CD11b and Intercellular Adhesion Molecule-1 Are Involved in Pulmonary Neutrophil Recruitment in Lipopolysaccharide-Induced Airway Disease

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To better define the roles of CD11b, CD11a, and one of their endothelial cell receptors, intercellular adhesion molecule-1 (ICAM-1), in the lower respiratory tract inflammatory response to inhaled lipopolysaccharide (LPS), we evaluated the physiologic and biologic response to inhaled LPS in mice receiving anti-CD11b antibody, anti-CD11a antibody, and anti–ICAM-1 antibody. Mice receiving anti-CD11b antibody had a dramatic reduction in pulmonary neutrophil recruitment compared with control mice (18,300 versus 143,000 cells/ml, and neutrophils 16.7% versus 77%), whereas mice receiving anti-CD11a antibody did not demonstrate a reduction in lavage cellularity. Mice receiving anti–ICAM-1 antibody also demonstrated a dose-dependent reduction in inflammatory cell recruitment to the alveolar space. Despite the significant reduction in inflammatory cell infiltrate in mice receiving either CD11b or ICAM-1 antibodies, there was no reduction in the development of airway hyperreactivity. These findings suggest that CD11b and ICAM-1 are important mediators of LPS-induced airway inflammation, but do not appear to be critical to the development of LPS-induced airway hyperreactivity.

Endotoxin or lipopolysaccharide (LPS) has been implicated in the pathogenesis of multiple forms of airway disease. The role of endotoxin in airway disease has been most thoroughly studied in the setting of occupational exposure to organic dusts, where the concentration of endotoxin in the bioaerosol is associated with the development and progression of airway disease (1, 2). Exposure to organic dusts causes a variety of acute respiratory problems, including wheezing, dyspnea, and decreased airflow (3). More recently, a role for endotoxin in the pathogenesis of asthma has been suggested by the relationship between endotoxin concentration in the domestic environment and its correlation to the clinical severity of asthma (4) and to the development of wheezing in infants (5). Recent studies have also shown that particulate matter from air pollution, which is strongly associated with the progression of airway disease (6), is contaminated with endotoxin (7). These findings suggest a potential role for endotoxin in air pollution–initiated airway disease.

Inhalation of endotoxin experimentally leads to rapid recruitment of neutrophils to the lower respiratory tract (8), accompanied by the development of airflow obstruction and airway hyperreactivity (AHR). In the traditional paradigm of airway inflammation, neutrophil recruitment has been causally linked to the development of increased airway resistance. The components of the pulmonary response to inhaled endotoxin have been studied using inhalation studies in humans (8, 9) and mice (8, 10, 11). The lower respiratory tract inflammatory response also includes production and release of proinflammatory cytokines (interleukin [IL]-1β, tumor necrosis factor [TNF]-α, and IL-6) and chemokines (IL-8 and macrophage inflammatory protein [MIP]-2) for up to 48 h. This cytokine response has been viewed as pivotal to the initiation and propagation of the neutrophil recruitment component; however, we have recently demonstrated that TNF-α and IL-1β may not be essential to the initiation of this process, but are produced and released as part of the generalized inflammatory response (12).

These findings suggest that mediators other than TNF-α or IL-1β govern the movement of the neutrophil from the vasculature to the airspace. The process of neutrophil emigration from the systemic circulation has been fairly well defined and occurs in a series of distinct phases, during which multiple families of leukocyte and endothelial cell adhesion molecules actively move the neutrophil from the postcapillary venules to the site of inflammation (13, 14). The role of each of these families of molecules has not been clearly defined in the pulmonary circulation, where neutrophil recruitment takes place from the pulmonary capillaries rather than the postcapillary venules. The importance of this difference in vessel type is that leukocytes in the pulmonary bed are already in prolonged contact with the endothelial cells as they pass through the narrower capillaries, whereas in postcapillary venules, the leukocyte must be attracted from the flowing stream of cells to the wall of the vessel, to establish contact with endothelial cells (15).

In addition, although the requirement for CD18 for neutrophil recruitment to systemic sites of inflammation has been demonstrated with a number of animal models (16, 17), pulmonary neutrophil recruitment has been distinguished from many models of systemic inflammation by the existence of both CD18-dependent and CD18-independent migration pathways. The goal of the current investigation was to specifically address the role of two of the β_2 integrins, CD11a/CD18 and CD11b/CD18, in pulmonary neutrophil recruitment in response to inhaled LPS, using function-blocking antibodies to the α chains of these molecules. Intercellular adhesion molecule-1 (ICAM-1), a primary endothelial receptor for the β_2 integrins, was concurrently studied. We demonstrate that use of a monoclonal antibody to ICAM-1 and an antibody to CD11b led to a dramatic reduction in pulmonary neutrophil recruit-

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Abbreviations: airway hyperresponsiveness, AHR; enzyme-linked immunosorbent assay, ELISA; intercellular adhesion molecule-1, ICAM-1; interleukin, IL; lipopolysaccharide, LPS; macrophage inflammatory protein, MIP; tumor necrosis factor, TNF.

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ment after aerosolized LPS exposure, but had no effect on the development of bronchial hyperreactivity. Antibody to CD11a did not diminish pulmonary neutrophil recruitment or affect AHR under the same conditions.

Materials and Methods

General Protocol

Pulmonary function testing was performed 1 d before and immediately after a 4-h inhalation challenge with LPS. In a separate group of mice, the lower respiratory tract inflammatory response to inhaled LPS was evaluated by whole lung lavage, again immediately following the LPS inhalation challenge. The inflammatory response was analyzed by comparing alterations in cellularity and cytokine levels (TNF-α, IL-1β, and MIP-2) in whole lung lavage fluid.

