Perspective

CD11/CD18-Dependent and -Independent Neutrophil Emigration in the Lungs

How Do Neutrophils Know Which Route to Take?

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Neutrophil adhesion to pulmonary microvascular endothelial cells and migration into the distal air spaces of the lungs occur through at least two adhesion pathways: one that requires the leukocyte adhesion complex, CD11/CD18, and one that does not (1–10). Which pathway is selected appears to depend on the stimulus. The role of CD11/CD18 has been primarily established through the use of antibodies to block the function of this molecule. Neutrophil emigration in response to *Escherichia coli, E. coli* lipopolysaccharide (LPS), *Pseudomonas aeruginosa*, immunoglobulin (Ig)G immune complexes, interleukin (IL)-1, and phorbol myristate acetate occurs through adhesion pathways that require CD11/CD18 (1–7). In contrast, *Streptococcus pneumoniae*, Group B Streptococcus, *Staphylococcus aureus*, hyperoxia, C5a, and hydrochloric acid elicit neutrophil emigration through pathways not inhibited, despite blockade of the CD11/CD18 adhesion complex (1–10). Even when stimuli elicit emigration primarily through CD18-dependent pathways, anti-CD18 antibodies block neutrophil emigration by only 60 to 80%, leaving about 20 to 40% of neutrophils emigrating through CD18-independent pathways. An autopsy report of a child with complete deficiency of CD11/CD18 (leukocyte adhesion deficiency, type I) showed neutrophils and monocytes within the alveoli and small airways (11), suggesting that human neutrophils, as well as those of mice and rats, can use CD11/CD18-independent mechanisms of neutrophil emigration.

It seems important to note that this adhesion and migration most likely occurs after neutrophils are already sequestered within the pulmonary capillaries at sites of infection or injury. In contrast to the postcapillary venules of the systemic circulation, where neutrophil emigration commonly occurs, the pulmonary capillaries are the site of emigration in the pulmonary circulation, and rolling (selectin-mediated or other) does not occur (12). The initial sequestration of neutrophils appears to involve other mechanisms, and recognized adhesion molecules do not appear to play a role. The mechanisms underlying this sequestration have been discussed elsewhere (13–15) and will not be mentioned further here.

Initially, the CD11/CD18-independent adhesion pathway was thought to occur only in the lungs, perhaps because of the unique structure of the pulmonary capillary bed and the unique cell types present in the lungs, including alveolar macrophages. In the skin, anti-CD18 antibodies blocked neutrophil emigration in response to numerous stimuli, and the inhibition was complete (1, 16). However, Jaeschke and colleagues and Kubes and associates found that sequestration of neutrophils within the sinusoids of the liver during endotoxemia does not require either CD11/CD18 or the selectins (17–19). The hepatic sinusoids are thus another site where alternative pathways are important, and other sites may yet be identified.

Because monoclonal antibodies block only one site on the CD11/CD18 heterodimer, there was always the concern that another site on this complex was mediating this so-called CD11/CD18-independent neutrophil emigration. Dr. Arthur L. Beaudet generated mice with a complete deficiency of the CD18 molecule (20, 21). However, similar to the patients, these mice have extraordinarily high neutrophil counts measuring 5 to 40 or more times higher than wild-type mice, even as neonates (20, 21). Neutrophil emigration into either *E. coli* LPS or *S. pneumoniae* pneumonia was actually increased compared with wild-type mice. However, the increase was not as great as the increase in circulating count, making these data extremely difficult to interpret because the relationship between circulating and emigrating neutrophils is complex and not linear (21). In an attempt to circumvent this problem, wild-type mice were lethally irradiated, and their bone marrows were reconstituted with a mixture of CD18 null- and wild-type stem cells obtained from 14-d fetuses. These studies showed that in response to *E. coli* LPS, the CD18 null neutrophils showed a defect in their emigration into the air spaces compared with wild-type neutrophils in the same mouse, whereas CD18 null neutrophils showed no defect in emigration induced by *S. pneumoniae* (22). These studies indicate that the CD11/CD18-independent emigration observed using blocking antibodies was truly independent and did not require any part of the CD11/CD18 leukocyte complex.

The studies presented in the paper by Mackarel and colleagues in this issue of the *American Journal of Respiratory Cell and Molecular Biology* are, to our knowledge, the

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Abbreviations: formylmethionyl leucylphenylalanine, FMLP; intracellular adhesion molecule, ICAM; interferon-γ, IFN-γ; lipopolysaccharide, LPS; leukotriene B_4, TB_4; tumor necrosis factor α, TNF-α.

