.

For our study, we recruited 18 healthy volunteers in a dose-escalation study[23] of 1 ng/kg, 2 ng/kg, and 4 ng/kg deposited into a segment of the right middle lobe. In the highest-dose group, imaging was performed both pre- and post-endotoxin. An example of the images obtained is shown in Figure 4. Figure 5 shows that the rate of [^{18}F]FDG uptake was higher in the 4 ng/kg dose group than in either of the other two dosing groups. Additionally, the rate of [^{18}F]FDG uptake was also higher in the affected lung regions of the right middle lobe than in control regions drawn on the contralateral unaffected left lung. Finally, the data also suggest that the measurements are reproducible in the absence of an intervention, since there was no significant difference in the rate of

Table 1. Comparison of Antiinflammatory Drug Testing Approaches in Sepsis and the Proposed Approach for Inflammatory Lung Diseases Such as CF

	sepsis	inflammatory lung disease
preclinical	ip[a] endotoxin in mice, cytokine biomarkers	ib[b] endotoxin, FDG-PET biomarker
phase I	iv[c] endotoxin, cytokine biomarkers	ib endotoxin, FDG-PET biomarker
phase II	sepsis patients, cytokine/physiologic biomarkers	lung disease patients, FDG-PET biomarker
phase III	sepsis patients, outcomes (death, ICU[d] LOS[e])	lung disease patients, outcomes (pulmonary or other function)

[a] Intraperitoneal. [b] Intrabronchial. [c] Intravenous. [d] Intensive care unit. [e] Length of stay.

[^{18}F]FDG uptake in the unaffected left lung between the baseline and post-treatment measurements in the highest dose group.

Correlating imaging data with the BAL data from our studies gives some information about the sensitivity of FDG-PET to detect inflammation. In healthy individuals, the concentration of neutrophils in BAL is approximately 10^3−10^4 cells/mL.[38] In our two human studies, which included stable patients with cystic fibrosis and healthy volunteers before and after the instillation of airway endotoxin, the rate of pulmonary [^{18}F]FDG uptake was increased in inflamed lungs compared to that in normal lungs, with the neutrophil concentrations in BAL ranging from 10^5 to 10^8 cells/mL.[22,23] Likewise, Konstan et al. reported that the mean concentration of neutrophils in BAL was approximately 10^6 cells/mL in patients with clinically mild cystic fibrosis. Therefore, this still limited set of data suggests that an increase in airway neutrophilia of 100 to 1000 times that measured in normal lungs will result in a detectable imaging signal and that this level of inflammation often corresponds to clinically mild disease.[39]

FDG-PET Imaging as a Biomarker in Phase I Clinical Trial Evaluations of Antiinflammatory Drugs. Altogether, these various studies suggest the possibility of a new testing paradigm (Table 1). Such a strategy would be analogous to the current standard practice of first testing the effects of potentially useful immunomodulatory drugs for sepsis in normal volunteers after the *intravenous* infusion of small doses of endotoxin before embarking upon a study in patients.[40−44] Thus, in studies relevant to inflammatory lung disease, novel therapies would first be tested in appropriate animal models using FDG-PET imaging as a biomarker of the inflammatory response. Promising candidate drugs would then be tested in normal human volunteers after bronchial instillation of endotoxin and then screened in a small group of actual patients. In each case, FDG-PET imaging would be used as an important biomarker of the inflammatory response. Drugs that showed efficacy in these studies could then be moved along to large multicenter phase III studies to demonstrate improvements in clinical outcomes such as overall pulmonary function. Thus, use of FDG-PET imaging as a biomarker of the inflammatory response has the potential to significantly alter the way novel drugs are developed and evaluated for patients with cystic fibrosis or other lung diseases characterized by neutrophilic inflammation.

Future Directions. Until recently, PET images, such as those shown in Figure 5, have been obtained without the benefit of direct correlation with high-resolution anatomic imaging. With the development of combination PET/CT scanners, it may now be possible to provide better anatomic correlation for areas of mild pulmonary inflammation. However, exact coregistration would require respiratory gating during the PET data acquisition to eliminate the effects of respiratory motion, and at present, this feature is not readily available in most current PET/CT scanners. The development of new PET tracers for imaging inflammation may provide additional information about other components of the inflammatory response. For instance, [^{11}C]-PK11195 is a compound that binds to peripheral benzodiazepine receptors, which are expressed on macrophages when activated. Preliminary studies in animal models of lung inflammation demonstrate that [^{11}C]PK11195 can be used to image the macrophage response.[45,46] Jones et al. also combined [^{18}F]FDG and [^{11}C]PK11195 PET imaging

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in patients with chronic obstructive pulmonary disease and asthma and showed that it may be possible to differentiate in vivo the neutrophilic and macrophage components of the inflammatory response.[45−48] Jones et al. have also demonstrated that [^{11}C]proline may be useful in imaging active pulmonary fibrosis, a sometimes devastating consequence of severe or prolonged inflammation in the lungs.[48]

In conclusion, PET imaging may not only be useful as a quantifiable biomarker to evaluate new antiinflammatory agents in drug development but may also offer novel ways of noninvasively studying different components of the inflammatory response in animal models of lung disease and in patients. As instrumentation continues to improve, and as new tracers are developed, we anticipate many more studies that will better characterize the pulmonary inflammatory process in vivo through imaging.

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