Mice

C57BL/6J (CD11a and CD11b experiments) and C3H/HeBFeJ (ICAM-1 experiments) mice, 6–10 wk old, were purchased from Jackson Laboratories (Bar Harbor, ME). All animals had free access to food and water except during the 4-h endotoxin exposure period. Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Iowa.

Monoclonal Antibodies

Mice were injected intravenously with anti-CD11b at a dose of 130 μg (Clone M1/70), anti-CD11a at a dose of 130 μg (clone M17), just before exposure, or anti–ICAM-1 at doses of 100 μg or 150 μg (Clone 3EL; PharMingen, La Jolla, CA), 1 h before exposure to aerosolized LPS. Dosing was based on previous studies using the same antibodies (18–21). Control mice were injected with the same dose of rat or hamster IgG, the isotype matched control.

LPS Inhalation Challenge

Exposures were performed in a 20-liter exposure chamber with a Collison nebulizer (CH Technologies, Westwood, NJ) delivering the aerosolized LPS. The mass median aerodynamic diameter of particles produced using this system is 1.4 μm, which results in an aerosol distribution with ~ 4% of particles reaching the alveolar region. The LPS concentrations generated by the aerosols during the exposure period were assayed using the chromogenic *Limulus* amoebocyte lysate assay (QCL-1000; BioWhittaker, Inc., Walkersville, MD) with sterile pyrogen-free labware and a temperature-controlled microplate block and microplate reader (405 nm), as previously described (3). Briefly, four separate samples were taken during each 4-h exposure period by drawing air from the exposure chamber through 47-mm binder-free glass microfiber filters (EPM-2000; Whatman, Mainstone, UK) held within a 47-mm stainless in-line air sampling filter holder (Gelman Sciences; Ann Arbor, MI). LPS was extracted from the filters with pyrogen-free water at room temperature with gentle shaking. The extracts were then serially diluted and assayed for endotoxin. Endotoxin concentrations ranged from 2.6–3.8 $\mu g/m^3$. This concentration of endotoxin was chosen because it represents a sufficient challenge of LPS that does not result in a maximal inflammatory response (10).

Assessment of Pulmonary Function

Mice were placed in 80 ml whole body plethysmographs (Buxco Electronics, Inc.; Troy, NY) ventilated by bias airflow at 0.2 liters/min. This unit was interfaced with differential pressure transducers, analog-to-digital converters, and a computer. The breathing patterns and pulmonary functions of each individual mouse were monitored over time. Direct measurements were made of respiratory rate, pressure change within the plethysmograph, and "box flow," which is the difference between the animal's nasal airflow and the flow induced by thoracic movement; this difference varies in the presence of airflow obstruction because of pulmonary compression (due to forced expirations). The Buxco system measures both the magnitude of the box pressure variations and the slope of the box pressure. Airway resistance is estimated by exposing mice to increasing doses of methacholine and recording the enhanced pause pressure (P_{enh}), where $P_{enh} = [T_e$ (expiratory time)/40% of T_r (relaxation time) $- 1 \times P_{ef}$ (peak expiratory flow)/ P_{if} (peak inspiratory flow) $\times 0.67]$. The validity of P_{enh} as a measure of bronchoconstriction or airway hyperresponsiveness has been examined (22). Pulmonary function was measured at baseline and after inhaled methacholine challenge with doses of 5, 10, and 15 mg/ml delivered using a DeVilbiss nebulizer.

Lung Lavage and Tissue Processing

Immediately following completion of the endotoxin exposure, animals were killed by CO_2 inhalation to perform lavage. The trachea was isolated and cannulated with PE-90 tubing, and 1-ml aliquots of normal saline were infused into the lungs by gravity, with a total volume of 6 ml infused. The lavage fluid was centrifuged at 200 × *g* for 5 min. The supernatant was then decanted and stored at –70°C until further use. The cell pellet is resuspended with Hanks' balanced salt solution, and a small aliquot is used for counting cells with a hemocytometer. Another aliquot of the cell suspension was spun onto a slide using a cytocentrifuge (Shandon, Southern Sewickley, PA). The cytospun cells were stained with Diff Quik Stain Set (Harleco, Gibbstown, NY), air-dried, and coverslipped for counting with light microscopy.

Cytokine Protein Analysis

Commercially available kits (R&D Systems, Minneapolis, MN) were used according to manufacturer's instructions to determine concentrations of murine TNF-α, IL-1β, and MIP-2 in the lavage fluid. In all cases, a monoclonal antibody was used as a capture reagent in a standard sandwich enzyme-linked immunosorbent assay (ELISA). Standard curves were derived using known concentrations of the recombinant specific cytokine supplied by the manufacturer. These cytokines/chemokines were chosen because in previous experiments in LPS-induced airway disease, we have shown that these proteins are produced and released by macrophages and other inflammatory cells (8).

Statistical Analysis

The primary comparison under investigation was the effect of blockade of ICAM-1, CD11a, or CD11b on the pulmonary inflammatory response to inhaled LPS. Specifically, comparisons evaluated differences in whole lung lavage cellularity, cytokine expression, and airway resistance. Statistical comparisons were made using nonparametric statistics, specifically the Mann-Whitney U test (23).