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first demonstration of an *in vitro* system where CD11/CD18-independent pathways of neutrophil emigration have been identified. These investigators have shown that neutrophil migration across endothelial cells isolated from human pulmonary arteries required CD11/CD18 when in response to formylmethionyl leucylphenylalamine (FMLP), but did not require this adhesion complex when in response to IL-8 or leukotriene-B_4 (LTB_4). Furthermore, CD11/CD18-independent emigration did not require β1 integrins, P-selectin, E-selectin, or the activity of neutrophil elastase or metalloproteinases. These results are very exciting for several reasons. First, they suggest that CD11/CD18-independent emigration requires only particular chemokines or leukotrienes and pulmonary arterial endothelial cells, and not necessarily a variety of other mediators or cell types, except to generate these substances. Second, the results also suggest that structural aspects of the capillaries and the alveolar wall, such as the narrow capillary diameters, are also not required. Third, they provide an *in vitro* system for examining the regulation of CD11/CD18-independent emigration.

Comparison of this *in vitro* system with *in vivo* observations is important before pursuing extensive mechanistic studies. The lack of a role for selectins mimics well the situation *in vivo*, where selectins are often not required for either CD18-dependent or -independent emigration (23, 24). Unfortunately, though, to our knowledge no studies have been published examining the adhesion pathways utilized by neutrophils in response to IL-8, LTB_4, or FMLP instilled into the distal lung parenchyma. While *E. coli* and *E. coli* LPS induce CD18-dependent emigration—suggesting that FMLP might as well—we have learned from surprising experiences that assumptions are often incorrect and each stimulus requires individual examination. Finally, the authors provide data demonstrating that expression of the CD11/CD18 complex on neutrophils was increased only during migration in response to FMLP. This observation contrasts with those made *in vivo* using ultrastructural immunohistochemistry with colloidal gold labeling, which showed that CD18 expression was not increased on sequestered intracapillary neutrophils at sites of *E. coli* pneumonia but was increased in *S. pneumoniae* pneumonia (25). Furthermore, migrated neutrophils within the alveolar space expressed increased amounts of CD18 during emigration in response to either organism (25). These holes in our knowledge (or discrepancies in the observations) should not at all discourage pursuit of this *in vitro* system but do demonstrate the complexities of neutrophil emigration and acute inflammation and the need for thoughtful studies integrating *in vitro* and *in vivo* systems.

Mackarel and colleagues used human pulmonary arterial cells to assess the role of CD11/CD18 in neutrophil emigration (26). In fact, the major site of emigration within the distal lung tissue is the pulmonary capillaries. Although technical difficulties may preclude use of microvascular cells, many differences in phenotype between endothelial cells from different sites have been identified (27, 28). For example, differences in shape between pulmonary arterial, capillary, and venous endothelial cells have been described (29). Studies in collaboration with Troy Stevens at the University of South Alabama have shown that tumor necrosis factor-α (TNF)-α–treated rat pulmonary microvascular endothelial cells undergo a rapid increase in their apparent stiffness in response to neutrophils adhering to their surface, as measured by magnetic twisting cytometry (30). In contrast, TNF-α–treated rat pulmonary arterial endothelial cells do not have such a response, despite an equivalent increase in intracellular adhesion molecule 1 (ICAM-1) expression, a major ligand for CD11/CD18, suggesting differences in the ability of ICAM-1 to signal intracellularly between these two cell types. Future studies comparing the utilization of CD18-independent emigration through microvascular and arterial pulmonary artery cells will prove interesting.

TABLE 1

CD11/CD18-dependent and -independent neutrophil emigration

Stimuli eliciting primarily CD11/CD18-dependent neutrophil emigration
Escherichia coli
E. coli lipopolysaccharide
Pseudomonas aeruginosa
IgG immune complexes
IL-1
Phorbol myristate acetate
Stimuli eliciting CD11/CD18-independent neutrophil emigration
Streptococcus pneumoniae
Staphylococcus aureus
Group B Streptococcus
Hyperoxia
C5a
Hydrochloric acid

Two questions arise from this study and similar ones. First, what determines the selection of an adhesion pathway during the acute inflammatory response? Stimuli inducing the CD11/CD18-dependent pathway increase ICAM-1 expression, suggesting perhaps that these pathways would more accurately be called ICAM-1–dependent and –independent. TNF-α expression, as well as nuclear factor κB (NF-κB) translocation from the cytosol to nuclei, appears to correlate with the use of CD11/CD18-dependent pathways, and these molecules are known to induce production of ICAM-1. In contrast, *S. pneumoniae* appears to initially induce production of interferon-γ (IFN-γ), which does not induce ICAM-1 expression on cultured human pulmonary capillary endothelial cells (C. Doerschuk, submitted manuscript). IFN-γ–deficient mice show a defect in neutrophil emigration in response to *S. pneumoniae* but not to *E. coli* or *P. aeruginosa*, and IFN-γ receptor–deficient mice also demonstrate this defect. Therefore, the cytokines initially induced by a particular stimulus may determine which adhesion pathway is employed. How the organism or other stimulus initially "contacts" the host is likely to be critical in determining the pathways of host defense.

