Cell signaling underlying the pathophysiology of pneumonia

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The symposium addressed the emergence of inflammation in the context of the airway epithelial cell as a consequence of bacterial interactions with receptors for microbial products and host factors such as Pseudomonas aeruginosa. The speakers addressed activation of the transcription factor, NF-κB occurring as a consequence of bacterial interactions with specific receptors, such as the Toll-like receptors and the TNF receptor 1 (Prince), or as a consequence of cytokine induction (Mizgerd). Also considered were mechanisms of bacterial virulence in the clinical setting (Wiener-Kronish) and the role of alveolar-capillary signaling mechanisms in the initiation of lung inflammation.

PATHOGEN-HOST INTERACTIONS AT THE AIRWAY EPITHELIAL SURFACE

Complex signaling circuits are activated by bacterial contact with airway epithelial cells that initiate chemokine expression to recruit phagocytes to the region of infection. Bacterial ligands also activate anti-inflammatory signals, resulting in receptor shedding, cytokine neutralization, and macrophage recruitment. Toll-like receptors (TLR) are critical in signaling bacterial infection. The Prince group has addressed the role of TLR2, which is apically displayed on airway cells and is of particular interest since it can respond to diverse types of bacteria. The TLR2 repertoire is further broadened through association with asialoGM1, which provides the GalNAcGal receptor for Pseudomonas aeruginosa pili and flagella, as well as for many other pulmonary pathogens (27). AsialoGM1 and TLR2 are colocalized within lipid rafts and are actively mobilized to the apical surface of infected airway cells where they initiate activation of NF-κB, as well as activator protein-1, cAMP response element binding protein, and CCAAT enhancer-binding protein, and release of IL-8, granulocyte-macrophage colony-stimulating factor, IL-6, and MUC-2.

The TNF-α/TNF receptor 1 (R1) cascade (Fig. 1) is also activated in airway epithelial cells by bacteria, specifically by Staphylococcus aureus protein A, which is especially abundant on bacteria isolated from patients with pneumonia. Bacteria induce the mobilization of TNFR1 to the surface of the respiratory epithelial cell. The protein A-TNFR1 interaction is essential for the pathogenesis of staphylococcal pneumonia (5). Stimulation of TNFR1 induces chemokine (IL-8) and cytokine (IL-6) production by airway cells but also activates TNF-α-converting enzyme (TACE) or ADAM-17, a protease that targets the ectodomains of many substrates including TNF-α, TNFR1, and IL6Rα. TACE-mediated shedding of IL-6Rα and its association with free IL-6 and cell surface-associated gp130 initiates “transsignaling,” a mechanism that stops polymorphonuclear leukocyte (PMN) chemokine expression and induces macrophage chemokine, CCL2, production (6). These events trigger the resolution phase of an acute inflammatory response. TACE-associated release of TNFR1 also neutralizes free TNF-α and limits the potential for ongoing TNF signaling. Bacterial induction of the TNF cascade has important anti-inflammatory effects that serve to limit the extent of PMN recruitment. Thus the airway epithelium, even in the absence of signals from professional immune cells, actively participates in both the recruitment and regulation of PMN mobilization to areas of bacterial contamination.

BACTERIA-INDUCED CYTOKINE SIGNALING

Neutrophil recruitment and plasma extravasation contribute to both bacterial clearance and inflammatory injury (Fig. 2). These innate immune functions require the expression of adhesion molecules, chemokines, and other mediators, induced by receptors for microbial products and host factors such as cytokines. Bacteria-induced cytokine signaling determines the pathophysiological outcome of pneumonia.

Cytokines regulate expression of many relevant genes by activating transcription factors such as NF-κB. The Mizgerd group has shown that in response to bacterial stimuli in the lungs, two NF-κB proteins translocate to nuclei, RelA and p50 (10, 19). RelA is essential for the transcription of chemokines and adhesion molecules that mediate neutrophil recruitment, and interrupting RelA compromises the clearance of bacteria from mouse lungs (1). In contrast, p50 limits expression of these and other proinflammatory genes (16, 17). Interrupting p50 increases mortality by exacerbating acute lung injury despite effective bacterial clearance (16). Thus a healthy outcome from lung infection requires the balance of two NF-κB proteins, RelA and p50, with opposing actions on gene expression.

