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Role of the Polymorphonuclear Leukocyte: Interaction with Nosocomial Pathogens

Advances in our understanding of the role of neutrophils in lung defense have occurred in several cycles over the past century. By the turn of the century, the concept of an antibacterial defense mechanism intrinsic to the lung was well established. Although it was recognized by the early 1900s that polymorphonuclear leukocytes were prominent phagocytes in the lungs of persons dying of bacterial pneumonia, these cells were widely believed to play no role in the eradication of most bacterial challenges. The pulmonary antibacterial defenses were believed to be dependent on large, phagocytic mononuclear cells recruited to the air spaces from alveolar walls in response to bacteria. The phagocytic activity of mononuclear cells was believed to be sufficient for most challenges; circulating leukocytes were involved only when mononuclear cells were unable to handle the challenge (1).

Phagocytic Cells Involved in Pulmonary Bacterial Clearance

The concept of a pulmonary defense mechanism against bacteria was extended in a series of elegant studies on the histogenesis of pneumonia (2–11). While quantitative bacteriologic methods were not utilized in these studies, large numbers of polymorphonuclear leukocytes were demonstrated in alveolar spaces 1.5 hours after bacterial inoculation. The importance of these polymorphonuclear leukocytes in lung defense against bacteria was suggested by photomicrographs showing free polymorphonuclear leukocytes phagocytosing organisms in alveolar spaces (10, 11). With the development of quantitative aerosol inoculation techniques, it became possible to study the ability of the lung to clear bacteria over time (12–14). Aerosolized *Staphylococcus aureus* (5×10^4) did not evoke a polymorphonuclear leukocyte response. All intracellular organisms identified by light microscopy within 4 hours of inoculation were found inside alveolar macrophages (13, 15). Similar results were reported for *Proteus mirabilis* (13). These investigations concluded that the alveolar macrophage was the principle defender of the lung against bacteria.

The first observation that aerosol inoculation of bacteria could result in a polymorphonuclear leukocyte response was made in experiments in which approximately 8×10^4 *Pseudomonas aeruginosa* were inoculated into the lung (16). The phagocytic response has subsequently been quantified in studies using both histological and bronchoalveolar lavage techniques (17, 18). While polymorphonuclear leukocytes played no role in the pulmonary clearance of inocula of up to 4×10^6 *Staphylococcus aureus*, aerosols of gram-negative bacteria elicited an impressive polymorphonuclear leukocyte inflammatory response in bronchi and alveoli. An additional determinate of a polymorphonuclear leukocyte response was the inoculum size (18, 19). Following aerosol and bolus inoculation, both bacterial clearance and the magnitude of the polymorphonuclear leukocyte response were related to the number of bacteria deposited in the lung. Thus, the generation of a polymorphonuclear leukocyte response following bacterial inoculation was dependent on the bacterial species and the inoculum size. These studies indicated that both alveolar macrophages and polymorphonuclear leukocytes were involved in lung defense against pathogenic organisms. The relative importance of each phagocyte was uncertain. The functional significance of the recruited polymorphonuclear leukocytes was demonstrated by selective depletion of polymorphonuclear leukocytes. Neutropenic animals cleared inocula of *Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Haemophilus influenzae* (20–22). Neutropenic animals could not clear gram-negative organisms, even in the presence of antibiotics (20).

These observations demonstrate that a dual phagocytic system is involved in pulmonary antibacterial responses. While alveolar macrophages can clear certain inocula of bacteria, an inflammatory response is usually generated by pathogenic organisms. Following an inoculation with virulent organisms, polymorphonuclear leukocytes recruited from the circulation are required for effective clearance of the organisms. Thus, the ability to rapidly recruit polymorphonuclear leukocytes into the pulmonary parenchyma represents a major component of the early defense against most bacteria.

