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Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs

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The recruitment of leukocytes to an extravascular destination requires intercellular communication between tissue cells and leukocytes. The molecules mediating this intercellular communication play differing roles in recruiting different types of leukocytes, in response to different stimuli, in different tissues, and in different hosts. The present communication reviews the adhesion molecules, chemokines, other cytokines, and NF-κB proteins which regulate the recruitment of neutrophils elicited by bacteria in the lungs.

Key words: adhesion molecules / cytokines / innate immunity / NF-κB / pneumonia

Contextual specificity of leukocyte recruitment

Leukocyte recruitment requires signals that direct leukocytes out of the blood and into and through the tissue to a desired site. Infection, injury, and inflammation induce the elaboration of adhesion molecules and chemoattractants which guide the migration of leukocytes expressing surface receptors for these molecules (see References 1,2 for overview). Leukocyte recruitment is dependent on such intercellular signalling molecules, but the roles of particular molecules vary and are specific to multiple parameters, including the following:

Cell

The recruitment of different types of leukocytes within a given setting may be mediated by distinct sets of molecules. For example: during ocular onchocerciasis, the recruitment of neutrophils requires platelet-endothelial cell adhesion molecule (PECAM)-1 but not intercellular adhesion molecule (ICAM)-1 whereas the recruitment of eosinophils requires ICAM-1 but not PECAM-1;[3] after intratracheal instillation of recombinant chemokine, macrophage inflammatory protein (MIP)-2 recruits neutrophils[4] whereas monocyte chemoattractant protein (MCP)-1 recruits monocytes;[5] during *Streptococcus pneumoniae* pneumonia, blockade of multiple CC chemokines decreases the recruitment of monocytes but not of neutrophils.[6]

Tissue

Recruitment of a single type of leukocyte by a given stimulus may be mediated by distinct sets of molecules in different organs or tissues. For example: neutrophil recruitment elicited by *S. pneumoniae* requires E- or P-selectins and CD18 in abdominal tissues but not in the lungs;[7–9] eosinophil recruitment elicited by interleukin (IL)-4 requires vascular cell adhesion molecule (VCAM)-1 in the skin but not in the pleural space;[10] neutrophil recruitment elicited by IgG immune complexes requires complement C3 in the lungs but not in the skin.[11]

Stimulus

Recruitment of a single type of leukocyte in a given tissue may be mediated by distinct sets of molecules in response to different stimuli. For example: neutrophil recruitment in the lungs requires CD18 when elicited by *Escherichia coli* lipopolysaccharide (LPS) or *Pseudomonas aeruginosa*, but not when elicited by *S. pneumoniae*;[12] neutrophil recruitment in the lungs requires CD49b and CD49d when elicited by the chemokine KC, but not when elicited by LPS.[13]

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Host

Recruitment of a single cell-type within a specific tissue by a given stimulus may be mediated by distinct sets of molecules in different hosts (with differing genetic and/or environmental constraints). For example: neutrophil recruitment typically requires CD18 during acute *P. aeruginosa* pneumonia, but not if the host was previously infected in the same site with that organism;[14] neutrophil recruitment typically requires E- and P-selectins during acute dermatitis, but not if the hosts have spontaneous or experimental chronic dermal lesions elsewhere;[15] neutrophils express CCR1 and CCR2 receptors and respond to the chemokine MCP-1 in rats with chronic vasculitis, but not in naïve rats.[16]

As the above examples illustrate, mammals have a diverse set of molecular pathways available for responding to wide varieties of insults, injuries, and infections. In this respect, an element of 'specificity' underlies even 'innate' (non-specific) immune responses. This contextual specificity necessitates that molecules regulating leukocyte recruitment are studied in integrated, relevant, well-defined experimental systems. The present communication reviews the molecular signals which mediate the emigration of neutrophils in the lungs in response to bacteria or bacterial products in the air spaces.

