**E. coli** pneumonia induces CD18-independent airway neutrophil migration in the absence of increased lung vascular permeability

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E. coli pneumonia induces CD18-independent airway neutrophil migration in the absence of increased lung vascular permeability. Am J Physiol Lung Cell Mol Physiol 285:L879–L888, 2003. First published June 20, 2003; 10.1152/ajplung.00134.2003.—We examined the relationship between neutrophil (polymorphonuclear leukocyte (PMN)) influx and lung vascular injury in response to Escherichia coli pneumonia. We assessed lung tissue PMN uptake by measuring myeloperoxidase and transvascular PMN migration by determining PMN counts in lung interstitium and bronchoalveolar lavage fluid (BALF) in mice challenged intratracheally with E. coli. Lung vascular injury was quantified by determining microvessel filtration coefficient (Kf,c), a measure of vascular permeability. We addressed the role of CD18 integrin in the mechanism of PMN migration and lung vascular injury by inducing the expression of neutrophil inhibitory factor, a CD11/CD18 antagonist. In control animals, we observed a time-dependent sixfold increase in PMN uptake, a fivefold increase in airway PMN migration, and a 20-fold increase in interstitial PMN uptake at 6 h after challenge. Interestingly, Kf,c increased minimally during this period of PMN extravasation. CD11/CD18 blockade reduced lung tissue PMN uptake consistent with the role of CD18 in mediating PMN adhesion to the endothelium but failed to alter PMN migration in the tissue. Moreover, CD11/CD18 blockade did not affect Kf,c. Analysis of BALF leukocytes demonstrated diminished oxidative burst compared with leukocytes from bacteremic mice, suggesting a basis for lack of vascular injury. The massive CD11/CD18-independent airway PMN influx occurring in the absence of lung vascular injury is indicative of an efficient host-defense response elicited by E. coli pneumonia.

PMN migration from blood vessels to the airways occurs via a multistep process involving activation of adhesion proteins and their ligands on PMNs and endothelial cells. Adhesion proteins of the selectin family (E-selectin, L-selectin, and P-selectin) contribute to the capture and rolling of PMNs, whereas β2-integrins (members of CD11/CD18 family) and ICAM-1 induce firm adhesion of PMN to endothelial cells required for the transmigration (9, 18–20, 27).

Although in most cases, PMN migration into the lung is an essential immune response to infection, migration of PMNs in certain pathological states may also contribute to inflammatory lung injury (39, 43). An important question is whether PMN extravasation is differentially regulated during Escherichia coli pneumonia compared with bacteremia. We have previously shown bacteremia induced in mice by intraperitoneal injection of E. coli to result in PMN sequestration, transvascular migration, and respiratory burst dependent on the activation of CD11/CD18 integrins (43). Additionally, the bacteremia-induced PMN sequestration and migration were associated with a marked increase in lung microvascular permeability (9, 43), and inhibition of PMN adherence to endothelial cells prevented the lung vascular injury (16). However, it remains unclear whether CD11/CD18 integrins are required for the extravasation of PMNs when the bacterial infection is localized in the lung, as in pneumonia, and whether CD11/CD18 integrins also contribute to vascular injury during the process of migration. A previous study using irradiated C57BL/6 mice in which the hematopoietic system was reconstituted with fetal liver cells of CD18-deficient mice showed that the percentage of CD18-deficient PMN in bronchoalveolar lavage fluid was only about one-fourth of that in the blood 6 h after airway instillation of E. coli (26), thus suggesting the importance of CD18 in mediating PMN migration induced by E. coli pneumonia. However, in contrast, another study concluded that PMN migration was not reduced in CD18-deficient (CD18−/−) mice (27), suggesting a CD18-independent pathway for PMN migration during gram-negative pneumonia.