Results

Lung Inflammation

Lung lavage performed on control mice before exposure to endotoxin demonstrates between 28,000 and 30,000 cells/ml of lavage fluid, which are > 99% macrophages. Immediately following the inhalation challenge with aerosolized LPS, whole lung lavage demonstrates a greater than 5-fold increase in the total cell number, and a complete shift in the population of cells, to a predominantly neutro-

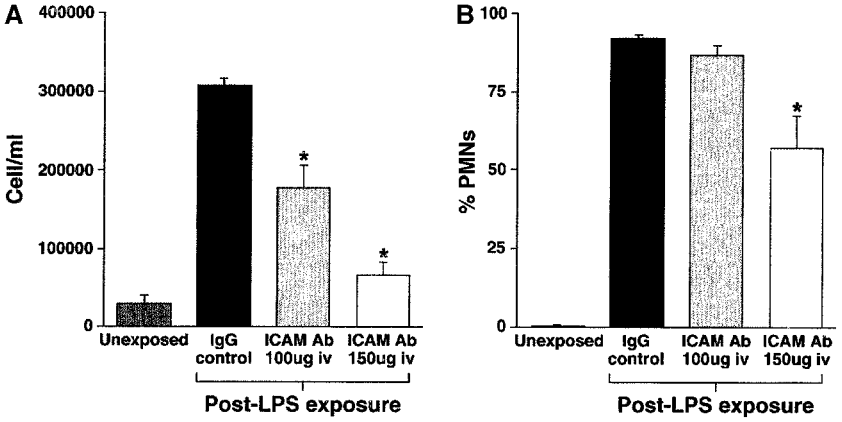

Figure 1. Whole lung lavage was performed to assess the cellular inflammatory response to inhaled LPS. Lung lavage fluid was assayed for the total number of cells (cell/ml) and the concentration of neutrophils (% PMNs) was calculated. Number of total cells/ml (*A*) and % PMNs (*B*) in the lung lavage fluid in mice unexposed to LPS, and after 4 h exposure to aerosolized LPS in mice receiving ICAM-1 antibody (100 or 150 μg intravenously), or isotype IgG as control. There was a dose-dependent decrease in the lung lavage cellularity with increasing ICAM-1 Ab dose. $*P < 0.05$, compared with IgG control.

philic infiltrate in the control mice (receiving isotype IgG). The use of ICAM-1 Ab at a dose of 100 μg intravenously, 1 h before LPS exposure, led to a significant reduction in the number of cells in the lung lavage fluid. An increased dose of 150 μg intravenously of anti–ICAM-1 resulted in a more marked reduction in cell recruitment as determined by whole lung lavage, with a significant reduction in the percentage of recruited neutrophils (Figures 1A and 1B). Mice receiving the higher dose of ICAM-1 antibody were noticeably more sluggish to wake up from the anesthetic used to give the injections, and then temporarily demonstrated some unusual neurologic behaviors, including episodic severe lethargy alternated with hyperactivity; however, no seizure activity was observed. Mice who received 130 μg of intravenous anti-CD11b antibody before LPS exposure had a dramatic reduction in cellular recruitment, with total cell counts unchanged from average pre-exposure levels. Similarly, the percentage of neutrophils in the lung lavage was reduced strikingly from 77% in the IgG control mice to 16.7% in mice receiving anti-CD11b antibody. Mice who received the anti-CD11a antibody before LPS exposure had no reduction in total number of cells recruited to the airspace or percentage of neutrophils, as compared with controls (Figure 2).

The lung lavage fluid was also assayed for the presence of inflammatory cytokines following exposure to aerosolized LPS, as an additional marker of the pulmonary inflammatory response, including levels of TNF-α, IL-1β, and MIP-2. Despite the dramatic reduction in inflammatory cell recruitment to the airspace after CD11b antibody, levels of the inflammatory cytokines TNF-α, IL-1β, and MIP-2 were unchanged between the groups (Table 1). Mice who received CD11a antibody had lavage levels of TNF-α, IL-1β, and MIP-2 similar to other groups. In mice receiving ICAM-1 Ab, TNF-α and IL-1β levels were similar; interestingly, lavage protein levels of MIP-2 were significantly increased in both groups receiving ICAM-1 antibody (Table 1).

Airway Physiology

Pulmonary function testing was initially performed on all mice before inhalation of LPS. There were no differences in P_{enh} values between the control mice and the mice receiving the antibodies at baseline (0 mg/ml methacholine) or following increasing doses of methacholine (Figure 3A). After exposure to aerosolized LPS for 4 h, all groups of mice demonstrated significant increases in P_{enh} at baseline (no methacholine) and with methacholine challenge. However, there were no differences between any of the experimental groups (Figure 3B), despite the dramatic reduction in neutrophil recruitment in mice receiving antibody to CD11b. Similarly, in the ICAM-1 blockade studies, inhalation of LPS led to an increase in airway resistance (P_{enh}) in all groups (compared with pre-exposure PFTs), including

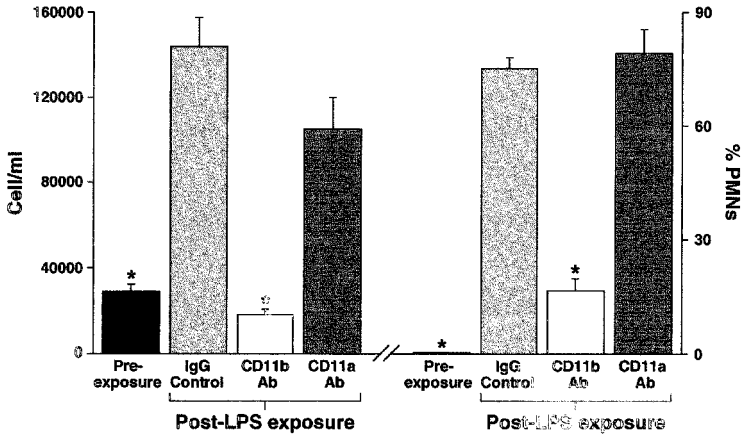

Figure 2. Whole lung lavage was performed to assess the cellular inflammatory response to inhaled LPS. Lung lavage fluid was assayed for the total number of cells (cell/ml) and the concentration of neutrophils (% PMNs) in mice before exposure to LPS (pre-exposure), and immediately after 4 h exposure to aerosolized LPS in mice receiving anti-CD11a antibody, anti-CD11b antibody (130 μg intravenously), or isotype IgG as control. Mice receiving anti-CD11b antibody had a marked reduction in both the total number of cells in lung lavage fluid and in the percentage of neutrophils. $*P < 0.05$, compared with IgG control.