Neutrophils in the lungs

Even in the absence of pulmonary inflammation, neutrophils are concentrated in the pulmonary capillaries compared to the systemic blood.[17] This concentration of neutrophils likely results from the narrow diameter of pulmonary capillaries relative to spherical neutrophils, suggesting that neutrophil transit through the pulmonary capillaries is dependent on cellular deformation. Inflammatory stimuli, such as bacteria in the lungs or blood, increase the numbers of neutrophils within the pulmonary capillaries. This accumulation results from neutrophil stiffening, trapping neutrophils within the pulmonary capillaries, and adhesion to endothelial cells, prolonging neutrophil retention. When inflammatory stimuli (such as microbes) are present in the alveolar air spaces, neutrophils emigrate from the pulmonary capillaries.[18] At least one anatomic pathway by which neutrophils emigrate during respiratory infection is between endothelial cells, through pre-existing holes in the sub-endothelial basement membrane, along the surface of interstitial fibroblasts to pre-existing holes in the sub-epithelial basement membrane, between type 1 and type 2 alveolar epithelial cells, and into the alveolar air space.[19,20] Some molecular interactions which induce neutrophils to make such journeys during respiratory infection are summarized in Figure 1 and discussed further below.

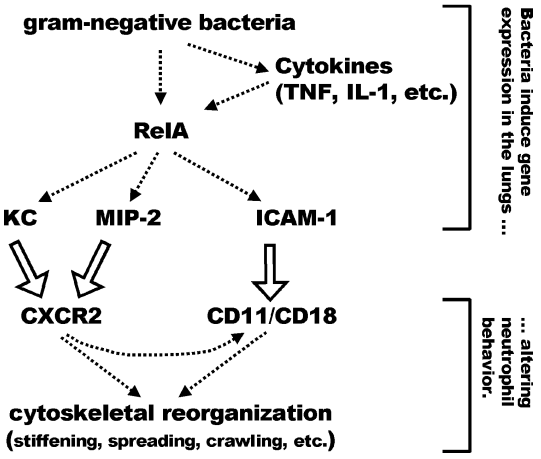

Figure 1. Molecular mechanisms by which neutrophil recruitment is stimulated by gram-negative bacteria in rodent lungs. Neutrophils constitutively express the receptors CXCR2 and CD11/CD18. Upon ligand binding, CXCR2 induces 'inside–out' signalling through integrins including CD11/CD18 (enhancing adhesion), and signalling pathways from both CXCR2 and CD11/CD18 induce cytoskeletal reorganizations directing neutrophil recruitment. The expression of ligands for these receptors, including the chemokines KC and MIP-2 and the adhesion molecule ICAM-1, is induced by gram-negative bacteria in the lungs and by cytokines elicited by these bacteria. Interrupting the function of CXCR2, KC, MIP-2, CD11/CD18, or ICAM-1 compromises neutrophil recruitment elicited by gram-negative bacteria or LPS in the lungs. The NF-κB subunit RelA activates gene transcription and is necessary for KC, MIP-2, and ICAM-1 expression and neutrophil recruitment elicited by bacterial LPS in the lungs.

Neutrophil recruitment during pneumonia: adhesion molecules

The recruitment of leukocytes requires them to transiently attach to endothelial cells and then subsequently to cells and substrates in the extravascular tissue. Cells attach to other cells and to extracellular matrix components using adhesion molecules.[1]

Selectins are lectin-like adhesion molecules which bind modified glycoconjugate ligands rapidly but briefly. Under flow conditions, selectin–ligand interactions result in rolling of neutrophils along endothelial surfaces, which can be important in emigration of neutrophils from postcapillary venules. However, rolling of neutrophils along the endothelium is unlikely to contribute to emigration from the pulmonary capillaries, since geometric constraints preclude such rolling.[17] In addition to facilitating rolling, selectins can also function as signal-inducing receptors.[21] Perhaps because of this, inhibition of selectin–ligand interactions compromises neutrophil emigration in the distal lung during some inflammatory reactions, such as that elicited by intrapulmonary IgG immune complexes.[22] Thus, selectins could be necessary for neutrophil emigration elicited by bacteria in the lungs, even in the absence of rolling requirements.