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In the present study, we found that intratracheal *E. coli* challenge induced robust PMN extravasation, which was 10-fold greater than following intraperitoneal *E. coli* challenge (43). To address the role of CD11/CD18 in the mechanism of migration, we conditionally expressed neutrophil inhibitory factor (NIF), a 41-kDa-specific CD11/CD18 antagonist glycoprotein (29) in the lung microvascular space using the E-selectin promoter (44). This strategy avoids the engagement of compensatory pathways that may be induced in CD18 knockout mice (27, 41) and obviates the deleterious effects of irradiation on cellular components of the immune system (15, 26). NIF, a product of Ancyllostoma caninum, inhibits PMN adhesion to endothelial cells by binding with high affinity to CD11b and low affinity to CD11a (23). Expression of NIF prevented the lung PMN sequestration, consistent with its role in inhibiting PMN adhesion to endothelial cells (42, 44). However, NIF expression failed to prevent the migration of PMNs after *E. coli* pneumonia, indicating that response occurred independently of CD11/CD18 integrins. Pulmonary vascular permeability only increased minimally, and the permeability response was not significantly altered by the CD11/CD18 blockade. We also found that the air space leukocyte respiratory burst was lower compared with cells obtained from bacteremic mice. The massive CD11/CD18-independent airway PMN influx elicited by *E. coli* pneumonia is indicative of an efficient host-defense response since migration occurred in the absence of significant lung vascular injury and tissue edema.

**MATERIALS AND METHODS**

*Mice.* The liposome-based gene delivery method was established in pathogen-free CD1 mice (males; 30–35 g body wt; Harlan, Indianapolis, IN) (42, 43); therefore, these mice were used in the present study to conditionally express NIF and to examine responses to intratracheal *E. coli* challenge. Mice were housed in pathogen-free conditions at the University of Illinois Animal Care Facility where they were treated in accordance with institutional guidelines. Before injection of transgene/liposome complex, mice were anesthetized with ketamine (60 mg/kg) and xylazine (2 mg/kg) in PBS.

**DNA/liposome preparation and in vivo gene transfer.** A mixture of dimethyldioctadecylammonium bromide (DDAB; Sigma Chemical, St. Louis, MO) and cholesterol (Calbiochem, La Jolla, CA) in chloroform was used to prepare the liposomes (43). Briefly, the mixture consisting of DDAB and cholesterol (1:1 M ratio) was dried using the Rotavapor R-124 (Brinkmann, Westbury, NY) and dissolved in 5% glucose. The lipid molecules were sonicated for 20 min. The construct pES-NIF in which NIF cDNA (a gift of Corvas, La Jolla, CA) is driven by the E-selectin promoter was made as described (21, 43). The pES-NIF construct (50 μg/mouse) and liposomes were combined at a ratio of 1 μg of DNA to 8 nmol of liposomes and injected into mice through a retro-orbital injection.

*E. coli* challenge. Mice received 1 × 10^8 live *E. coli* (ATCC 25922) in PBS (pH 7.4) via intratracheal instillation. This *E. coli* dosage did not result in death of mice within a 48-h period. Control mice were injected intratracheally with an equal volume of PBS.

**Western blot analysis.** Mice were euthanized and homogenized in PBS (pH 7.4) containing protease inhibitor cocktail (60 μl/10 ml PBS, Sigma) using a tissue grinder. The homogenates were centrifuged at 500 g for 10 min at 4°C. Supernatants were collected, and the protein concentration of each sample was measured with a Bradford-type Coomasie Plus Protein Assay (Pierce, Rockford, IL). An equal amount of protein from each sample (10–30 μg) was resolved in 4–12% NuPage Bis-Tris Gel (Invitrogen, Carlsbad, CA), and Western blotting was performed to determine expression of NIF.

**NIF antibody production.** New Zealand female rabbits were immunized with 20 μg per animal per injection of NIF protein (47 kDa). NIF protein was mixed (1:1) with complete Freund adjuvant and injected (2 ml/animal) as in Muller et al. (30). The antibody was obtained by (NH₄)₂SO₄ precipitation at different bleedings followed by absorption on a protein A/G affinity column as in Andrew and Titus (1). The specificity was established by 1) enzyme-linked immunosorbent assay against NIF and 2) immunoblotting of whole organ homogenates, followed by enhanced chemiluminescence detection. The antibody recognized a single band of ~47 kDa in all organs tested.

