Regulation of lipopolysaccharide-induced increases in neutrophil glucose uptake

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Schuster DP, Brody SL, Zhou Z, Bernstein M, Arch R, Link D, Mueckler M. Regulation of lipopolysaccharide-induced increases in neutrophil glucose uptake. Am J Physiol Lung Cell Mol Physiol 292: L845–L851, 2007.—The pathogenesis of many lung diseases involves neutrophil inflammation. Neutrophil functions, in turn, are critically dependent on glucose uptake and glycolysis to supply the necessary energy to meet these functions. In this study, we determined the effects of p38 mitogen-activated protein kinase and hypoxia-inducible factor (HIF)-1, as well as their potential interaction, on the expression of membrane glucose transporters and on glucose uptake in murine neutrophils. Neutrophils were harvested and purified from C37BL/6 mice and stimulated with lipopolysaccharide (LPS) in the presence or absence of specific p38 and HIF-1 inhibitors. Glucose uptake was measured as the rate of [3H]deoxyglucose (DG) uptake. We identified GLUT-1 in mouse neutrophils, but neither GLUT-3 nor GLUT-4 were detected using Western blot analysis, even after LPS stimulation. LPS stimulation did not increase GLUT-1 protein levels but did cause translocation of GLUT-1 from the cell interior to the cell surface, together with a dose-dependent increase in [3H]DG uptake, indicating that glucose uptake is regulated in these cells. LPS also activated both p38 and the HIF-1 pathway. Inhibitors of p38 and HIF-1 blocked GLUT-1 translocation and [3H]DG uptake. These data suggest that LPS-induced increases in neutrophil glucose uptake are mediated by GLUT-1 translocation to the cell surface in response to sequential activation of neutrophil p38 and HIF-1α in neutrophils. Given that neutrophil function and glucose metabolism are closely linked, control of the latter may represent a new target to ameliorate the deleterious effects of neutrophils on the lungs.

deoxyglucose; GLUT-1; p38; hypoxia-inducible factor-1

NEUTROPHILIC INFLAMMATION is a root cause of many lung diseases, including pneumonia, acute lung injury (ALI), acute respiratory distress syndrome (ARDS), cystic fibrosis (CF), and bronchiolitis obliterans syndrome (BOS), among others (38, 41, 45, 46). In each case, inflammatory stimuli initiate the recruitment of neutrophils to the lungs, where they can cause or contribute to lung tissue injury via increased production of reactive oxygen species and the release of preformed proteases. Quantifying neutrophilic inflammation during lung disease, as a biomarker of anti-inflammatory drug effects or prognosis, remains a major challenge in patients with lung disease. Non-invasive imaging with positron emission tomography (PET) and the fluorine-18-labeled glucose analog fluorodeoxyglucose ([18F]FDG) has been proposed as a valuable means of monitoring lung inflammation (3, 6, 14, 26–31, 33, 34, 39, 44, 48–50, 55). Recently, we have shown that changes in pulmonary glucose uptake can be monitored and quantified with FDG-PET imaging in mouse and canine models of lung injury, in a human model of focal pulmonary inflammation, and in patients with CF (8–10, 56, 57).

Although neutrophils may not be solely responsible for the increased signal observed during FDG-PET imaging of lung inflammation, we (56) and others (23, 24, 28, 30, 31) have repeatedly shown that activated neutrophils recruited to the lungs are nevertheless the dominant cell type. However, little is known about mechanisms regulating neutrophil glucose metabolism during pulmonary inflammation. If FDG-PET imaging is to be employed effectively as a new biomarker, it will be important to understand the mechanisms that drive tissue accumulation of the radiolabeled glucose analog during various states of lung inflammation.

Glucose uptake is facilitated by the GLUT family of membrane transporters. Although 14 isoforms have been identified in the human genome, glucose uptake per se is facilitated by GLUT-1, GLUT-3, and GLUT-4 in various tissues. GLUT-1 is constitutively expressed in many tissues, but LPS increases glucose uptake and GLUT-1 mRNA and protein levels in murine macrophages (18, 20). Whether or not a similar effect occurs in neutrophils has not been reported.