TABLE 1
Cytokine protein levels in lung lavage fluid after aerosolized LPS exposure

	TNF-α	IL-1β	MIP-2
IgG control*	889 ± 72.4	22.3 ± 3.2	981 ± 239
CD11b Ab	986 ± 173	26.4 ± 4.6	1030 ± 60.4
CD11a Ab	560 ± 78.3	19.6 ± 2.9	1145 ± 250
IgG control†	1604 ± 167	25.6 ± 3.4	1441 ± 356
ICAM-1 Ab (100 μg)	1541 ± 115	28.1 ± 5.5	2493 ± 389‡
ICAM-1 Ab (150 μg)	1391 ± 159	20.3 ± 2.76	2850 ± 419‡

Definition of abbreviations: ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; LPS, lipopolysaccharide; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-α.
* Control values for LPS exposure with CD11b and CD11a antibodies.
† Control values for LPS exposure with ICAM-1 antibody.
‡ Significantly increased over control value, $P < 0.05$.

control mice, and those receiving anti–ICAM-1 Ab intravenously at either 100 or 150 μg (Figures 4A and 4B). Both groups of data indicate that blockade of neutrophil recruitment to the alveolar space does not offer protection against the development of increased airway resistance as measured noninvasively (P_{enh}) following LPS inhalation.

Discussion

The current investigation is focused on the role of two of the leukocyte β_2 integrins, CD11a/CD18 and CD11b/CD18, and one of their endothelial cell receptors, ICAM-1, in mediating the lower respiratory tract inflammatory response after inhaled LPS exposure. We have demonstrated that acute functional blockade of CD11b or ICAM-1 using intravenous antibody delivered just before LPS exposure dramatically inhibits neutrophil recruitment to the alveolar space as measured by whole lung lavage. Interestingly, despite this profound reduction in the cellular inflammatory infiltrate to the alveolar space, there appear to be significant increases in AHR in these animals after LPS exposure, suggesting that LPS-induced inflammatory cell recruitment is not the sole cause of LPS-induced AHR.

In addition, in the animals with significantly decreased neutrophil recruitment, levels of the neutrophil chemokine MIP-2 in the lavage fluid were either unchanged or elevated. Previous studies have indicated that MIP-2 alone is not sufficient for neutrophil recruitment, demonstrating incomplete blockade of neutrophil recruitment to the lung by MIP-2 antibodies in response to various inflammatory stimuli (24). In addition, a rat model of glucocorticoid inhibition of neutrophil recruitment after LPS challenge similarly reported no change in lavage MIP-2 levels (25). However, the finding of elevated MIP-2 levels in the lavage fluid of mice undergoing ICAM-1 blockade is novel. Soluble ICAM-1 has been demonstrated to activate rat alveolar macrophages to produce MIP-2 (26), and to induce MIP-2 production by brain microvascular endothelial cells *in vitro* (27). In our studies, it is possible that antibody ligation of ICAM-1 on the surface of alveolar macrophages, endothelial cells, or epithelium stimulated increased MIP-2 production from one of these cell types.

The importance of the β-chain (CD18) of the β_2 integrin family is well-established not only in reference to the human disease, LAD I, but also in several models of acute systemic inflammation (17, 28). In the setting of pulmonary inflammation, certain inflammatory stimuli (*Escherichia coli*, *Pseudomonas aeruginosa*, immune complex injury) appear to be β_2 integrin–dependent, and other stimuli utilize a CD18-independent pathway (Gram-positive bacteria, hyperoxia, HCl-induced pneumonia) (29, 30). Although LPS has previously been considered to be a CD18-dependent stimulus, the source of the inflammatory stimulus as well as the nature of the stimulus is important. A recent report indicates that only a small component of PMN transmigration into the lung in a sepsis model, following intraperitoneal instillation *E. coli*, is CD18-dependent, whereas all of the changes in microvascular permeability in the lung depend on the β_2 integrins (31). Additionally, the bacterial/inflammatory stimulus in all of the previous animal studies has been instilled into the trachea in a model of a "pneumonic" process. The role of the β_2 integrins in inflammatory cell recruitment following aerosolized inhalation of LPS to the lower respiratory tract has not been studied, and the relative contributions of each of the α-subunits (CD11a, b, c, and d) are poorly understood, especially in pulmonary models of inflammation. Previous studies focused specifically on CD11b have had controversial results dependent on the method of blockade of CD11b and the inflammatory stimulus applied. Monoclonal antibody block-