**PMN sequestration assay.** Mouse lungs were homogenized in 5% hexadecyltrimethylammonium bromide (HTAB) in 50 mM phosphate buffer (pH 6; 5.0 ml HTAB/g tissue) for quantification of lung vascular PMN sequestration by myeloperoxidase (MPO) activity (43). The homogenates were centrifuged at 40,000 rpm for 30 min. The pellets were subjected to three cycles of freeze-thaw. Each cycle was followed by homogenization and centrifugation. Three supernatants were collected and mixed; 0.1 ml of the pooled sample was mixed 1:30 (vol/vol) with assay buffer (0.167 mg/ml of o-dianisidinehydrochloride and 0.0005% H₂O₂), and absorbance change was measured at 460 nm for 3 min. MPO activity was calculated as the change in absorbance over time.

**Morphometric analysis of lung interstitial PMN uptake.** We used a computer-based stereological method to quantify the number of PMNs in lung interstitial tissue as described by us (11). This assessment was made in a blinded fashion without knowledge of tissue sections, which were prepared by inflating the mouse lungs with 10% formalin and embedding in paraffin. Tissue blocks were sectioned to 5-μm thick and mounted onto glass slides. The hematoxylin and eosin-stained tissue sections were visualized using a high magnification objective with a high numerical aperture. The computerized optical counting system consisted of a microscope, computer-controlled x-y-z motorized stage, high-sensitivity video camera, computer-assisted image capture, and stereological software program from MicroBrightField (Colchester, VT). The instrumentation was calibrated before each measurement. The middle region (~30 mm²) of the top lobe of the left lung was outlined at low magnification (×1.25). At least 5% of the outlined region was measured with a systematic random design of counting frame using a ×100 oil-immersion objective with a 1.4 numerical aperture (9, 26). The total number of PMN in the outlined region of lung was determined using the formula: N = 2Q/V - SSF/ASF, where 2Q is the total number of PMN counted by optical evaluation using a random design procedure for all measurements. The area sampling fraction (ASF) is the counting frame (6,400 μm²), and the section sampling fraction (SSF) is the fraction of section sampled in the region of lung.

**Transalveolar PMN migration.** Bronchoalveolar lavage was performed by cannulating the trachea with a blunt-ended 21-gauge needle, instilling 0.6 ml of sterile PBS con-
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E-selectin-dependent NIF expression in lungs. Western blot analysis of lungs of mice in which the empty vector was delivered showed no expression of NIF (Fig. 1). In another group, at the 48-h after delivery of pE5-NIF construct, the mice were challenged with intratracheal E. coli (10⁸) instillation. NIF expression was evident at 1 h after challenge, peaked at 6 h, and decreased by 12 h (Fig. 1).

Lung vascular PMN sequestration and interstitial uptake induced by intratracheal E. coli challenge. After intratracheal E. coli, lung tissue MPO activity in control mice increased significantly within 3 h after challenge and increased sixfold at the 6-h time point. In mice expressing NIF, MPO activity also increased within 3 h, but the responses were significantly reduced by 25 and 80% of control levels at the 3- and 6-h time points, respectively (Fig. 2A). Morphometric analysis of PMN in the lung interstitium demonstrated a significant time-dependent 18-fold increase at 6 h after intratracheal E. coli challenge (Fig. 2B). In contrast to vascular PMN sequestration, the interstitial PMN uptake was not affected by the expression of NIF (Fig. 2B).

Air space PMN migration induced by intratracheal E. coli challenge. Analysis of bronchoalveolar lavage fluid used to assess transvascular PMN migration demonstrated that ~90% of the cells in bronchoalveolar lavage fluid were PMN, as determined by flow cytometry. The total number of PMN was counted in bronchoalveolar lavage samples from each time point. The bronchoalveolar lavage fluid was then collected and measured for the total number of PMN by using forward and side scatter characteristics to gate over the PMN population. The results are reported as the total number of cells counted in the gated PMN population from the bronchoalveolar lavage fluid from four mice at each time point.