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The early response cytokines TNF-α and IL-1 (α and β) activate NF-κB. A lack of signaling either from TNF-α or from both IL-1α and IL-1β is of little consequence during Escherichia coli or Streptococcus pneumoniae pneumonias (10, 18, 20). Unlike mice deficient in one or the other pathway, mice without signaling receptors for all three early response cytokines have pronounced changes during pneumonia, and their phenotypes differ depending on the infecting organism. In response to E. coli, there are modest defects in NF-κB activation or bacterial clearance, but mice are significantly protected from inflammatory injury (15). In contrast, during S. pneumoniae pneumonia, interrupting these signaling pathways substantially decreases NF-κB activation, innate immunity gene expression, and bacterial clearance (10). Thus, for both bacteria examined, TNF-α and IL-1 have essential but overlapping roles. However, the signaling pathways dependent upon them and their functional significance are microbe specific.

Other cytokines and transcription factors are also important during pneumonia. For example, IL-6 is essential to neutrophil recruitment and bacterial clearance (9). Activation of STAT3 depends in part on IL-6 during E. coli pneumonia (9), and STAT3 protects lungs from injury (8). Although IL-6 and STAT3 are functionally significant, their regulation and mechanisms of action during pneumonia remain to be determined. Finally, cytokines are influential beyond transcription, and posttranscriptional regulation of innate immunity genes will likely emerge as critical to the pathophysiology of pneumonia.

P. AERUGINOSA INFECTION: CLINICAL CONSIDERATIONS

The use of clinically isolated P. aeruginosa in experiments is prudent because strains isolated from patients have more genetic diversity than strains utilized in the laboratory. P. aeruginosa is a useful bacteria to use in experiments of acute lung injury because it is commonly found in patients who are ventilated and who have acute lung injury (3), and it is

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Fig. 1. Staphylococcus aureus protein A activates the classical TNF-α signaling cascade by interacting with TNF receptor 1 (R1) displayed on the surface of airway cells resulting in chemokine, cytokine, and mucin expression. In addition, protein A stimulates the protease activity of TACE (tumor necrosis factor-converting enzyme or ADAM-17), which cleaves and releases soluble (s) TNFR1 from the airway surface. sTNFR1 is then available to neutralize free protein A and TNF-α ligands. TRADD, TNFR-associated death domain protein; TRAF, TNF receptor-associated factor; RIP, receptor-interacting protein; ATF, activating transcription factor.

Fig. 2. Neutrophil recruitment and plasma extravasation, as observed above 24 h after infection of mouse lungs, contribute to bacterial clearance and inflammatory injury and require the expression of adhesion molecules, chemokines, and other mediators regulated by bacteria-induced cytokine signaling.

3 Presented by Jeanine Wiener-Kronish.
associated with a higher mortality in patients with nosocomial pneumonia than other bacteria (2).

*P. aeruginosa* has extensive genetic variability, allowing it to thrive in a wide variety of environments. The available genome sequence information on *P. aeruginosa* provides information on one *P. aeruginosa* strain, PAO1, a laboratory strain. Comparisons of strains to PAO1 have shown that a more virulent strain, PA14, has two pathogenicity islands, PAPI-1 and PAPI-2 (7). Notably, the large PAPI-1 island is absent from PAO1, and only part of PAPI-2 is in PAO1 (30). Four genomic islands have been identified in *P. aeruginosa*: PAGI-1, PAGI-2, PAGI-3, and the flagellum island (4). These islands can confer virulence characteristics, and they appear to be only in certain strains of *P. aeruginosa* (4).

ExoU appears to be on an 80-kb island (29), and although it appears that the genomic content of different isolates of *P. aeruginosa* genes that encode virulence determinants are highly conserved, strains appear to contain either exoS or exoU but not both genes. Strains that contain exoU are associated with a poor clinical outcome and increased rates of mortality (22), hence the rationale for evaluating specific strains associated with lung injury in patients and comparing them to strains from patients without lung problems.

Bacteriophages can also bring genetic information to various bacterial strains. Two prophages, Bacto1 and PFI, were found in the PAO1 genome (28).

Besides the pathogenicity islands or genomic islands, *P. aeruginosa* has multiple other mechanisms for virulence. Cell-associated virulence factors include pili, flagella, lipopolysaccharide, and a variety of fimbrial-related molecules (23). *P. aeruginosa* also produces products via the type I and type II secretory systems. These include hydrogen cyanide, proteases, neuraminidase, and elastase. These products are produced by the bacteria and released into the environment. *P. aeruginosa* also intoxicates eukaryotic cells by directly injecting toxins into the cytosol via the type III secretion system. The four toxins injected include ExoS, ExoT, ExoY, and ExoU. In vivo ExoU toxicity can be suppressed with phospholipase A$_2$ (PLA$_2$) inhibitors, suggested that ExoU has phospholipase A$_2$ activity (23). ExoU possesses a conserved domain homologous to patatin, a soluble storage protein of potato tubers. Interestingly, patatin is mainly stored in plant vacuoles as an inactive enzyme and is activated upon environmental stress or pathogenic infection. ExoU also has residues that align with human cytosolic phospholipase A$_2$ (cPLA$_2$) and calcium-independent PLA$_2$ (24). On the basis of site-specific mutagenesis of S142 or D344 to alanine, the prediction is that ExoU belongs to a family of enzymes that utilize a catalytic dyad (24). The substrates for ExoU appear to be phospholipids and neutral lipids (24). It is believed that the amino-terminal half of ExoU contains the catalytic domain. Notably, ExoU requires a host cell factor, which so far has not been identified (24).

