Cytokines · 2

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Cytokines and lung inflammation: mechanisms of neutrophil recruitment to the lung

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Inflammation within the lung represents the host's response to several insults including trauma, infection, autoimmune disorders, adult respiratory distress syndrome (ARDS) associated with sepsis or multiorgan failure, cancer, allograft rejection, and ischaemia reperfusion injury. Although historically the lung has been perceived as an organ primarily involved in gas exchange, its role in mediating host defence has only recently been appreciated. The airway, with its mucociliary clearance and the extensive alveolar capillary membrane composed of both immune and non-immune cells, functions as an important barrier against inhaled and haematogenous challenges. The pulmonary response to these inflammatory stimuli ultimately affects host survival since the lung must maintain its structural integrity for gas exchange.

The capacity of the lung to generate an acute inflammatory response is necessary to ensure adequate clearance of offending agents. For example, the host response to a bacterial pneumonitis is characterised by a fulminate, acute inflammatory reaction. The histopathology of this pneumonia is composed of proteinaceous exudate and massive neutrophil infiltration leading to consolidation of the lung. Once the inciting agent is cleared, the inflammatory reaction resolves and normal repair and tissue remodelling occurs. This re-establishes normal lung function without the development of chronic pulmonary fibrosis. In contrast, an exuberant acute inflammatory reaction such as ARDS may culminate in severe lung injury which ultimately affects host survival. Although the basic mechanisms operative in mediating this acute pulmonary inflammation remain to be fully elucidated, the participation of both immune and non-immune cells in the generation of reactive oxygen metabolites, lipids, and protein mediators of inflammation are essential to the full expression of this inflammatory response. In this review we will focus on recent advances in inflammation research that address mechanisms of cytokine induced neutrophil recruitment into the lung during the pathogenesis of acute pulmonary inflammation.

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Mechanisms operative in the recruitment of neutrophils into the lung

The recruitment of neutrophils to sites of acute lung injury is dependent upon a complex series of events which includes the following steps: endothelial cell activation and expression of endothelial cell derived neutrophil adhesion molecules, neutrophil-endothelial cell adhesion, neutrophil activation and expression of neutrophil derived adhesion molecules, neutrophil diapedesis, and neutrophil migration beyond the vascular barrier via established chemotactic gradients.

INTERACTIONS BETWEEN NEUTROPHILS AND ENDOTHELIAL CELLS

Adhesion interactions between neutrophils and endothelial cells is a prerequisite event for successful neutrophil extravasation at sites of inflammation. Only within the last decade has there been significant progress in understanding the basic underlying mechanisms of neutrophil-endothelial cell interaction. The development of monoclonal antibody technology and the discovery of patients with inherited leucocyte adhesion deficiency (LAD) have provided insight into the molecular mechanisms of neutrophil-endothelial cell adhesion events. Patients with LAD have a significant circulating leucocytosis and recurrent clinical episodes of sepsis which are characterised by the failure to form "pus" at sites of infection. This inherited disease is usually fatal in childhood.[1 2] The molecular basis for the disease results from genetic mutations within the leucocyte β_2 integrin family (mutations of the common β_2 subunit).[1 2]

NEUTROPHIL DERIVED ADHESION MOLECULES

The leucocyte β_2 integrin adhesion molecule family consists of a complex group of heterodimeric glycoproteins that are expressed only on the surface of leucocytes. The three members of this leucocyte integrin family have a variable α chain and a constant β chain with the following cluster designations: CD11a/CD18, CD11b/CD18, and CD11c/CD18.[2–6]

While CD11a/CD18 is expressed on all leucocytes, only CD11b/CD18 (Mac-1) appears to be significantly expressed on neutrophils.[2-6] Neutrophils have a substantial pool of CD11b/CD18 present within secondary and tertiary granules.[5-7] Activation of neutrophils by activating or chemotactic factors results in a rapid translocation of CD11b/CD18 to the cell surface.[5 6] The ligand/receptor for neutrophil derived CD11b/CD18 is the split product of complement, iC3b, and the intracellular adhesion molecule 1 (ICAM-1) found on the surface of both immune and non-immune cells.[6] Studies investigating the role of leucocyte β_2 integrins during the pathogenesis of acute lung injury have employed specific monoclonal antibodies against either the variable α chain or the constant β chain of the CD11b/CD18 complex. These studies have shown that anti-CD11b and anti-CD18 antibodies are critical in attenuating neutrophil dependent pulmonary vascular permeability, haemorrhage, and neutrophil accumulation in the lung.[8-11]

