Cell signaling underlying the pathophysiology of pneumonia

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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 in the airway induce cross-compartmental signaling that leads to inflammatory consequences. 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.

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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3 Presented by Jeanine Wiener-Kronish.
**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 Ca²⁺ 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 alveolo-capillary region (Fig. 3). By means of catheter and micropuncture techniques, respectively, these authors separately loaded alveolar epithelial and capillary endothelial cells with the Ca²⁺ fluorophore, fura 2. Ratiometric imaging provided quantifications of the cytosolic epithelial and endothelial Ca²⁺ concentrations.

Microinjection of a TNF-α bolus in the alveolus generated rapid Ca²⁺ 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.**

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**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 leukocyte 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