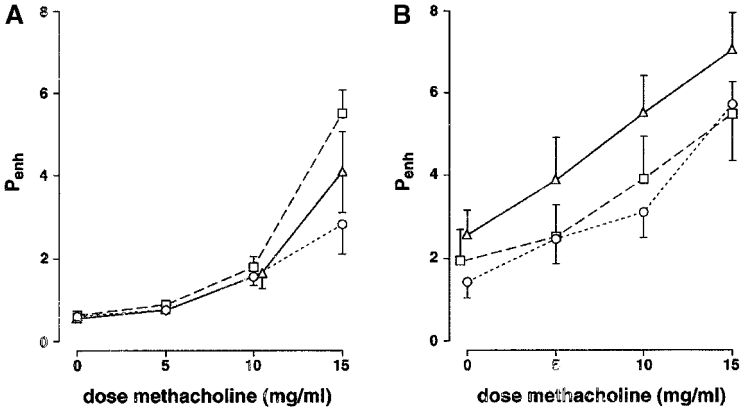

Figure 3. Airway resistance before and after exposure to aerosolized LPS. P_{enh} was measured prior to exposure to LPS (*A*) and immediately following exposure for 4 h to aerosolized LPS (*B*) at baseline (no methacholine) and with gradually increasing doses of methacholine challenge in control mice (*open circles*), mice receiving anti-CD11b antibody (*open squares*), and mice receiving anti-CD11a antibody (*open triangles*). All mice demonstrated increased airway resistance following LPS exposure, and there were no significant differences between the groups.

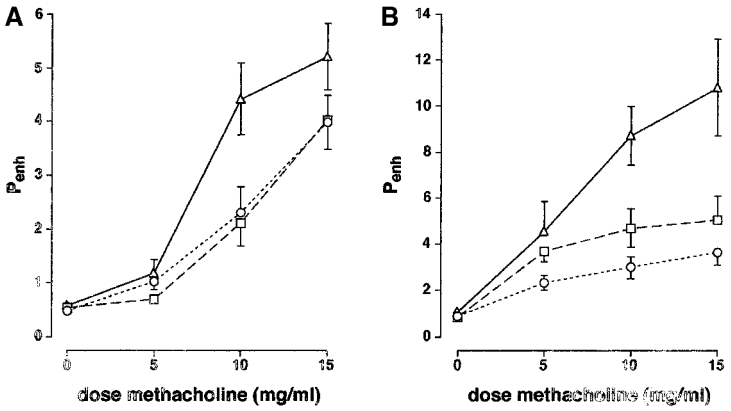

Figure 4. Airway resistance before and after exposure to aerosolized LPS. P_{enh} was measured prior to exposure to LPS (*A*) and immediately following exposure for 4 h to aerosolized LPS (*B*) at baseline (no methacholine) and with gradually increasing doses of methacholine challenge in control mice (*open circles*) and in mice receiving anti-ICAM-1 antibody 100 μg intravenously (*open squares*) or 150 μg intravenously (*open triangles*). All mice demonstrated increased airway resistance following LPS exposure, and there were no significant differences between the groups.

ade of CD11b led to reduction in neutrophil recruitment in both a peritonitis model (32), and a rat *E. coli* pneumonia model (33). However, Lu and coworkers concluded that CD11b/CD18 was not necessary for effective neutrophil emigration in peritonitis, using the $CD11b^{-/-}$ mutant mice (18), and these results were supported by more recent studies using monoclonal antibodies to block CD11b in rabbit peritonitis (34). This investigation demonstrates the requirement for CD11b/CD18 for neutrophil recruitment after exposure to aerosolized LPS, as mice receiving anti-CD11b antibody had a reduction in cellular recruitment to pre-exposure levels.

In the current investigation, antibody to CD11a did not have any effect on the number of recruited inflammatory cells in the alveolar space. This is in contrast to several previous studies demonstrating at least a partial requirement for CD11a/CD18 for neutrophil emigration to the lung after intratracheal endotoxin (35) or *P. aeruginosa* (19). However, these were models of a pneumonic process with tracheal installation of the bacteria or bacterial product, as compared with the inhalation exposure currently reported.

In animal studies involving pulmonary uptake of a particle or pathogen, there have been a number of reports which demonstrate that intratracheal instillation is not equivalent to aerosol exposure (36, 37). Inhalation of aerosolized particles generates a more homogeneous distribution throughout the lower respiratory tract, although a large percentage of the delivered dose may be retained in the nose and upper airways. Intratracheal instillation has been demonstrated to more reliably deliver a specific dose to the lung, but the distribution may be very nonhomogeneous, with fewer particles reaching the lung periphery. There are also distinct differences in clearance and retention when administered by instillation versus inhalation. Although intratracheal instillation has been accepted as an alternative approach in certain models, aerosol inhalation by the spontaneously breathing animal is likely to provide a better model for airway diseases acquired from environmental exposures. The role of ICAM-1 in acute lung inflammation caused by aerosolized pathogens/pollutants has not been studied; however, ICAM-1 has been demonstrated to participate in pulmonary neutrophil emigration following intratracheal instillation of *P. aeruginosa* (19) or LPS (35, 38). In addition to models of bacterial pneumonia, antibody to ICAM-1 has been shown to reduce neutrophil recruitment in phorbol-ester–induced lung inflammation (39), immune-complex mediated–lung injury (40), and several model systems of ischemia–reperfusion (41, 42). Our findings demonstrate partial blockade of neutrophil recruitment by anti–ICAM-1 following aerosolized exposure to LPS. There are a number of potential explanations for this incomplete blockade. First, as we demonstrated a dose-dependent reduction, our highest dose of antibody may not have been sufficient to block enough of the expressed ICAM-1 molcules, and we were limited from attempting increasing doses by the unusual toxicity of the antibody. Second, ICAM-1 serves as the endothelial ligand for several molecules expressed by leukocytes. During neutrophil recruitment, both CD11a/CD18 and CD11b/CD18 bind ICAM-1 before transmigration, but at different sites on the ICAM-1 molcule. Our antibody may bind to an epitope, which only prevents attachment of one of these neutrophil integrins (13, 14). Finally, the expected redundancy within the system may allow for upregulation of alternate endothelial cell adhesion molecules under these circumstances.