This hypothesis was tested by comparing neutrophil emigration elicited by *S. pneumoniae* in the lungs of wild type (WT) mice and mice deficient in both E- and P-selectins, which are typically expressed by endothelial cells at sites of inflammation. In contrast to acute neutrophil emigration elicited in the peritoneal cavity by *S. pneumoniae* or thioglycollate[8] or in the skin by croton oil,[15] neutrophil emigration elicited by *S. pneumoniae* in the lungs is not compromised by the combined deficiency of E- and P-selectins.[9] Furthermore, fucoidin, which inhibits the remaining selectin molecule (L-selectin, expressed by leukocytes), does not decrease neutrophil emigration in *S. pneumoniae*-infected lungs of mice deficient in both E- and P-selectins.[9] Thus, *S. pneumoniae* in the lungs elicits neutrophil emigration which does not require selectins.

Integrins are heterodimeric adhesion molecules composed of transmembrane α and β chains. The β_2 chain (CD18), expressed exclusively by leukocytes, pairs with 1 of the 4 α chains of the CD11 family. CD11/CD18 molecules mediate firm adhesion to endothelial cells by binding diverse ligands including ICAM-1. CD11/CD18 adhesion molecules are essential to neutrophil emigration in many settings.[1] Studies using function-blocking antibodies suggest that CD11/CD18 adhesion molecules contribute to neutrophil recruitment in the lung, but the relative role of CD11/CD18 is specific to the stimulus initiating inflammation. Blocking antibodies against CD11/CD18 decrease neutrophil emigration to ~30% of control values after instillation of *E. coli*, *E. coli* LPS, or *P. aeruginosa*,[7,23,24] whereas they do not affect emigration elicited by *S. pneumoniae*, Group B *Streptococcus*, or *Staphylococcus aureus*.[7,24,25]

Mice with a gene-targeted deficiency of CD18[26] provide an independent tool, subject to different sets of limitations than blocking antibodies, for studying CD11/CD18 function *in vivo*. Leukocytes from CD18-deficient mice do not express CD11/CD18. In contrast to expectations from the blocking antibody studies, CD18-deficient mice demonstrate a significant increase in numbers of emigrated neutrophils compared to WT in response to either *E. coli* or *S. pneumoniae* in the lungs.[27] These results definitively confirm that CD11/CD18-independent pathways for neutrophil emigration can be elicited by bacteria in the lungs, but they fail to demonstrate a role for CD11/CD18-dependent pathways, perhaps due to phenotypic alterations from CD18 deficiency.

CD18 deficiency escalates peripheral blood neutrophil counts, due to roles of CD11/CD18 in regulating both immune and hematopoietic functions.[28] This peripheral blood neutrophilia makes it difficult or impossible to collect appropriate control values or to derive an 'expected' value for emigrated neutrophils in tissues of CD18-deficient animals. In order to directly compare the abilities of WT and CD18-deficient neutrophils to emigrate in response to stimuli in the lungs, mice with both types of neutrophils circulating in their blood were generated. C57BL/6 mice were lethally irradiated, and their hematopoietic systems were reconstituted from mixtures of WT and CD18-deficient fetal liver cells. After intratracheal instillation of *E. coli* LPS or of *P. aeruginosa*, a smaller fraction of emigrated neutrophils were CD18-deficient compared to the fraction of circulating neutrophils,[12] indicating that CD18-deficient neutrophils are compromised in their ability to emigrate compared to WT cells. In contrast, after instillation of *S. pneumoniae*, there were no significant differences in the fractions of emigrated and circulating neutrophils that were CD18-deficient,[12] indicating that CD18-deficient neutrophils are as capable as WT neutrophils of emigrating in response to this stimulus. Thus, different techniques of inhibiting CD11/CD18 function (blocking antibodies and gene targeting), with disparate sets of limitations, each result in the conclusion that neutrophil emigration in the lungs can be elicited via CD11/CD18-dependent pathways and via CD11/CD18-independent pathways. The data to date suggest that gram-negative bacteria in the lungs induce predominantly CD11/CD18-dependent emigration, and gram-positive bacteria

in the lungs induce predominantly CD11/CD18-independent emigration.