The mechanism(s) by which LPS might exert its effects on glucose uptake in neutrophils or other phagocytes is unknown. A possible candidate is the transcription factor hypoxia-inducible factor (HIF)-1, which is known to be a potent inducer of “hypoxia-responsive genes” [including glut-1 (21)]. HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β. Ordinarily, the HIF-1α chain is minimally detectable, if at all, under normoxic conditions, because it is rapidly ubiquitinated and degraded in the proteosome. With hypoxia, HIF-1α is stabilized and the HIF-1α/β complex can translocate to the nucleus. In addition to hypoxia, however, inflammatory stimuli like LPS have been reported to stabilize HIF-1α even under normoxic conditions (36). Although HIF-1α has recently been detected in neutrophils during hypoxia (53), and the importance of HIF-1α in regulating cell function in neutrophils and other myeloid cells has been demonstrated by targeted interruption

1 The formal gene symbol for the family of glucose transport facilitators (protein symbol GLUT) is SLC2A, but we will, as have others, use the lowercase italicized form “glut” to indicate the gene and the uppercase form for the protein.

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of the HIF-1α gene (13), its role in mediating glucose transport in neutrophils has not been studied.

Finally, any effect that LPS may have on the HIF-1 pathway will require one or more intermediate signaling molecules. Although TNF-α would be an obvious candidate, previous studies by our group have shown no abrogation of the LPS effect on glucose uptake by TNF-α blockade (56). An alternative is the mitogen-activated protein kinase (MAPK) p38, a member of a family of stress-activated protein kinases transducing extracellular signals into intracellular responses that has been implicated in the recruitment of neutrophils to sites of inflammation as well as their subsequent activation (15, 25). The HIF-1 pathway can be activated by p38 (16, 47), but once again, the interaction of these molecules in neutrophils has not been studied previously. Thus the purpose of the present investigation was to determine the role of p38 and HIF-1, as well as their potential interaction, on GLUT-1 expression and glucose uptake in murine neutrophils.

METHODS

Reagents. All experiments were approved by the Animal Studies Committee of Washington University in St. Louis. Wild-type C57BL/6 mice, 8–11 wk old, were used in these studies. LPS (lipopolysaccharide from Escherichia coli 055:B5) was obtained from Sigma (St. Louis, MO). The p38 MAPK inhibitors SB-203580 and SB-202190 (2), which inhibit p38 by binding to the ATP site (37), were obtained from EMD Biosciences (Calbiochem). The HIF-1α inhibitors NSC-134754 and NSC-643735 (7) were obtained from the National Cancer Institute through their Developmental Therapeutic Program. Both compounds inhibit HIF-1 activation due to deoxyerumine mesylate, but only the former inhibits activation by hypoxia. Antibodies to the membrane glucose transporters GLUT-1 and GLUT-4 were generated by members of our group (M. Mueckler) and have been fully characterized for use in Western analyses (22). An antibody to GLUT-3 was generously provided by Dr. Sherin Devaskar (David Geffen School of Medicine, UCLA, CA). An antibody to the body. Confocal microscopy was performed using a Zeiss LSM 510 laser scanning microscope with a ×63 oil-immersion planapocromat objective. For quantitation, slides were prepared and coded so that the grader did not know the experimental condition under which the neutrophils were obtained. Samples from the different preparations were evaluated at the same time under identical conditions of laser brightness and other instrument settings. Single optical sections (0.3-μm thickness) taken tangentially to the cell surface were collected and saved as computer image files from the middle of ~50 cells/slide. The percentage of the total cells examined showing any evidence for GLUT-1 in the cell membrane (ring staining) was recorded.

Statistical analysis. Group data are expressed as means ± SD. Statistical significance was set at \( P < 0.05 \). The SigmaStat 3.1 program (Systat Software) was used for these calculations.

RESULTS

Dose response to LPS. Mice were administered intraperitoneal LPS in doses ranging from 0.2 to 20 μg/g body wt. These doses have previously been shown to produce dose-related increases in mortality during oleic acid-induced acute lung injury in mice and are associated with increased \([18F]FDG\) uptake in murine neutrophils.
upregulation by the lungs (56). One hour after LPS administration, cells were harvested from bone marrow and incubated with [3H]DG. Figure 1A shows that glucose uptake (measured as the rate of [3H]DG uptake) in these cells increased in a dose-dependent fashion.

In separate studies, neutrophils were harvested after Percoll purification from the bone marrow of mice and then incubated with varying concentrations of LPS (Fig. 1B). Again, glucose uptake increased progressively in response to increasing concentrations of LPS in the incubation medium. Since these data are normalized to cell protein concentrations, they suggest that glucose uptake per cell was increasing in a concentration-dependent manner in response to LPS. These data, then, demonstrate that neutrophil glucose uptake can be modified by inflammatory stimuli such as LPS and that glucose uptake is likely a regulated phenomenon.