The role of ICAM-2 in pulmonary neutrophil recruitment, for example, has not been well described, but is a known ligand for CD11a/CD18, and its tissue distribution with high concentration in vascular endothelium makes it an important molecule for further study in this system (43). Constitutive expression of ICAM-2 is significantly higher than that of ICAM-1; however, it is reportedly not further upregulated by inflammatory mediators (44). Further study of the interaction of the CD11/CD18 family with ICAM-2 in pulmonary systems is certainly warranted.

The findings presented here question the relationship between neutrophil recruitment and AHR following inhalation of endotoxin. In the traditional view of airway diseases (including asthma and chronic bronchitis), airway inflammation—characterized by cellular infiltrate, release of inflammatory mediators, and increased airway secretions—and AHR have been suggested to be causally linked (45, 46). However, more recently a number of studies question this traditional paradigm whereby the induction of inflammatory cells and mediators directly initiates AHR. In an allergic murine asthma model, Henderson and colleagues demonstrated that soluble IL-4 receptor blocked eosinophil infiltration into the airway, without reduction in AHR to methacholine challenge (47). This discordance between

airway eosinophilia and AHR has also been reported in other allergic asthma models, using ovalbumin as well as more common aeroallergens, such as house dust mite allergen, as the initiating antigens (48, 49). Similarly, in acute, nonallergic models of airway inflammation and hyperresponsiveness, it appears that neutrophil influx may not always be directly linked to the development of AHR (50). The current investigation supports the hypothesis that inflammatory cell recruitment and AHR are independently initiated components of the pulmonary response to inhaled endotoxin exposure.

The more significant question raised by our findings is the determination of whether chronic airway remodeling caused by repeated exposure to aerosolized toxins can be altered by preventing recurrent neutrophil recruitment to the airways. Hoshino and coworkers (51) demonstrated that inhaled corticosteroid treatment in patients known to have asthma led to inhibition of airway infiltration by inflammatory cells and reduced the thickness of the lamina reticularis of the basement membrane. This patient population experienced improvement in respiratory symptoms. In our laboratory, using a chronic murine model of aerosolized endotoxin exposure, we have demonstrated that endotoxin-resistant mice (C3/HeJ), who do not have inflammatory cell recruitment in response to acute or chronic inhalation of endotoxin, also do not develop chronic airway remodeling, unlike the C3H/HeBFeJ mice, who develop significant airway thickening after chronic endotoxin exposure (52). However, to date there are no studies focused on prolonged blockade of adhesion molecules, or the role of adhesion molecules in tissue damage and airway remodeling.

The contribution of each of the β_2 integrin molecules to pulmonary neutrophil emigration from the vasculature is an extremely complex process that is in need of further investigation. The results of the current studies refocus attention on CD11b and one of its receptors, ICAM-1, as important components of the adhesion cascade in pulmonary inflammation in response to aerosolized LPS, and raise a number of important questions about the observed dichotomy between cellular inflammation and AHR.