Recruitment of Polymorphonuclear Leukocytes

Polymorphonuclear leukocytes are rare in the air spaces of normal lungs (23) and represent less than 2% of the cells present in normal bronchoalveolar lavage fluid. It is likely that the presence of bacteria

in alveolar spaces triggers the generation of chemotactic factors, which are responsible for polymorphonuclear leukocyte accumulation in the lung within the air spaces or the interstitium of the lung. Chemotactic activity has been demonstrated in bronchoalveolar lavage fluids following intrapulmonary inoculation of both gram-positive and gram-negative organisms (22, 24, 25). Increases in chemotactic activity preceded the accumulation of polymorphonuclear leukocytes. Furthermore, the number of polymorphonuclear leukocytes and the amount of chemotactic activity in bronchoalveolar lavage fluids could be correlated. Thus, the generation of chemotactic factors in the pulmonary air spaces appears to mediate the rapid recruitment of polymorphonuclear leukocytes.

Potential chemotactic factors are shown in Table 1. The role of the complement system in polymorphonuclear leukocyte recruitment has been evaluated using congenic C5 sufficient (C5+) and C5 deficient (C5−) mice, which differ only at the locus that determines the presence or absence of the C5 molecule (26). Products of the C5 molecule have been shown to be important in polymorphonuclear leukocyte recruitment in murine lungs following inoculation with gram-positive and gram-negative bacteria (27, 28). The mechanism responsible for cleavage of C5 in the lung is unknown. Bacteria might generate C5 fragment by activation of an alternative pathway. Alternatively, proteinases derived from alveolar macrophages or neutrophil granules might cleave C5 without activation of the remainder of the complement pathway (29, 30).

Chemotaxins other than C5 fragments have also been demonstrated to be important in polymorphonuclear leukocyte recruitment following bacterial challenge. No differences in polymorphonuclear leukocyte recruitment were found in C5+ and C5− mice following inoculation of 10^6 or 10^7 *Staphylococcus aureus* (25). Additionally, following gram-negative bacterial challenge C5-mice generated significant but delayed polymorphonuclear leukocyte responses that were associated with significant levels of chemotactic activity in bronchoalveolar lavage fluid (22, 28). The chemotaxin(s) involved in the recruitment of polymorphonuclear leukocytes following challenge with *Staphylococcus aureus* probably include alveolar macrophage-derived chemotactic factor for neutrophils activity (31, 32) and/or macrophage-generated products of the lipoxygenase pathway (33–36). Additionally, alveolar macrophages generate platelet-activating factor (37, 38) and plasminogen activator (39), which may function as chemotaxins. Secreted products from recruited polymorphonuclear leukocytes may also have important roles (40–43), particularly in the later phases of the response. Finally, chemotaxins generated by bacteria might be also involved (44–46).

In summary, recruitment of polymorphonuclear leukocytes following bacterial challenge to the lung involves multiple chemotaxins. The importance of these chemotaxins likely varies for differing bacterial species. Additionally, it is likely that more than one pathway is involved for any given bacterium. Complement components are important in the initial 4 to 6 hours of the response for most but not all bacteria. Chemotactic factors other than complement are present in the air spaces. These factors are important in the later (12–24 h) phases of the response and are probably involved in the early responses as well.

While the major components involved in the generation of the inflammatory response are being identified, large gaps in our knowledge exist. Little is known about the regulatory events that control cell-secreted chemotaxins. Further studies of the genetic regulation of these chemotactic factors as well as other amplifying and inhibiting factors are required. Additionally, the physical properties of chemotaxins have not been studied. Studies to assess lipid solubility, molecular size, charge and diffusability are needed. Until these studies are performed, it will be difficult to determine whether chemotactic factors can actually cross the alveolar epithelium and pulmonary interstitium and reach the circulation to attract inflammatory cells.

Table 1: Potential chemotactic factors involved in neutrophil recruitment.

Complement pathway
C5A, C5A des Arg
Fibrinolytic/kinin pathway
kallikrein
plasminogen activator
Macrophages
AMCFN
5-HETE
11-HETE
leukotriene B_4
platelet activating factor
Granulocyte products
cell-derived, stable conversion of C5 to C5a
Products of bacterial growth

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