Second, what neutrophil–endothelial adhesion molecules, if any, are used during CD11/CD18-independent adhesion? Most recognized adhesion molecules have been excluded. P-selectin, E-selectin, L-selectin, and ICAM-1 are not required, neither singly nor in combination. Re-

cent studies have also excluded VLA-4/VCAM-1 interactions, since blockade of VLA-4 mediates only a small fraction of CD11/CD18-independent neutrophil emigration (C. Doerschuk, submitted manuscript). PECAM-1 is also not required. Novel adhesion molecules may be involved, and the *in vitro* system described by Mackarel and colleagues represents an excellent opportunity to identify them. Alternatively, no adhesion molecules may be needed for neutrophil migration within the tight constraints of the pulmonary capillaries, where capillary diameters measure 5 to 9 μm and neutrophils measure 6.5 to 8 μm in diameter when spherical (31). Malawista and colleagues (32) have described a novel *in vitro* system in which neutrophil adhesion and chemotaxis along glass is examined in response to products released by lysis of red blood cells. CD11/CD18 was required when the space between the coverslip and the slide measured over 14 μm, but when the space was less than 14 μm, chemotaxis occurred despite blockade of CD11/CD18 (32). The authors called this "chimneying" and compared it to migration through three-dimensional matrices. However, electron microscopic studies have shown that neutrophils attached to the walls of capillaries may sometimes fill the lumen, but they are often flattened along one side of the capillary wall. These flattened ones still appear to be migrating, despite not touching both walls because there are pseudopods extending between endothelial cells (33, 34). Designing studies to test the hypothesis that adhesion molecules are not required *in vivo* and/or that the geometry of the capillary wall is sufficient has been difficult. However, the study by Mackarel and colleagues elegantly demonstrates that neutrophils can adhere and migrate through CD11/CD18-independent mechanisms without being constrained within a narrow space.

Finally, all CD11/CD18-independent adhesion may not occur through the same mechanisms. Numerous different mechanisms may be involved. For example, a single unifying hypothesis linking the requirement for IFN-γ in CD11/CD18-independent emigration and the induction of CD11/CD18-independent emigration by IL-8 or LTB_4 in the *in vitro* studies (or by C5a *in vivo* [2]) is not immediately obvious. Furthermore, the link between either of these situations and the chimneying effect (32) is also not apparent. In addition, whether the 20 to 40% of neutrophils that emigrate through CD11/CD18-independent pathways during inflammatory responses primarily mediated through CD11/CD18 use the same molecules and mechanisms as neutrophil emigration through purely CD11/CD18-independent pathways is also unclear. Finally, the different stimuli which induce CD11/CD18-independent emigration in the lungs, including *S. pneumoniae*, hydrochloric acid, and hyperoxia, seem unlikely to either signal through or induce a common adhesion pathway, although this possibility can not be excluded. It seems too early to generalize about stimuli and pathways, although to postulate that gram-negative organisms induce CD11/CD18-dependent neutrophil emigration, whereas gram-positive organisms elicit CD11/CD18-independent pathways is tempting.

The paper by Mackarel and colleagues is important in its careful description and initial characterization of a CD11/CD18-independent pathway of neutrophil emigration across pulmonary arterial endothelial cells. Mechanistic studies to understand the molecules involved in this pathway are likely the next step. Efforts in our laboratory are focused on identifying gene transcripts that are increased during CD11/CD18-independent but not CD11/CD18-dependent emigration using both differential display and gene microarray technology, in the hopes of better understanding these pathways. Finally, these mechanisms of neutrophil emigration have been studied only during the very acute events in the initiation of innate immunity and host defense. Whether similar mechanisms mediate host defense at later times or in chronic and recurrent inflammation remains to be determined.

Note: Morland and colleagues have since demonstrated that IL-8 and sputum elicit CD18-independent neutrophil emigration through human umbilical vein endothelial cells while FMLP induces CD18-dependent pathways of emigration (35).

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