Measurement of macrophage inflammatory protein-2 in bronchoalveolar lavage fluid by ELISA. Mice were killed by intraperitoneal anesthetic at designated time points. Bronchoalveolar lavage was performed by cannulating the trachea with a blunt-ended 21-gauge needle, instilling 0.6 ml of sterile PBS containing 1 mM EDTA, and collecting the fluid by gentle aspiration. Approximately 1 ml of lavage fluid was retrieved per mouse and stored at −20°C for measurement of cytokine levels (13). Murine macrophage inflammatory protein-2 (MIP-2) concentration was quantitated by a modification of a double ligand method as described (13). Antibody was generously supplied by Dr. Ted Standiford (Univ. of Michigan, Ann Arbor, MI). The ELISA method was able to detect murine MIP-2 concentrations ≥25 pg/ml. The ELISA did not cross-react with IL-2, IL-4, IL-6, interferon-γ, or with other members of the murine chemokine family, including murine JE/monocyte chemoattractant protein-1, growth-regulated oncogene, or epithelial cell-derived neutrophil attractant-78 (13).

Pulmonary microvascular permeability. Capillary filtration coefficient (Kcap) was determined using pulmonary microvascular permeability to liquid as described (9). Briefly, after the standard 20-min equilibration perfusion, the outflow pressure was rapidly elevated by 10 cmH₂O for 2 min. The lung wet weight change in a ramp-like fashion, reflecting net fluid extravasation. At the end of each experiment, lungs were dissected free of nonpulmonary tissue, and lung dry weight was determined. Kcap (ml·min⁻¹·cmH₂O⁻¹·dry g⁻¹) was calculated from the slope of the recorded weight change normalized to the pressure change and to lung dry weight.

As another approach for the assessment of leakage of pulmonary microvessels, we determined the rate of pulmonary edema formation by continuously monitoring the lung wet weight change. Lung weight change of lungs obtained from different groups was followed for 90 min after beginning of perfusion (described above). Because the perfusate albumin concentration was constant at the onset of perfusion and pulmonary arterial pressure did not change during the 90-min monitoring period, rate and magnitude of the increase in lung wet weight (i.e., attainment of a new isogravimetric state) provided another index of permeability of pulmonary microvessels.

Survival studies in mice. At 48 h after the injection of pE5-NIF/liposome complex, mice were challenged with a dosage of 1 × 10⁸ live E. coli per mouse intratracheally for mortality analysis. CD1 mice not receiving injection of NIF construct were used as controls. A 50% survival rate after E. coli challenge was determined in all groups.

Statistical analysis. Data are expressed as means ± SE. Comparisons between experimental groups were made by paired t-tests with a significance value set at P < 0.05.

RESULTS

E-selectin-dependent NIF expression in lungs. Western blot analysis of lungs of mice in which the empty vector was delivered showed no expression of NIF (Fig. 1). In another group, at the 48-h after delivery of pE5-NIF construct, the mice were challenged with intratracheal E. coli (10⁸) instillation. NIF expression was evident at 1 h after challenge, peaked at 6 h, and decreased by 12 h (Fig. 1).

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Air space PMN migration induced by intratracheal E. coli challenge. Analysis of bronchoalveolar lavage fluid used to assess transvascular PMN migration demonstrated that ~90% of the cells in bronchoalveo-
lar lavage fluid were PMN at 6 h after intratracheal challenge of *E. coli*, and the migration was increased fivefold at this time (Fig. 3A). The transalveolar PMN response peaked at 6 h after intratracheal *E. coli* challenge. Expression of NIF did not significantly interfere with the migration of PMN into the air space; i.e., the migration response showed only a 10% decrease (Fig. 3A). A similar pattern of PMN migration also occurred in the total number of PMNs in bronchoalveolar lavage fluid at these time points in control and NIF-expressing mice (Fig. 3B).