Effects of LPS on GLUT-1. GLUT-1 was detected in neutrophils by Western analysis (Fig. 2), but an increase in protein levels in response to LPS incubation was not detected even after 24 h. Neither GLUT-3 nor GLUT-4 was detected in either unstimulated or LPS-stimulated neutrophils (data not shown).

Subsequently, we used confocal laser scanning microscopy to define the intracellular localization of GLUT-1 in the presence or absence of LPS (Fig. 3). In neutrophils without exposure to LPS, GLUT-1 immunofluorescence was observed throughout the cell cytoplasm. However, in cells incubated with LPS, GLUT-1 consistently redistributed toward the cell surface.

Effect of p38 MAPK inhibition on neutrophil glucose uptake. Also as reported by others (1), we found that LPS caused significant phosphorylation (activation) of p38 (Fig. 4). To test whether the p38 activation has an effect on glucose uptake, neutrophils were incubated with specific p38 inhibitors and stimulated with LPS. The expected LPS-induced increase in glucose uptake was absent when neutrophils were first incubated with the specific p38 inhibitors SB-202190 or SB-203580 (Fig. 5). Incubation with the inhibitors themselves had no significant effect on glucose uptake. Since these p38 inhibitors act as competitive inhibitors of ATP binding, p38 protein levels in neutrophils were not affected (data not shown). However, translocation of GLUT-1 to the plasma membrane was abrogated by the p38 inhibitor SB-203580 (Fig. 3).

Effect of HIF-1α inhibition on neutrophil glucose uptake. Incubation of neutrophils with LPS also increased the concentration of the transcription factor HIF-1α (Fig. 6). The expected LPS-induced increase in glucose uptake was significantly but not completely blocked by the HIF-1α inhibitor NSC-134754 but not at all by NSC-643735 (Fig. 7). The former, but not the latter, inhibitor has previously been shown to inhibit HIF-1α activation by hypoxia (7). Neither HIF-1α inhibitor had a significant intrinsic effect on glucose uptake (Fig. 7).

As with p38 inhibition, translocation of GLUT-1 was consistently blocked by the HIF-1α inhibitor NSC-134754 (Fig. 3). However, whereas HIF-1α inhibition had no effect on activated p38 protein levels by Western analysis (data not shown), p38 inhibition significantly decreased HIF-1α expression (Fig. 6), suggesting that HIF-1α acts downstream from p38.

DISCUSSION

The main new findings of this study are 1) LPS stimulation of mouse neutrophils causes a dose-dependent increase in [3H]DG uptake, suggesting that glucose uptake is regulated in

Fig. 1. Effect of LPS on the rate of [3H]deoxyglucose (DG) uptake in mouse neutrophils harvested and purified from mouse bone marrow femurs after intraperitoneal exposure to increasing doses of LPS (A) or after bone marrow neutrophils were harvested and then incubated with increasing concentrations of LPS (B). *P < 0.05 compared with no LPS. +P < 0.05 compared with the lowest dose or concentration of LPS. #P < 0.05 compared with 100 ng/ml LPS. n = 4–5 per group.

Fig. 2. A: Western blot of glucose transporter GLUT-1 from mouse neutrophils. Twenty-five micrograms of total protein were loaded per lane. For the first 3 lanes, neutrophils were harvested from the femur marrow of mice and incubated for 1 h in Opti-MEM without LPS (quiescent, Q) or in the presence of LPS (1 μg/ml) for 1 or 7 h before being solubilized in protein extraction buffer. For the fourth lane, neutrophils were obtained via peritoneal lavage 24 h after intraperitoneal LPS (20 μg/g). Blots were probed with anti-GLUT-1 antibody and then stripped and reprobed for β-actin. B: densitometric evaluation of the Western blots. n = 3 per group.
these cells; 2) GLUT-1 appears to be the dominant, and perhaps the only, glucose transporter expressed in mouse neutrophils; 3) LPS-induced increases in neutrophil glucose uptake are associated with translocation of the glucose transporter GLUT-1 from the cell interior to the cell surface, rather than increased GLUT-1 protein levels; 4) both p38 and HIF-1α inhibitors block [3H]DG uptake and the translocation of GLUT-1; and 5) p38 inhibition is associated with reduced levels of HIF-1α. Altogether, these results suggest that LPS acts through p38 to stabilize HIF-1α which in turn mediates GLUT-1 translocation, allowing increased glucose uptake. In this way, neutrophils can increase their energy supply for such functions as chemotaxis or the respiratory burst.