ICAM-1, a ligand for CD11/CD18 and a member of the immunoglobulin gene superfamily, is expressed at low levels basally on pulmonary capillary endothelial cells and is further induced in response to LPS in the lungs.[29,30] Blocking ICAM-1 function with antibodies, or decreasing its expression with antisense oligonucleotides, decreases neutrophil emigration elicited by LPS or *P. aeruginosa* in the lungs.[23,31] Thus, ICAM-1 mediates neutrophil emigration in the lungs elicited by these gram-negative bacterial stimuli.

Two independent lines of mice with insertions in the ICAM-1 gene,[32,33] designed to eliminate its expression, resulted in the discovery that multiple ICAM-1 gene products arise from alternative splicing.[34,35] Some of the alternatively spliced forms appear to be LPS-inducible and especially abundant in the lungs.[34] Neutrophil emigration elicited by LPS in the lungs is not altered by gene targeting that eliminates full length ICAM-1 but spares subsets of alternatively spliced forms,[31] suggesting that the alternatively spliced forms may perform essential functions of ICAM-1 in mediating this neutrophil emigration. It is also possible that other, ICAM-1-independent pathways are responsible for the surprising lack of effect of ICAM-1 gene targeting on neutrophil emigration elicited by LPS in the lungs. The potential roles of alternatively spliced forms of ICAM-1 in regulating neutrophil recruitment remain to be determined.

Additional adhesion molecules also contribute to neutrophil emigration elicited by bacterial stimuli in the lungs. In response to LPS, neutrophil recruitment into the pulmonary air spaces is diminished by blocking antibodies against CD29, CD49e, or CD49f, suggesting that the β_1 integrins very late antigen (VLA)-5 (CD49e/CD29) and VLA-6 (CD49f/CD29) contribute to this process.[13] The β_1 integrin ligands essential to neutrophil emigration elicited by LPS in the lungs remain to be identified, but VLA-5 and VLA-6 may mediate neutrophil recruitment by binding fibronectin and laminin and facilitating transit through the interstitium.[13] The glycosylphosphatidylinositol-anchored urokinase receptor (uPAR) can mediate adhesion to substrates and can alter the adhesion and signalling properties of CD11/CD18 and other adhesion molecules, in addition to acting as a receptor for urokinase-type plasminogen activator (uPA).[36] The genetic deficiency of uPAR, but not of uPA, decreases neutrophil emigration elicited by *P. aeruginosa* in the lungs,[37] suggesting that uPAR facilitates neutrophil emigration independent of its interactions with uPA, perhaps by directly or indirectly regulating cellular attachment. Finally, neutrophil emigration elicited by gram-positive bacteria in the lungs has not yet been demonstrated to be dependent on any adhesion molecule, to the author's knowledge. Such neutrophil recruitment may require novel, yet to be identified adhesion molecules.

Neutrophil recruitment during pneumonia: chemokines

Chemokines are chemotactic cytokines that stimulate the directed migration of cells expressing their cognate receptors.[2] Chemokines also facilitate adhesion, by inducing intracellular signalling pathways which result in conformational changes in integrins such as CD11/CD18, promoting firm adhesion to their ligands. Chemokines are classified according to their primary structure, and peripheral blood neutrophils express receptors for chemokines of the α (CXC) family which contain the glutamic acid-leucine-arginine (ELR) motif. Humans and rodents have genes for similar but nonidentical sets of ELR^+ CXC chemokines. ELR^+ CXC chemokines stimulate neutrophil chemotaxis *in vitro*, and intratracheal instillation of the ELR^+ CXC chemokine MIP-2 recruits neutrophils into the pulmonary air spaces *in vivo*.[4]

