Epithelium, Tear Down This Wall!

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The presence of bacteria within epithelial-lined compartments, such as the lung, elicits rapid recruitment of innate immune cells. In this issue of *Cell Host & Microbe*, Chun and Prince (2009) report that epithelial cells facilitate this process by reorganizing their intercellular junctions to enhance immune cell transmigration.

Polymorphonuclear leukocytes (PMNs), or neutrophils, are the first inflammatory cells to be recruited in response to infection or tissue damage. The significant role played by these innate immune cells is emphasized by the fact that PMN deficiency, whether due to genetic defects, disease, or therapy, is associated with increased risk of infection. Such infections often involve the lungs, which are continuously exposed to airborne microbes. Thus, the ability of PMNs to access the airspace in order to contain and destroy potential pathogens is critical to health.

To reach the alveolar airspaces, or any other tissue site, PMNs must first migrate from the blood stream across the vascular endothelium. The process of transendothelial migration, or migration across a monolayer of endothelial cells, has been defined in detail. Initially, tethering is accomplished by interactions between selectins on the apical surface of endothelial cells and surface carbohydrates of the PMN. PMNs then roll and are ultimately arrested on the endothelial surface by β2-integrin-mediated adhesion. Migration then occurs either through an endothelial cell, the transcellular route, or between adjacent endothelial cells, the paracellular route.

Transepithelial migration—which is required for PMNs to cross the alveolar epithelium of the lung, intestinal epithelium of the gut, and tubular epithelium of the kidney—occurs by processes similar to transendothelial migration, although there are several major differences (Zemans et al., 2008). First, because PMNs within the tissues have already crossed the endothelium, they are, by definition, at least partially activated before reaching the epithelium. Second, because the interstitial tissue the PMNs encounter after leaving the vessels is relatively static, the initial tethering and rolling adhesive interactions are unnecessary. In addition, transepithelial migration occurs exclusively via the paracellular route rather than paracellular and transcellular routes, as is the case in endothelium. There are also differences in the polarity of migration, as PMNs first encounter the apical membrane of endothelial cells, while the basolateral membrane is the initial site of interaction with epithelium. Finally, selectins (which are critical to early phases of transendothelial migration) do not appear to be involved in transepithelial migration, and some PMN surface proteins, such as CD44, enhance binding to endothelial cells to enhance migration but, conversely, limit transepithelial migration. Despite these differences, it is striking that many surface adhesive molecules involved in transendothelial migration are also important to transepithelial migration.

From the perspective of the PMN, the greatest distinction between endothelium and epithelium may be the nature of the junctions between adjacent cells. Intercellular junctions between endothelial cells are rather loose and, particularly when inflammatory mediators are present, readily allow PMN transmigration. In contrast, epithelial cells, which are charged with maintaining a barrier separating internal and external milieus, are joined to one another via the tight junction. While tight junctions may, in a tissue-specific manner, allow ions and small solutes to pass between cells, they normally exclude particles as large as cells. It has therefore been suggested that PMN-derived signaling molecules, oxidants, and proteases may trigger local dissolution of the tight junction. While data supporting this hypothesis are weak, it is noteworthy that PMNs tend to cross in small groups, perhaps at sites where the tight junction has been disrupted. Transepithelial PMN migration also appears to preferentially occur at sites where three epithelial cells meet: so-called tricellular tight junctions, which have a unique structure that may be amenable to passage of cells (Burns et al., 2003). However, the signaling events that "open" the tight junction to allow PMNs to pass have not been previously defined.

In this issue, Chun and Prince shed some light on the molecular mechanisms by which pulmonary epithelial cells accommodate paracellular PMN transmigration (Chun and Prince, 2009). Their work takes advantage of the observations, in experimental animals and human subjects, that intrapulmonary instillation of lipotechoic acid or lipopolysaccharide (ligands for Toll-like receptor (TLR) 2 and TLR4, respectively) results in rapid recruitment of PMNs into the alveolar space (Hoogerwerf et al., 2008). This has been thought to be primarily due to direct chemoattractant effects of bacterial products and the IL-8 released by airway epithelium in response to TLR ligation (Chun and Prince, 2006; Koff et al., 2008). For example, ligation of TLR2, which is expressed on the apical surface of airway epithelia, triggers intracellular Ca^{2+} release that leads to IL-8 secretion (Chun and Prince, 2006). In the present study, Chun and Prince hypothesized that TLR2 ligation might also induce airway epithelium to modulate their intercellular junctions in order to facilitate PMN transmigration. The authors show that apical exposure of cultured airway epithelial cells to heat-killed *Pseudomonas aeruginosa* or a synthetic TLR2 ligand caused marked decreases in tight junction-associated occludin as well as adherens junction-associated E-cadherin. TLR2 activation was associated with activation of the intracellular Ca^{2+}-dependent protease calpain, and in vitro analyses showed that calpain is able