**Generation of MIP-2 in bronchoalveolar lavage fluid.** We measured MIP-2 levels in the bronchoalveolar lavage fluid of mice challenged with either intratracheal or intraperitoneal *E. coli* to compare whether MIP-2 was generated differentially after *E. coli* pneumonia. MIP-2 concentration in bronchoalveolar lavage fluid increased at 1 and 6 h after intratracheal *E. coli* challenge (Fig. 4). Generation of MIP-2 in bronchoalveolar lavage fluid was highest at the 6-h time point in mice after intratracheal *E. coli*. In contrast, bronchoalveolar lavage fluid MIP-2 concentration was low or undetectable after intraperitoneal *E. coli* challenge at both time points (Fig. 4).
We determined the effects of CD11/CD18 blockade with conditional NIF expression on the mortality of mice challenged with a lethal dose of intratracheal E. coli (10). In the control group of mice challenged with E. coli, 50% survived at 8 h after the challenge, whereas 50% of the NIF-expressing mice were alive at the 12-h time point (Fig. 7). All control mice died by 12 h after the challenge, whereas 40% of the mice expressing NIF survived after the same E. coli challenge.

DISCUSSION

We determined in these studies 1) the relationship between PMN extravasation and the development of lung microvessel injury and 2) the contribution of CD11/CD18 integrin to PMN migration response and lung microvessel injury after E. coli pneumonia. Our previous study in a bacteremia model in which mice were challenged with intraperitoneal E. coli showed marked lung vascular PMN sequestration secondary to PMN adhesion but with relatively few PMN migrating into the air space (i.e., at peak, ~10% of cells in the recovered bronchoalveolar lavage fluid were PMN) (43). Moreover, the PMN migration was associated with a fourfold increase in lung microvessel permeability (9). In the present study, we show that a sublethal intratracheal challenge of E. coli (in which the same number of bacteria were used as in the intraperitoneal model) resulted in a fivefold greater PMN airway migration response with ~90% of cells migrating into the air space being PMNs. Interestingly, there was a relatively small increase in lung vascular permeability after E. coli pneumonia in spite of the massive extravasation of PMNs.

We measured the differences in the oxidative respiratory burst of bronchoalveolar lavage leukocytes as a possible explanation for these findings. Reactive oxygen species have been demonstrated to increase endothelial permeability by either acting as signaling molecules to induce paracellular gaps or at high concentrations, causing direct oxidative injury (12, 17, 24). Our data demonstrate that bronchoalveolar lavage leukocytes from bacteremic mice were maximally activated at 3 h after challenge. Moreover, the cells could not be further stimulated with PMA at a concentration that produces a maximal respiratory oxidative burst. In contrast, the oxidative burst of bronchoalveolar lavage leukocytes from intratracheal E. coli-challenged mice was significantly reduced (~60% of the maximum), and these cells could be further stimulated. Although there was much greater influx of PMNs after E. coli pneumonia, the diminished respiratory burst of these PMNs may account for the reduced lung vascular injury and edema formation. In contrast, PMNs in Kf,c at either the 6- or 12-h time points after intratracheal E. coli challenge (Fig. 6A). Intratracheal E. coli infection as well as NIF expression also had no significant effect on changes in isogravimetric lung wet weight at either the 1- or 12-h time points after challenge (Fig. 6B).

Mortality studies with lethal dosage of E. coli. To assess the effects of intratracheal instillation of E. coli on lung microvessel injury. Airway infection resulted in a small increase in lung microvessel permeability by 1 h. At 6 h after intratracheal challenge, Kf,c increased 60% over the value in unchallenged lungs (Fig. 6A). This change in Kf,c at 6 h after intratracheal E. coli was significantly less than the 400% increase observed after intraperitoneal E. coli challenge in mice (43). Expression of NIF did not significantly reduce the
airways of bacteremic mice appeared to be maximally activated, which may be a factor in the marked lung vascular injury (16, 43).

Further evidence of this differential host inflammatory response is evident by the production of MIP-2, the murine homolog of IL-8, in the air space of E. coli pneumonia mice. Chemokines such as MIP-2 are released after activation of alveolar macrophages (5, 35, 36) and contribute to the normal trafficking of PMNs. It has been shown that the chemotactic gradient established by MIP-2 is an important determinant of transalveolar PMN migration (3, 40). Thus in an E. coli pneumonia model, PMN may be attracted to the site of infection through the generation of chemokines such as MIP-2, but PMN may need to be activated locally by pathogens and the release of other mediators.