Recent investigations have identified an additional group of adhesion molecules that mediate endothelial adhesion independently of leucocyte β_2 integrin. These adhesion molecules have been identified as selectins or LEC-CAMs (lectin, epidermal growth factor, complement, cellular adhesion molecules).[7 12 13] The three members of the selectin family that are important in mediating neutrophil-endothelial cell adhesion include L-selectin (leucocyte endothelial cell adhesion molecule 1; LECAM-1), E-selectin (endothelial leucocyte adhesion molecule 1; ELAM-1), and P-selectin (granule membrane protein-140; GMP-140 or platelet activation dependent granule to external membrane; PADGEM). While L-selectin is constitutively expressed on the cell surface of leucocytes, the expression of E-selectin must be induced on the surface of activated endothelial cells by either endotoxin, tumour necrosis factor α (TNFα) or interleukin 1 (IL-1), whereas P-selectin is rapidly expressed after release from either the α granules of platelets or Weibal-Palade bodies of endothelial cells.[14] L-selectin, originally described as the lymphocyte homing receptor (MEL-14), has been identified on human neutrophils.[12 13] While the binding of neutrophils to endothelium appears to be dependent upon either the leucocyte β_2 integrin family or L-selectin molecules, recent studies have shown the importance of the combination of these adhesion molecules in neutrophil-endothelial cell adhesion and transendothelial migration. Neutrophil derived L-selectin interaction with activated endothelium is an early event leading to margination or "rolling" under conditions of heightened shear force.[13 15 16] Neutrophil derived β_2 integrins apparently have no effect on the early margination. However, they appear to be essential in promoting stable intravascular adhesion and transendothelial cell migration.[6 7 16]

ENDOTHELIAL CELL DERIVED ADHESION MOLECULES

ICAM-1, a member of the immunoglobulin supergene family, originally described as the counter-receptor for CD11a/CD18, is an important ligand/receptor for CD11b/CD18 expressed on neutrophils. ICAM-1 is found constitutively expressed on endothelial cells and is upregulated in response to endotoxin, TNF, or IL-1.[5 6] It is also present on the surface of mononuclear phagocytes and other non-immune cells such as fibroblasts and epithelial cells.[6] This adhesion molecule has an important role in mediating neutrophil dependent inflammatory events in the lung.[6] Neutralising monoclonal antibodies to either CD11b, CD18, or ICAM-1 in an animal model of lung injury have been shown to attenuate neutrophil dependent injury.[9] These findings support the notion that neutrophil derived β_2 integrins and endothelial cell derived ICAM-1 are essential in neutrophil dependent lung injury.

E-selectin is synthesised and expressed on the surface of endothelial cells in response to endotoxin, TNF, or IL-1.[12 13 15-18] This cell surface expression of E-selectin appears to be more rapid than the expression of endothelial cell derived ICAM-1.[6] In contrast, P-selectin is rapidly mobilised to the surface of endothelial cells following exposure to split products of complement, thrombin, or histamine.[2-14] Both E-selectin and P-selectin are important endothelial surface adhesion molecules for the promotion of neutrophil adhesion, and this mechanism is dependent upon the recognition of sialylated derivatives of the Lewis X oligosaccharide (sLe^x) on the L-selectin molecule.[12 15-19] These findings provide evidence that ligand/receptor relationships between neutrophil derived L-selectin and the vascular selectins E-selectin and P-selectin may be an essential mechanism of neutrophil extravasation at sites of acute lung inflammation.

A potential paradigm for neutrophil extravasation has recently been proposed that incorporates three or more sequential steps.[16] Firstly, activation of the endothelium in response to the local generation of TNF or IL-1 leads to expression of endothelial cell derived selectins and ICAM-1. Constitutively present, neutrophil derived L-selectin interacts with vascular selectins leading to the "rolling" effect. Secondly, local generation of neutrophil specific chemotactic cytokines such as interleukin 8 (IL-8) results in the activation of neutrophils in the intravascular compartment, upregulation of neutrophil derived β_2 integrins, and concomitant shedding of cell surface L-selectin. Thirdly, the interaction of neutrophil derived β_2 integrin with its receptor/ligand ICAM-1 results in the rapid arrest and activation dependent neutrophil adhesion. The subsequent steps leading to diapedesis and migration beyond the vascular compartment may be dependent upon both continued expression of β_2 integrins and the movement along a neutrophil specific (IL-8) chemotactic cytokine concentration gradient.