The mechanisms underlying the control of glucose uptake in neutrophils are fundamentally important if changes in imaging signal during FDG-PET imaging of lung inflammation are to be understood. Previous studies show that parenterally administered LPS causes increased [18F]FDG uptake in the lungs of both mice and dogs and that this increase is related to neutrophil numbers recovered from bronchoalveolar lavage (12, 56). Furthermore, data from dogs (12) suggest that neutrophils must first be activated, for instance by LPS, before glucose uptake is increased, because neutrophils that penetrate into the airspaces during ALI do not show increased uptake in the absence of LPS stimulation. As shown in Fig. 1 of the current study, increases in glucose uptake are dose and concentration dependent in vivo and in vitro, respectively. These findings support the idea that glucose uptake in neutrophils is regulated and that not only the number of neutrophils but also their state of activation ultimately contribute to the signal detected by FDG-PET imaging.

p38 MAPK, HIF-1α, and GLUT-1 all are expressed in neutrophils. Our conclusion that LPS acts through p38 and HIF-1α to mediate increases in glucose uptake via GLUT-1 translocation depends of course on showing that these key molecules are expressed in neutrophils. Jones et al. (31) suggested that increased [18F]FDG uptake by neutrophils is by and large due to priming from stimuli like TNF-α (32). However, in a previous study (56), we found that TNF-α inhibition did not affect LPS-induced increases in lung glucose uptake, and studies in TNF-α receptor-deficient mice confirmed these observations (56). On the other hand, p38 is well known as a stress-activated MAPK that transduces extracellular signals into intracellular responses; it has been implicated in the recruitment of neutrophils to sites of inflammation as well as their subsequent activation (15, 25). p38 expression and activation by LPS in neutrophils has been documented previously by others (1) and is corroborated now (Fig. 2).

HIF-1α, on the other hand, has only recently been detected in neutrophils when stabilized by hypoxia (53). As shown in Fig. 6, we now demonstrate that inflammatory signals like LPS also are capable of stabilizing the concentration of this mole-
creases in glucose uptake.

GLUT-1, but this effect must be mediated by

those regulating glucose transport and glycolysis (13, 42).

Least well studied in neutrophils are the various GLUT
isoforms. We found that GLUT-1, but not GLUT-3 or
GLUT-4, is expressed in these cells (Fig. 2) even after LPS
stimulation. Furthermore, GLUT-1 protein levels failed to
change in response to LPS stimulation, either in vitro or in vivo
(Fig. 2). Thus neutrophils possess the necessary machinery to
link LPS stimulation with increased glucose uptake (e.g., Figs.
1B, 5, and 7) via GLUT-1, but this effect must be mediated by
a mechanism other than increased GLUT-1 transcription.

p38 and HIF-1 activation are linked to increased glucose
uptake. The importance of both p38 and HIF-1 in mediating
changes in neutrophil glucose uptake was demonstrated in this
study by the use of small molecule inhibitors. LPS-induced
increases in neutrophil glucose uptake were completely
blocked by two well-characterized inhibitors of p38 (Fig. 5).
Likewise, the recently identified HIF-1 inhibitor NSC-134754
(7) also significantly reduced changes in glucose uptake in
response to LPS (Fig. 7). A second inhibitor of HIF-1, how-
ever, failed to alter changes in glucose uptake after LPS.
Interestingly, this pattern of inhibition also has been reported
previously (7). Both inhibitors block HIF-1 activity and
HIF-1α protein expression induced by deferoxamine, but only
NSC-134754 (the compound used in the current study) inhib-
hited HIF-1 activity and HIF-1α protein expression and in-
creased GLUT-1 expression in response to hypoxia (7). To-
gether, these data suggest that HIF-1, like p38, regulates
LPS-induced increases in glucose uptake in mouse neutrophils
in response to LPS and that LPS affects HIF-1 via the same
pathway as hypoxic stimulation.

p38 and HIF-1 mediate GLUT-1 translocation in response
to LPS. Even without a significant change in protein levels,
changes in the cellular distribution of GLUT-1 or in its effi-
ciency as a transporter could facilitate glucose uptake after LPS
stimulation. In Fig. 3, we show that GLUT-1 translocated from
the cytoplasm to the cell membrane after incubation with LPS.
Malide et al. (40) made similar observations about the cellular
distribution of GLUT-1 in human macrophages stimulated in
vitro by phorbol myristate acetate (PMA) or N-formyl-methionyl-
leucyl-phenylalanine (fMLP). Furthermore, as shown in Fig. 3,
the effects of the p38 and HIF-1α inhibitors on glucose uptake
are mirrored by their effects on GLUT-1 translocation; i.e.,
LPS failed to cause a redistribution of GLUT-1 when neutro-
phils were preincubated with the p38 inhibitor SB-203580 or
the HIF-1α inhibitor NSC-134754.