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References

1. Kennedy, S. M., D. C. Christiani, E. A. Eisen, D. H. Wegman, I. A. Greaves, S. A. Olenchock, T.-T. Ye, and P.-L. Lu. 1987. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am. Rev. Respir. Dis.* 135:194–200.
2. Schwartz, D. A., K. J. Donham, S. A. Olenchock, W. Popendorf, D. S. van Fossen, L. F. Burmeister, and J. A. Merchant. 1995. Determinants of longitudinal changes in spirometric functions among swine confinement operators and farmers. *Am. J. Respir. Crit. Care Med.* 151:47–53.
3. Manfreda, J., V. Holford-Strevens, M. Cheang, and C. P. W. Warren. 1986. Acute symptoms following exposure to grain dust in farming. *Environ. Health Perspect.* 66:73–80.
4. Michel, O., J. Kips, J. Duchateua, F. Vertongen, L. Robert, H. Collet, R. Pauwels, and R. Sergysels. 1996. Severity of asthma is related to endotoxin in house dust. *Am. J. Respir. Crit. Care Med.* 154:1641–1646.
5. Park, J. H., D. R. Gold, D. L. Spiegelman, H. A. Burge, and D. K. Milton. 2001. House dust endotoxin and wheeze in the first year of life. *Am. J. Respir. Crit. Care Med.* 163:322–328.
6. Dockery, D. W., C. A. Pope, X. Xu, J. D. Spengler, J. H. Ware, M. E. Fay, B. G. Ferris, and F. E. Speizer. 1993. An association between air pollution and mortality in six US cities. *N. Engl. J. Med.* 329:1754–1759.
7. Becker, S., J. M. Soukup, M. I. Gilmour, and R. B. Devlin. 1996. Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. *Toxicol. Appl. Pharmacol.* 141:637–648.
8. Deetz, D. C., P. J. Jagielo, T. J. Quinn, P. S. Thorne, S. A. Bleuer, and D. A. Schwartz. 1997. The kinetics of grain dust-induced inflammation of the lower respiratory tract. *Am. J. Respir. Crit. Care Med.* 155:254–259.
9. Clapp, W. D., S. Becker, J. Quay, J. L. Watt, P. S. Thorne, K. L. Frees, X. Zhang, C. R. Lux, and D. A. Schwartz. 1994. Grain dust-induced airflow obstruction and inflammation of the lower respiratory tract. *Am. J. Respir. Crit. Care Med.* 150:611–617.
10. Schwartz, D., P. Thorne, P. Jagielo, G. White, S. Bleuer, and K. Frees. 1994. Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. *Am. J. Physiol.* 267:L609–L617.
11. Jagielo, P., P. Thorne, J. Kern, T. Quinn, and D. Schwartz. 1996. Role of endotoxin in grain dust-induced lung inflammation in mice. *Am. J. Physiol.* 270:L1052–L1059.
12. Moreland, J. G., R. Fuhrman, C. Wohlford-Lenane, T. Quinn, E. Benda, J. Pruessner, and D. Schwartz. 2001. TNF-α and IL-1β are not essential to the inflammatory response in LPS induced airway disease. *Am. J. Physiol.* 280:L173–L180.
13. Carlos, T., and J. Harlan. 1994. Leukocyte-endothelial adhesion molecules. *Blood* 84:2068–2101.
14. Malik, A., and S. Lo. 1996. Vascular endothelial adhesion molecules and tissue inflammation. *Pharmacol. Rev.* 48:213–229.
15. Hogg, J., and C. Doerschuk. 1995. Leukocyte traffic in the lung. *Ann. Rev. Physiol.* 57:97–114.
16. Issekutz, T. 1995. Leukocyte adhesion and the anti-inflammatory effects of leukocyte integrin blockade. *Agents Actions* 46:85–96.
17. Thiagarajan, R., R. Winn, and J. Harlan. 1997. The role of leukocyte and endothelial adhesion molecules in ischemia-reperfusion injury. *Thromb. Haemost.* 78:310–314.
18. Lu, H., C. W. Smith, J. Perrard, L. Tang, S. B. Shappell, M. L. Entman, A. L. Beaudet, and C. M. Ballantyne. 1997. LFA-1 is sufficient in mediating neutrophil emigration in Mac-1-deficient mice. *J. Clin. Invest.* 99:1340–1350.
19. Qin, L., W. M. Quinlan, N. A. Doyle, L. Graham, J. E. Sligh, F. Takei, A. L. Beaudet, and C. M. Doerschuk. 1996. The roles of CD11/CD18 and ICAM-1 in acute *Pseudomonas aeruginosa*-induced pneumonia in mice. *J. Immunol.* 157:5016–5021.
20. Scheynius, A., R. Camp, and E. Pure. 1993. Reduced contact sensitivity reactions in mice treated with monoclonal antibodies to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. *J. Immunol.* 150:655–663.
21. Saban, M. R., R. Saban, D. Bjorling, and M. Haak-Frendscho. 1997. Involvement of leukotrienes, TNF-alpha, and the LFA-1/ICAM-1 interaction in substance P-induced granulocyte infiltration. *J. Leukoc. Biol.* 61: 445–451.
22. Hamelmann, E., J. Schwarze, K. Takeda, A. Oshiba, G. L. Larsen, C. G. Irvin, and E. W. Gelfand. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 156:766–775.
23. Rosner, B., and A. Munoz. 1988. Autoregressive modelling for the analysis of longitudinal data with unequally spaced examinations. *Stat. Med.* 7:59–71.
24. Bless, N. M., R. L. Warner, V. A. Padgaonkar, A. B. Lentsch, B. J. Czermak, H. Schmal, H. P. Friedl, and P. A. Ward. 1999. Roles for C–X-C chemokines and C5a in lung injury after hindlimb ischemia-reperfusion. *Am. J. Physiol.* 276:L57–63.
25. O'Leary, E. C., and S. H. Zuckerman. 1997. Glucocorticoid-mediated inhibition of neutrophil emigration in an endotoxin-induced rat pulmonary inflammation model occurs without an effect on airways MIP-2 levels. *Am. J. Respir. Cell Mol. Biol.* 16:267–274.
26. Schmal, H., B. J. Czermak, A. B. Lentsch, N. M. Bless, B. Beck-Schimmer, H. P. Friedl, and P. A. Ward. 1998. Soluble ICAM-1 activates lung macrophages and enhances lung injury. *J. Immunol.* 161:3685–3693.
27. Otto, V. I., U. E. Heinzel-Pleines, S. M. Gloor, O. Trentz, T. Kossmann, and M. C. Morganti-Kossmann. 2000. sICAM-1 and TNF-alpha induce MIP-2 with distinct kinetics in astrocytes and brain microvascular endothelial cells. *J. Neurosci. Res.* 60:733–742.
28. Vedder, N. B., R. K. Winn, C. L. Rice, E. Y. Chi, K. E. Arfors, and J. M. Harlan. 1988. A monoclonal antibody to the adherence-promoting leukocyte glycoprotein, CD18, reduces organ injury and improves survival from hemorrhagic shock and resuscitation in rabbits. *J. Clin. Invest.* 81:939–944.
29. Doerschuk, C. M., R. K. Winn, H. O. Coxson, and J. M. Harlan. 1990. CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. *J. Immunol.* 144:2327–2333.
30. Mizgerd, J. P., H. Kubo, G. J. Kutkoski, S. D. Bhagwan, K. Scharffetter-Kochanek, A. L. Beaudet, and C. M. Doerschuk. 1997. Neutrophil emigration in the skin, lungs, and peritoneum: different requirements for CD11/CD18 revealed by CD18-deficient mice. *J. Exp. Med.* 186:1357–1364.