CYTOKINES MODULATING NEUTROPHIL RECRUITMENT INTO THE LUNG: TNF, IL-1, IL-1 RECEPTOR ANTAGONIST, AND IL-8

Cytokines comprise a diverse group of biologically active polypeptides that are instrumental in the evolution of an acute inflammatory response. To illustrate potentially important cytokine networks operative in the lung that mediate neutrophil recruitment we have focused our discussion on early response cytokines (IL-1 and TNF), IL-1 receptor antagonist (IRAP), and the potent neutrophil activating/chemotactic factor IL-8.

Although biochemically different, TNF and IL-1 have pleiotropic and overlapping effects on several cellular functions.[20-25] At sites of local inflammation these cytokines are important as early response mediators in regulating cellular function and dictating events leading to the initiation, maintenance, and repair of tissue injury. In contrast, the exaggerated systemic release of TNF and IL-1 can result in multiorgan injury and increased host morbidity and mortality. TNF and IL-1 therefore have a broad spectrum of biological activity that influences the outcome of an inflammatory response. Several studies have found a significant correlation between serum TNF and IL-1 levels and the severity of ARDS and mortality due to multiorgan failure.[26-30] In addition, inhibition of endogenously produced TNF during bacteria induced septic shock has been shown to attenuate significantly the pathogenesis of multiorgan injury and mortality.[29 31] Interestingly, our laboratory has studied the endogenous expression and regulation of TNF from a murine model of endotoxaemia and has shown that TNF is rapidly produced after an LD_{100} infusion of endotoxin.[32] Peak levels of TNF were seen within one hour, followed by a rapid decline to relatively unmeasurable levels by eight hours. Similar findings have been seen in human volunteer subjects injected with low doses of endotoxin.[33] Although these results suggest that TNF is under strict regulation, the overexpression of TNF and IL-1, in the context of septic shock, are potent inflammatory cytokines that can trigger a cascade of events leading to the accumulation of pulmonary neutrophils and acute lung injury.

Recent investigations of an IL-1 inhibitor have led to the isolation, purification, cloning, and expression of an IL-1 receptor antagonist (IL-1ra).[35-40] IL-1ra is a 22 kDa polypeptide that has 40% homology with IL-1β and has been shown to be produced by peripheral blood monocytes or monocytic tumour cell lines in response to either endotoxin or adherent IgG.[35 39 40] The inhibitory activity of IL-1ra appears to be at the level of competitive occupation of the IL-1 receptor without evidence for agonist activity.[35 39 40] In vitro IL-1ra has been shown to inhibit IL-1 induced neutrophil adhesion to endothelial cells.[35 39 40] In vivo it has been shown to be a potent inhibitor of *Escherichia coli* mediated septic shock and lung injury.[41] Interestingly, IL-1ra has been found to attenuate the neutrophilic alveolitis associated with the intratracheal administration of endotoxin or IL-1.[42] These findings suggest that IL-1ra has an important immunomodulating influence on IL-1 dependent inflammation, and its production in the lung may play a part in the pathogenesis of acute lung injury.

The salient feature of TNF and IL-1 dependent acute inflammation in the lung appears to be the sequestration of neutrophils and neutrophil dependent lung injury. Although TNF and IL-1 were initially reported to be chemotactic for neutrophils,[43] recent studies by several investigators have now shown that neither IL-1 nor TNF has direct chemotactic activity for neutrophils in vitro.[44] These findings would suggest that cytokine networks may be operative in vivo that are dependent upon the initial expression of early response cytokines (TNF and IL-1) followed by the generation of distal mediators that influence neutrophil chemotaxis. Confusion in this area has been clarified by the isolation, purification, cloning, and expression of IL-8. IL-8 belongs to a unique supergene family that includes a number of peptide analogues that have in common four conserved cysteine residues in identical location, with the first pair of cysteines separated by one amino acid.[45 46] The other members of this C-X-C chemokine supergene family that appear to have significant neutrophil chemotactic activity include neutrophil activating peptide 2 (NAP-2) which is formed by proteolytic processing from platelet basic protein or connective tissue activating peptide III (CTAP-III) released from platelet α granules,[45-47] GROα which was originally described as a mitogen for human melanoma cells,[48] and ENA-78 which has recently been isolated from a pulmonary epithelial cell.[47] Two other members of this family include platelet factor 4 (PF-4) and interferon (IFN)γ inducible peptide 10 (γIP-10).[45 46]