p38 vs. HIF-1 inhibition of glucose uptake. As shown in
Figs. 5 and 7, p38 inhibition completely blocked LPS-induced
increases in neutrophil glucose uptake, whereas HIF-1 inhibi-
tion had a significant but lesser effect. Its possible that the
reduced effect of HIF-1α inhibition compared with p38 inhibi-
tion could simply represent an inadequate concentration of
the HIF-1 inhibitor, but higher concentrations of the HIF
inhibitors were precluded because they were toxic to neutro-
phils in culture. However, there are other, intriguing possi-
bilities. For instance, studies by others show that p38 MAP kinase
inhibition can prevent insulin-stimulated glucose uptake but
not basal uptake or insulin-stimulated GLUT-4 translocation of
the transporter to the cell surface in 3T3-L1 adipocytes and L6

HIF-1α

Heavy chain

Fig. 5. Effect of the p38 MAPK inhibitors on [3H]DG uptake in mouse
neutrophils in response to LPS (1 μg/ml). The second bar demonstrates that
incubation with the drug vehicle DMSO had no intrinsic effect on the rate of
[3H]DG uptake. Both p38 inhibitors completely blocked LPS-induced in-
creases in glucose uptake. n = 5 per group. *P < 0.05 compared with means
of other groups.

Fig. 6. Effect of LPS on HIF-1α stabilization in neutrophils as determined by
Western analysis after immunoprecipitation. Representative example (from
n = 3) of Western blots. Note that in the presence of the p38 MAPK inhibitor
SB-203580, HIF-1α levels were markedly reduced, despite stimulation with
LPS.

Fig. 7. Effect of HIF-1α inhibitors on the rate of [3H]DG uptake in mouse
neutrophils in response to LPS (1 μg/ml). The second bar demonstrates that
incubation with the drug vehicle DMSO had no intrinsic effect on the rate of
[3H]DG uptake. Only NSC-134754 decreased the rate of [3H]DG uptake (*P <
0.05 compared with both control and LPS + NSC-134754 conditions; +P <
0.05 compared with control) in response to LPS exposure. This result is
consistent with the failure of NSC-643735 to inhibit HIF-1α stabilization in
response to hypoxia but not to deferoxamine (see text). n = 5 per group.
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muscle cells (19, 51), suggesting that the primary effect of p38 in these systems is to phosphorylate and activate the transporters once they become inserted into the cell membrane. Our data (Fig. 5) could be interpreted similarly. Furthermore, even though HIF-1 inhibition of glucose uptake was incomplete (Fig. 7), both p38 and HIF-1 inhibition were able to block GLUT-1 translocation (Fig. 3). Thus one explanation for the disparate effects of p38 inhibition vs. HIF-1 inhibition on glucose uptake is that the p38 inhibitors blocked both p38-induced translocation and an increase in the intrinsic activity of GLUT-1, whereas HIF-1 inhibition only blocked translocation. Such a mechanism would be consistent with a report by Tan et al. (52) showing a reduced Km for glucose uptake in human neutrophils in response to incubation with either fMLP or PMA.

Another possibility is that p38 inhibitors block the effects of LPS on GLUT isoforms other than GLUT-1, whereas HIF-1 only affects GLUT-1. Although GLUT-1, GLUT-3, and GLUT-4 have been reported to be present in human granulocytes and GLUT1 and GLUT-3 have been identified in human monocytes and lymphocytes (17, 35), only GLUT-1 has been reported in mouse macrophages (4, 18). We identified GLUT-1 in mouse neutrophils (Fig. 2), but not GLUT3 or GLUT4, even after LPS stimulation.

Finally, it will be important to investigate in future studies whether the results described after LPS stimulation also are observed after neutrophils are activated in other ways, e.g., by tissue injury in the absence of LPS.

In summary, the data in this study suggest that LPS-induced increases in neutrophil glucose uptake are mediated by GLUT-1 translocation to the cell surface in response to sequential activation of neutrophil p38 and HIF-1α. In addition, p38 also may affect the intrinsic activity of this glucose transporter. Given that neutrophil function is closely linked to glycolysis, the control of neutrophil metabolism may represent a new target to manipulate the deleterious effects of neutrophils in a variety of clinical circumstances. An improved understanding of how neutrophil metabolism is regulated also will be important to interpret FDG-PET imaging when used to quantify lung inflammation or to evaluate the effects of novel anti-inflammatory agents (11).

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