ELR^+ CXC chemokines are essential to neutrophil emigration induced by bacteria or LPS in the lungs. Blocking antibodies against either KC or MIP-2 decrease neutrophil recruitment elicited by intrapulmonary LPS in rats,[38,39] suggesting that each of these chemokines is independently essential for maximal neutrophil recruitment in this setting. During pulmonary infection with *Klebsiella pneumoniae*, blocking the function of either MIP-2 or Lungkine inhibits neutrophil recruitment in the lungs of mice,[40,41] again suggesting independent roles for different ELR^+ CXC chemokines. During *Legionella pneumophila* pneumonia, neutrophil recruitment is inhibited to a greater degree by blocking the receptor CXCR2 (which recognizes both KC and MIP-2) than by simultaneously blocking both KC and MIP-2, suggesting that CXCR2 ligands other than KC and MIP-2 contribute to neutrophil recruitment during this infection.[42] Thus, multiple ELR^+ CXC chemokines have essential independent

roles in regulating neutrophil emigration induced by bacterial stimuli in the pulmonary air spaces.

The reasons behind independent requirements for multiple ELR$^+$ CXC chemokines are not obvious. The directed migration of neutrophils from within the pulmonary capillaries, between endothelial cells, across the interstitium, between epithelial cells, and into the alveolar air spaces may require sequential interactions with different chemokines present in distinct anatomic locations. Microenvironments with unique chemokine characteristics may result from differential chemokine expression by local cells, or differential retention or presentation of chemokines by cells or matrix components.[43–45]

Other classes of chemokines may also mediate neutrophil recruitment elicited by bacterial stimuli in the lungs. Blocking antibodies against the β family (CC) chemokine MIP-1α decrease neutrophil emigration induced by LPS in rats,[46] although it is unclear whether the essential role for MIP-1α in this setting involves signalling to neutrophils or to other cells. Receptors for and responsiveness to CC chemokines are increased in neutrophils by treatment with the cytokines interferon (IFN)-γ, tumor necrosis factor (TNF)-α, or granulocyte-macrophage colony stimulating factor (GM-CSF) *in vitro*.[47–49] Since IFN-γ, TNF-α, and GM-CSF are elaborated during bacterial pneumonias, CC chemokines may directly influence neutrophil functions during infection. Chronic inflammation in rats (induced by adjuvant immunization) stimulates circulating neutrophils to express the receptors CCR1 and CCR2 and to migrate to the CC chemokine MCP-1 both *in vivo* and *in vitro*,[16] demonstrating that altered receptor expression opens new pathways for neutrophil recruitment. Furthermore, circulating neutrophils in human patients with sepsis have decreased surface expression of CXCR2 and decreased *in vitro* responses to several ELR$^+$ CXC chemokines,[50] suggesting that altered receptor expression may bar otherwise available pathways for neutrophil recruitment. Thus, the roles of specific chemokines in regulating neutrophil recruitment will likely change during the progression of pulmonary infection and may be affected by other inflammatory diseases.

Neutrophil recruitment during pneumonia: TNF and IL-1

During bacterial pneumonia, multiple stimuli may induce the expression of chemokines and adhesion molecules in the lungs. The earliest signalling events are likely initiated by receptors recognizing and responding to bacterial products such as LPS.[51] Subsequently, mediators elaborated by the host, such as the cytokines TNF and IL-1, may amplify, propagate, and prolong the expression of these essential genes. For some lung inflammations, such as that induced by the intrapulmonary formation of IgG immune complexes, TNF-α and IL-1β are each independently essential for neutrophil emigration in the lungs.[52]