IL-8, an 8·0 kDa polypeptide, is initially synthesised as a 99-amino acid precursor with a characteristic leader sequence of 22 amino acids that subsequently undergoes proteolytic *N* terminal cleavage to either 77, 72, or 69 amino acid forms.[45 46] In addition to being a potent chemoattractant and activating cytokine for neutrophils, it has a 10 to 100 fold increase in potency as a lymphocyte chemotaxin.[45 46] IL-8 maintains its biological activity in the presence of significant changes in pH and resists mild proteolytic degradation compared with other known chemotactic factors. This suggests that the production of IL-8 at in vivo sites of acute inflammation may have prolonged biological activity for the recruitment of neutrophils. As is the case with other known neutrophil chemotactic factors, IL-8 can activate neutrophils via a GTP binding protein. This process is dependent on both Ca^{2+} and protein kinase C.[45 46] While original investigations isolated IL-8 from peripheral blood monocytes,[45 49] subsequent studies have identified the expression of this cytokine from several other cellular sources. Some of these cells are analogous to the

major cellular constituents of the alveolar capillary wall including endothelial cells,[50] fibroblasts,[51 52] epithelial cells,[53] alveolar macrophages,[54] and neutrophils.[55 56] IL-8 production by these cells is stimulus specific. Endothelial cells, alveolar macrophages, and neutrophils produce IL-8 in response to endotoxin, TNF, or IL-1, whereas pulmonary fibroblasts and epithelial cells synthesise this cytokine only in response to the host derived stimuli TNF or IL-1. These findings support the notion that non-immune cells of the lung, once thought of as "targets" to TNF or IL-1, actually participate as effector cells in the production of a potent neutrophil activating/chemotaxin. Recently, Metinko and colleagues[57] have shown that hyperoxia can lead to an induction of IL-8 gene expression with a four fold increase in IL-8 production by mononuclear cells compared with normoxic conditions. In addition, endotoxin was found to significantly potentiate this hyperoxic response. These findings suggest that, under conditions simulating Gram negative bacteria induced lung injury, the addition of an oxidant stress such as supplemental oxygen may have a potentiating influence on the production of a potent neutrophil chemotactic cytokine.

Conclusion: potential scenario for neutrophil recruitment into the lung

Several scenarios can be considered for the recruitment of neutrophils to the lung during either local inflammation (pneumonia) or systemic inflammation that results in acute lung injury compatible with ARDS. In Gram negative bacterial pneumonia the release of endotoxin stimulates the alveolar macrophage to produce IL-8, TNF, and IL-1. TNF or IL-1 act in either an autocrine or paracrine fashion to stimulate contiguous non-immune cells to express adhesion molecules for neutrophils. IL-8 expression results in upregulation of neutrophil derived CD11a/CD18 complex and simultaneous shedding of cell surface L-selectin. The maintenance of an IL-8 dependent chemotactic gradient by both the immune and non-immune cellular constituents of the alveolar capillary membrane provides the chemotactic signal for an amplified recruitment of neutrophils into the alveolar space. In contrast, endotoxaemia in the context of septic shock results in the exaggerated systemic release of early response cytokines, TNF and IL-1, and the chemotactic cytokine, IL-8. In addition, endotoxaemia together with cytokines (TNF, IL-1, and IL-8) results in lung microvascular endothelial cell activation, neutrophil aggregation, neutrophil-endothelial cell adhesion and activation. This subsequently leads to lung microvascular injury, augmented vascular permeability and oedema formation, enhanced neutrophil adhesion, and production of IL-8 by the cellular constitutents of the alveolar capillary wall. This acute lung injury may be further accentuated by oxygen supplementation leading to potentiated oxidant stress, formation of reactive oxygen intermediates, and augmented production of IL-8. IL-8 generated in this manner upregulates neutrophil CD11a/CD18 complex and establishes a chemotactic gradient, leading to recruitment and activation of neutrophils contributing further to lung injury. Continuing research in the area of adhesion molecule expression and cytokine production and regulation should therefore provide a more precise mechanism of the events that mediate acute pulmonary inflammation.

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