to specifically degrade occludin and E-cadherin. Consistent with the functional importance of this calpain-dependent occludin and E-cadherin proteolysis, the calpain inhibitor calpeptin markedly inhibited PMN migration across cultured airway epithelium. The relevance of this phenomenon was validated in vivo by showing that *Tlr2*$^{-/-}$ or calpeptin-treated mice were deficient in airway PMN recruitment induced by *P. aeruginosa* infection. Consistent with the proposed role of TLR2-dependent calpain activation, calpeptin treatment did not further reduce PMN recruitment in *Tlr2*$^{-/-}$ mice. Thus, activation of TLR2 in airway epithelium results in calpain-dependent modulation of tight junction and adherens junction structure and facilitates PMN transmigration (Figure 1).

Chun and Prince's observations represent the first example in which epithelial intercellular junctions are modified in a manner that enhances transepithelial migration of immune cells. It is likely that this process also occurs in other epithelia, although the details may differ at specific sites, such as the gut, where some tolerance to TLR ligands is required to prevent an inappropriate response to the intestinal microbiota. What is perhaps even more surprising is the suggestion that the tight junction protein occludin may be involved in this process. Although it was the first transmembrane tight junction protein discovered, the lack of an obvious intestinal, renal, or pulmonary epithelial phenotype in *occludin*$^{-/-}$ mice has caused many to question the importance of this protein (Saitou et al., 2000). However, this is controversial, because others have correlated specific endocytosis of occludin with renal and intestinal

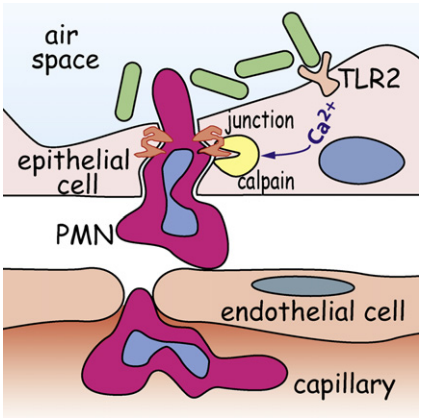

Figure 1. Migration of PMNs into the Alveolar Air Space

As described in the text, PMNs must first exit the capillary before migrating across the alveolar epithelium. In this issue, Chun and Prince (2009) demonstrate that activation of apical TLR2 causes increased intracellular Ca^{2+}, calpain activation, and degradation of the apical junctional complex proteins occludin and E-cadherin. This facilitates transepithelial PMN migration.

epithelial barrier dysfunction (Clayburgh et al., 2005; Shen and Turner, 2005). Nonetheless, the absence of a barrier defect in *occludin*$^{-/-}$ mice is consistent with Chun and Prince's observation that, despite proteolysis of some occludin and E-cadherin molecules, the barrier to ion and solute flux created by airway epithelia remains intact after TLR2 activation. One possible explanation is that occludin is most critical in restricting paracellular movement of large, cell-sized objects.

In order to further our understanding of the biological process discovered by Chun and Prince, it may be fruitful for future studies to ask whether epithelial surface molecules known to be involved in PMN transmigration are also modified by TLR2 activation, and if ligands for other TLRs are able to replace or enhance the effects of TLR2 agonists. It would, for example, be of interest to determine whether lipopolysaccharide-mediated TLR4 activation induces PMN transmigration by mechanisms that are similar to or distinct from TLR2 ligands. Thus, while questions remain, the observation that TLR2 activation causes airway epithelia to accommodate paracellular PMN migration revises the role of intercellular junctions in this process from bystander to active participant.

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