Each of 2 different receptors for TNF-α, TNF receptor 1 (TNFR1) and TNFR2, induces signalling and gene expression *in vitro* (reviewed in Reference 53). TNFR1 is preferentially activated by soluble forms of TNF-α, whereas TNFR2 is preferentially activated by TNF-α that is presented on cellular surfaces.[54] During pulmonary infection with *E. coli*, the deficiency of both TNFR1 and TNFR2 results in greater numbers of neutrophils in the lungs compared to WT.[55] Decreased killing of intrapulmonary bacteria is observed during *E. coli* pneumonia in TNFR1/TNFR2-deficient mice,[55] suggesting that increased emigration may have resulted from increased bacterial stimuli in the lungs. Many,[55–59] but not all,[60,61] studies demonstrate bacterial killing in the lungs to require TNF-α signalling. Studies of neutrophil emigration in response to bacteria or bacterial LPS in the lungs show varying effects of inhibiting TNF-α signalling, including increased emigration,[55,61] no effect on emigration,[59,60,62,63] or decreased emigration.[56,58,61,64]

Some investigators report varied responses within their studies, which may help illuminate biologically relevant sources of this variability. Skerrett *et al.* observed that neutrophil emigration elicited by aerosolized LPS was diminished by TNFR1 deficiency whereas that elicited by aerosolized *P. aeruginosa* was not,[61] and Peschon *et al.* observed that neutrophil emigration elicited by repeated intranasal challenge with *Micropolyspora faeni* antigen was diminished by TNFR1/TNFR2 deficiency whereas that elicited by an intranasal insufflation of LPS was not.[63] Thus, TNF signalling may have different roles in mediating neutrophil emigration in response to different stimuli. Ulich *et al.* observed soluble TNFR1 to diminish neutrophil emigration 6 h after LPS instillation, but not at 4 or 12 h after LPS instillation.[64] And Laichalk *et al.* observed that an inhibitor based on soluble TNFR2 significantly diminished neutrophil emigration 48 h after

instillation of *K. pneumoniae*, but not 24 h after instillation.[58] Thus, TNF-α signalling may be essential to neutrophil emigration for very limited time frames, which also may differ across stimuli. Considered together, these studies suggest that, in some settings, TNF-α plays a limited but essential role in neutrophil emigration elicited by bacterial stimuli in the lungs. However, altogether, these studies provide substantial evidence for TNF-independent pathways for neutrophil emigration in response to bacterial stimuli in the lungs.

Like TNF-α, IL-1 binds to 2 different receptors, the type I IL-1 receptor (IL1R1) and the type II IL-1 receptor (IL1R2). IL1R2 does not elicit second messenger signalling or cellular responses such as gene expression (see Reference 65 for review); only IL1R1 induces signals from IL1α or IL-1β. Although IL1R1, TNFR1, and TNFR2 associate with diverse adapter proteins, their signalling pathways partially overlap, eliciting common transcription factors and similar patterns of gene expression (e.g. see References 66, 67). Thus, IL-1 and IL1R1 may mediate similar functions as TNF-α and its receptors, and may facilitate the emigration of neutrophils in the lungs in the absence of TNF-α signalling.

To begin testing this hypothesis, neutrophil emigration induced by *E. coli* in the lungs was compared in WT mice and mice deficient in both TNFR1, the primary receptor for soluble TNF-α, and IL1R1, the only receptor for IL-1α and IL-1β. In contrast to TNFR1/TNFR2 deficient mice[55] and to mice deficient in IL1R1 alone,[68] mice deficient in both TNFR1 and IL1R1 exhibit a significant defect in *E. coli*-induced neutrophil emigration.[68] These data suggest that downstream signals which can be elicited by either TNFR1 or IL1R1 are required for neutrophil emigration elicited by *E. coli* in the lungs.