31. Gao, X., N. Xu, M. Sekosan, D. Mehta, S. Y. Ma, A. Rahman, and A. B. Malik. 2001. Differential role of CD18 integrins in mediating lung neutrophil sequestration and increased microvascular permeability induced by *Escherichia coli* in mice. *J. Immunol.* 167:2895–2901.
32. Rosen, H., and S. Gordon. 1987. Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro and inflammatory cell recruitment *in vivo*. *J. Exp. Med.* 166:1685–1701.
33. Zeni, F., C. Parent, R. Correa, C. Natanson, B. Freeman, J. Fontana, M. Quezado, R. L. Danner, Y. Fitz, S. Richmond, E. Gerstenberger, S. M. Banks, and P. Q. Eichacker. 1999. ICAM-1 and CD11b inhibition worsen outcome in rats with *E. coli* pneumonia. *J. Appl. Physiol.* 87:299–307.
34. Rutter, J., T. James, D. Howat, A. Shock, D. Andrew, P. De Baetselier, J. Blackford, J. Wilkinson, G. Higgs, B. Hughes, and M. Robinson. 1994. The in vivo and in vitro effects of antibodies against rabbit β2-integrins. *J. Immunol.* 153:3724–3733.
35. Tang, G., J. E. White, P. D. Lumb, D. A. Lawrence, and M.-F. Tsan. 1995. Role of endogenous cytokines in endotoxins- and interleukin-1–induced pulmonary inflammatory response and oxygen tolerance. *Am. J. Respir. Cell Mol. Biol.* 12:339–344.
36. Leong, B. K., J. K. Coombs, C. P. Sabaitis, D. A. Rop, and C. S. Aaron. 1998. Quantitative morphometric analysis of pulmonary deposition of aerosol particles inhaled via intratracheal nebulization, intratracheal instillation or nose-only inhalation in rats. *J. Appl. Toxicol.* 18:149–160.
37. Foster, W., D. Walter, M. Longphre, K. Macri, and L. M. Miller. 2001. Methodology for the measurement of mucociliary function in the mouse by scintigraphy. *J. Appl. Physiol.* 90:1111–1118.
38. Kumasaka, T., W. M. Quinlan, N. A. Doyle, T. P. Condon, J. Sligh, F. Takei, A. L. Beaudet, C. F. Bennett, and C. M. Doerschuk. 1996. Role of the intercellular adhesion molecule-1 (ICAM-1) in endotoxin-induced pneumonia evaluated using ICAM-1 antisense oligonucleotides, anti-ICAM-1 monoclonal antibodies, and ICAM-1 mutant mice. *J. Clin. Invest.* 97:2362–2369.
39. Barton, R., R. Rothlein, J. Ksiazek, and C. Kennedy. 1989. The effect of anti-intercellular adhesion molecule-1 on phorbol-ester-induced rabbit lung inflammation. *J. Immunol.* 143:1278–1282.
40. Mulligan, M. S., G. P. Wilson, R. F. Todd, C. W. Smith, D. C. Anderson, J. Varani, T. B. Issekutz, M. Miyasaka, T. Tamatani, and M. Myasaka. 1993. Role of beta 1, beta 2 integrins and ICAM-1 in lung injury after deposition of IgG and IgA immune complexes. *J. Immunol.* 150:2407–2417. [Published erratum appears in *J. Immunol.* 150:5209.
41. Horgan, M., M. Ge, J. Giu, R. Rothlein, and A. Malik. 1991. Role of ICAM-1 in neutrophil-mediated lung vascular injury after occlusion and reperfusion. *Am. J. Physiol.* 261:H1578–H1584.
42. Seekamp, A., M. S. Mulligan, G. O. Till, C. W. Smith, M. Miyasaka, T. Tamatani, R. F. Todd, and P. A. Ward. 1993. Role of β2 integrins in ICAM-1 in lung injury following ischemia-reperfusion of rat hind limbs. *Am. J. Pathol.* 143:464–472.
43. Xu, H., I. Tong, A. De Fougerolles, and T. Springer. 1992. Isolation, characterization, and expression of mouse ICAM-2 complementary and genomic DNA. *J. Immunol.* 149:2650–2655.
44. Staunton, D., M. Dustin, and T. Springer. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 339:61–64.
45. Moreno, R. H., J. C. Hogg, and P. Pare. 1986. Mechanics of airway narrowing. *Am. Rev. Respir. Dis.* 133:1171–1180.
46. Bousquet, J., P. Jeffery, W. Busse, M. Johnson, and A. Vignola. 2000. Asthma: from bronchoconstriction to airways inflammation and remodeling. *Am. J. Respir. Crit. Care Med.* 161:1720–1745.
47. Henderson, W. J., E. Chi, and C. Maliszewski. 2000. Soluble IL-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma. *J. Immunol.* 164:1086–1095.
48. Henderson, W. R., E. Y. Chi, R. K. Albert, S. J. Chu, W. J. E. Lamm, Y. Rochon, M. Jonas, P. E. Christie, and J. M. Harlan. 1997. Blockade of CD49d (α4 integrin) on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. *J. Clin. Invest.* 100:3083–3092.
49. Tournoy, K. G., J. C. Kips, C. Schou, and R. A. Pauwels. 2000. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin. Exp. Allergy* 30:79–85.
50. Sun, J., and K. Chung. 1997. Airway inflammation despite loss of bronchial hyper-responsiveness after multiple ozone exposures. *Respir. Med.* 91:47–55.
51. Hoshino, M., Y. Nakamura, J. J. Sim, Y. Yamashiro, K. Uchida, K. Hosaka, and S. Isogai. 1998. Inhaled corticosteroid reduced lamina reticularis of the basement membrane by modulation of insulin-like growth factor (IGF)-I expression in bronchial asthma. *Clin. Exp. Allergy* 28:568–577.
52. George, C., H. Jin, C. Wohlford-Lenane, M. O'Neill, J. Phipps, P. O'Shaughnessy, J. Kline, P. Thorne, and D. Schwartz. 2001. Endotoxin responsiveness and subchronic grain dust-induced airway disease. *Am. J. Physiol.* 280:L203–L213.