Previous studies have used three approaches to address the role of CD11/CD18 in the mechanism of infiltration of PMN in E. coli pneumonia: monoclonal antibodies directed against CD11/CD18 (32), CD18−/− mice (27), and CD18 null PMN (26). Antibodies against CD18 prevented acute PMN migration during E. coli pneumonia (6, 32), whereas PMN migration persisted in mice deficient in CD11/CD18 (CD18−/−) (27), indicating that CD11/CD18-independent pathways for PMN infiltration are used during acute pulmonary inflammation. However, a study in C57BL/6-irradiated mice in which the hematopoietic system was reconstituted with fetal liver cells of CD18−/− mice showed that the percentage of CD18-deficient PMN in bronchoalveolar lavage fluid was 25% of that in blood 6 h after airway instillation of E. coli (26), thus suggesting the importance of CD18 in mediating PMN migration induced by E. coli pneumonia. These conflicting findings may be the result of the approaches used to assess the role of CD11/CD18-dependent PMN migration. It is

**Fig. 5.** A: flow cytometric analysis of oxidative respiratory burst of leukocytes in BALF of mice challenged with intraperitoneal or intratracheal 10⁸ E. coli. With the use of a dihydrorhodamine 123 (Rho123)-based flow cytometry assay, mean fluorescence intensity (MFI) was measured in leukocytes from BALF of mice over a time course after either intraperitoneal or intratracheal E. coli challenge. Mice challenged with intraperitoneal E. coli had increased MFI, peaking at 3 and 6 h (P < 0.05). In contrast, mice challenged with intratracheal E. coli had significantly reduced MFI at the 3- and 6-h time points (P < 0.05). Ex vivo stimulation with phorbol 12-myristate 13-acetate (PMA) led to increased MFI of leukocytes isolated from mice challenged with intratracheal E. coli for 6 h. Bars indicate means ± SE (n = 4). *P < 0.05, intratracheal E. coli challenge vs. intraperitoneal E. coli challenge. #P < 0.05, 6-h intratracheal E. coli challenge vs. 6-h intratracheal E. coli challenge with ex vivo PMA stimulation. B: histograms of fluorescence using Rho123-based flow cytometry assay of leukocytes from BALF. Leukocytes with ex vivo stimulation with PMA after the 6-h E. coli intratracheal or intraperitoneal challenge were compared with leukocytes obtained from mice challenged for 6 h with E. coli intratracheally or intraperitoneally alone. Leukocytes from intraperitoneally challenged mice had no change in fluorescence when stimulated ex vivo with PMA. In contrast, leukocytes from mice challenged with intratracheal E. coli for 6 h had a right shift in fluorescence after ex vivo stimulation with PMA. The fluorescence histogram from leukocytes from unchallenged mice is displayed for comparison.
possible that the regulation of adhesion pathways differs after inhibition of CD11/CD18 by antibodies compared with genetic deletion in CD18−/− mice. Neutralizing anti-CD18 antibodies may have other effects, such as cross-linking of the antigen, which could modify the adhesion pathway (7, 25, 44). CD18 deficiency may result in activation of compensatory mechanisms, and there are known phenotypic alterations, including soft tissue infections and neutrophilia, which may confound comparisons with the effects of anti-CD18 antibodies (34). Interpretation of results from CD18−/− mice is also problematic because of the marked neutrophilia seen in these mice (27). The number of circulating PMN in CD18−/− mice with E. coli pneumonia was increased eightfold compared with control mice. Thus the lack of effect in blocking PMN migration may be attributed to the neutrophilia. Because lethal irradiation of mice can also have complex effects on the host-defense response (15, 26), it is possible that the impaired migration of CD18-null PMN may be a result of a compromised inflammatory response. We also assessed the role of CD11/CD18 integrins in the mechanism of airway migration of PMN in response to E. coli pneumonia in an attempt to resolve this controversy. We used a novel NIF expression strategy to assess the role of CD11/CD18 in mediating PMN migration in