Nearly 2,000 strains of *P. aeruginosa* from ~200 neonates, pediatric, and adult intubated, ventilated patients have been collected. Strains and the communities of bacteria in the endotracheal aspirates of these patients are being characterized. Strains of *P. aeruginosa* that are newly acquired in the hospital differ from those that patients have chronically. Virulence of the strains from patients with and without lung injury is being evaluated.

**ALVEOLAR-CAPILLARY SIGNALING IN THE INITIATION OF LUNG INFLAMMATION**

The initiation of pneumonia induced by inhaled bacteria is attributable to the transmission of an inflammatory signal from the distal airway to the vascular compartments. Thus introduction of bacteria such as *P. aeruginosa*, or of bacterial products in the airway, induces leukocyte migration into the alveolus within 3–4 h (26). This time course matches the time course in which chemokines such as TNF-α are secreted from alveolar macrophages, thereby providing the chemotactic signal for leukocyte recruitment (11, 13, 26). Because the airway and vascular compartments are separated by epithelial and endothelial barriers that restrict and, possibly, inhibit direct diffusion of chemokines between the compartments, it follows that the chemotactic signal has to be vectorially transmitted by cross-compartmental signaling.

The Bhattacharya group considered the role of the cytosolic Ca$^{2+}$ as the conveyor of the inflammatory signal from the alveolus to the adjoining capillary (12). The group established the isolated, blood-perfused rat lung for real-time, optical imaging of the alveocapillary region (Fig. 3). By means of catheter and micropuncture techniques, respectively, these authors separately loaded alveolar epithelial and capillary endothelial cells with the Ca$^{2+}$ fluorophore, fura 2. Ratiometric imaging provided quantifications of the cytosolic epithelial and endothelial Ca$^{2+}$ concentrations.

Microinjection of a TNF-α bolus in the alveolus generated rapid Ca$^{2+}$ increases not only in the alveolar epithelium but also in the capillary endothelium, revealing alveolar-capillary signal transmission (12). A potential artifact of the micropuncture technique, namely alveolar leakage, was ruled out by a set of studies that included the demonstration that the TNF-α effect could be blocked by pretreating the alveolus with an antibody that

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*Presented by Jahar Bhattacharya.*

**Fig. 3.** Alveolar-capillary region of a rat lung viewed by 2-photon microscopy. A, alveolus; V, venular capillary. Endothelial cells (arrow) lining a venular capillary and epithelial cells (double arrows) lining the alveolar wall are clearly evident (courtesy Jens Lindert).
blocks ligation of TNF-α receptor, TNFR1. Pretreating the capillary with the antibody failed to block the signaling effect induced by alveolar TNF-α. Furthermore, TNF-α injected in the capillary failed to induce epithelial Ca²⁺ increases in the alveolus. These findings point to the vectorial specificity of the TNF-α-induced alveolar-capillary signal transmission.

A consequence of the alveolar-capillary signaling was the surface expression of the leucocyte adhesion receptor, P-selectin, in capillary endothelium. Endothelial P-selectin is normally held in intracellular vesicles. Increase of Ca²⁺ or H₂O₂ causes P-selectin expression (21), which marks proinflammatory endothelial activation. Alveolar TNF-α increased capillary P-selectin expression in adjoining capillaries within 5 min (12), indicating that the cross-compartmental inflammatory signaling is rapid.

Characteristically, the alveolar epithelial Ca²⁺ response consisted of high-amplitude Ca²⁺ oscillations that were absent in the capillary endothelial response. This difference reflected different patterns of Ca²⁺ mobilization in the two cell types. Thus, while the epithelial response was consistent with Ca²⁺ release from endoplasmic reticular stores, the damped endothelial response pointed to direct Ca²⁺ entry. Externally applied arachidonate causes direct Ca²⁺ entry (14). Consistent with this possibility, pretreating the alveolus with inhibitors of the arachidonate precursor cPLA₂ blocked the TNF-α-induced alveolar-capillary Ca²⁺ transmission (12). Details of signaling intermediates downstream of cPLA₂, in particular the role of oxidants (25), are under evaluation.

REFERENCES