Fig. 6. A: time course of microvessel liquid permeability (measured as capillary filtration coefficient, Kf,c) in lungs of control and pES-NIF-transduced (eNIF) mice after intratracheal E. coli challenge. Kf,c values were determined (see MATERIALS AND METHODS) at times indicated on the abscissa. Peak Kf,c value increased ~60% above control at 6 h. NIF expression did not significantly affect the response. Bars indicate means ± SE (n = 5). *P < 0.05 compared with control (no intratracheal E. coli challenge). B: time course of changes (Δ) in isogravimetric lung water content. Values were obtained at 0, 1, and 12 h in control mice challenged with intratracheal E. coli or in mice that had been preinjected with pES-NIF/liposome complex 48 h before the intratracheal E. coli challenge. Lung wet weight was continuously monitored (see MATERIALS AND METHODS) at the postchallenge times indicated. Points indicate means ± SE (n = 5). No significant changes from baseline or between the groups were observed.

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response to E. coli pneumonia. NIF binds to CD11b and CD11a in activated PMN and fully blocks PMN adhesion to endothelial cells in response to a variety of stimuli, including PMA and lipopolysaccharide (20, 44). The degree of inhibition is similar to anti-CD18 antibodies and greater than either anti-CD11a or anti-CD11b antibody (21). We induced the conditional expression of NIF in lung endothelial cells by transduction of a construct consisting of NIF cDNA driven by the endothelial cell-specific E-selectin promoter as described by us (43). Thus NIF expression was induced after the intratracheal E. coli challenge. NIF expression employing this strategy is also endothelial cell restricted because the E-selectin promoter was used to drive NIF expression (43). This approach avoids the confounding effects of anti-CD18 antibodies, genetic deletion of CD18, and altered trafficking of CD18-null PMN in irradiated mice. We showed that NIF was expressed within 1 h after challenge with E. coli, consistent with the time course of E-selectin expression (42). In a previous study, we showed that NIF expression in this manner prevented lung vascular PMN sequestration after E. coli-induced gram-negative bacteremia (9). However, after intratracheal E. coli challenge, NIF expression also prevented lung vascular PMN sequestration but had little effect on PMN extravasation. The effect of NIF in preventing lung vascular PMN sequestration is consistent with its action in blocking CD18 and inhibiting PMN adhesion (8, 28).

We conclude from these observations that activation of CD11/CD18 integrins during E. coli pneumonia plays a role in the sequestration of PMNs, but a CD11/CD18 pathway is not required for migration of PMNs. Thus the findings are consistent with previous observations in CD18−/− mice that CD11/CD18-independent pathways are used for PMN migration during acute lung inflammation (27).

There are possible limitations that need to be considered. One is that NIF is selective in blocking CD11b function; however, we have previously shown that NIF antagonizes both CD11a and CD11, and it is as effective in blocking PMN adhesion as anti-CD18 antibodies (21). We observed that NIF expression reduced pulmonary vascular PMN sequestration during E. coli pneumonia, consistent with blockade of CD18. Another concern is related to assessment of PMN migration using bronchoalveolar lavage. The study of PMN migration by examining bronchoalveolar lavage fluid may favor those cells that are unable to adhere to the airway epithelium and thus are differentially recovered by lavage compared with normal PMN. Thus we also carried out morphometric evaluation of PMN migration by counting PMN in the lung interstitium. These results indicated that NIF did not prevent PMN extravasation, consistent with the bronchoalveolar lavage data.

We also demonstrated that the mice in which CD11/CD18 blockade was induced by NIF expression during E. coli pneumonia had a survival advantage. We observed that 100% of control mice were dead at 12 h after a lethal intratracheal E. coli challenge when 40% of NIF-expressing mice were alive. The mechanism of increased survival in NIF-expressing mice after a lethal intratracheal E. coli challenge is an intriguing question. One possibility is that NIF-expressing mice are protected from PMN-induced tissue injury because NIF prevents CD18-dependent PMN activation responses. The survival advantage may also be the result of a robust host-defense response elicited by the CD11/CD18-independent PMN migration. However, increased bacterial clearance as a possible explanation of reduced mortality is unlikely to be the case since CD11b blockade has been shown to reduce bacterial clearance (32). This interesting observation of prolonged survival in the CD18-blockaded mice may be the result of many factors, including enhanced PMN activation responses or apoptosis, which will need to be further addressed in a detailed manner.
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DISCLOSURES

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