Both TNFR1 and IL1R1 induce the nuclear translocation of NF-κB transcription factors (discussed below), and the nuclear accumulation of NF-κB in the lungs during IgG immune complex inflammation requires both TNF and IL-1 signalling.[69] However, the nuclear accumulation of NF-κB in the lungs is not detectably different between WT and TNFR1/IL1R1 deficient mice during *E. coli* pneumonia,[68] suggesting that NF-κB translocation in the lungs does not require these receptors during this infection and that the decrease in neutrophil emigration in TNFR1/IL1R1 deficient mice does not result from diminished NF-κB translocation in lung cells. In contrast, NF-κB translocation in the liver during *E. coli* pneumonia is significantly inhibited by TNFR1/IL1R1 deficiency,[68] indicating that these cytokine receptors are essential for this response. NF-κB translocation in the liver may contribute to maximal neutrophil emigration induced by bacteria in the lungs, since liver-derived acute phase proteins such as serum amyloid A and complement C3 are dependent on NF-κB[70–72] and can facilitate neutrophil emigration and activation.[73–76] In addition, the pulmonary expression of the chemokine KC, but not MIP-2, is compromised by TNFR1/IL1R1 deficiency.[68] Since KC is essential to maximal emigration elicited by *E. coli* LPS in the lungs,[38] the decreased expression of KC in TNFR1/IL1R1 mice may also contribute to the decreased neutrophil emigration in response to intrapulmonary *E. coli*.

It is notable that much of the local inflammatory response induced by *E. coli* in the lungs, including approximately half of the neutrophil emigration, half of the KC expression, and all of the MIP-2 expression and NF-κB translocation, was unabated by the deficiency of both TNFR1 and IL1R1. These data suggest either that much of the initial inflammatory response to *E. coli* in the lungs does not require signalling by TNF-α or IL-1 or that other receptors are mediating signals in response to these cytokines. TNFR2 is capable of signalling from TNF-α, especially membrane-bound TNF-α,[54] and hence TNF-α signalling via TNFR2 may contribute to neutrophil emigration, NF-κB translocation, and gene expression in this setting. Ongoing experiments are designed to examine the inflammatory responses to intrapulmonary bacteria and LPS in gene-targeted mutant mice with combined deficiencies of all three signalling receptors for these early response cytokines (TNFR1, TNFR2, and IL1R1).

Neutrophil recruitment during pneumonia: other cytokines

Signaling from other cytokines can also regulate neutrophil recruitment elicited by bacterial stimuli in the lungs. Neutrophil emigration elicited by *K. pneumoniae* in the lungs is diminished by genetic deficiency of the IL-17 receptor;[77] IL-17 may facilitate emigration by increasing the number of neutrophils available in the blood and/or by increasing chemokine expression in the lungs.[77] In contrast to the other mediators discussed above, IL-6 limits LPS-elicited neutrophil emigration in

the lungs. Exogenous IL-6 decreases LPS-elicited neutrophil recruitment in rat lungs,[78] and IL-6-deficiency increases LPS-elicited neutrophil recruitment in mouse lungs.[79] IL-6 may limit LPS-elicited neutrophil emigration by decreasing the pulmonary expression of TNF-α, MIP-2, and other cytokines.[79]

Neutrophil recruitment during pneumonia: NF-κB

The coordinated expression of adhesion molecules and cytokines required for neutrophil recruitment may be mediated in part by transcription factors that bind to promoter elements common to their genes. Genes for ICAM-1, KC, MIP-2, and many other neutrophil receptor ligands contain functional κB sites in their upstream untranslated regions (see Reference 80 and references therein), suggesting that these genes (and, hence, neutrophil recruitment) may be regulated by the NF-κB family of transcription factors. NF-κB proteins are inhibited by IκB proteins under basal conditions. The intratracheal instillation of *E. coli* LPS results in the degradation of IκB-α and IκB-β and the nuclear translocation of the NF-κB subunits p65 (RelA) and p50 (Reference 81 and unpublished observations).

Gene-targeted interruption of RelA results in embryonic lethality,[82] which was hypothesized to result from an essential role for RelA in the prevention of apoptosis induced by TNF-α.[83] Combining RelA deficiency with the gene-targeted deficiency of either TNF-α or TNFR1 confirmed this hypothesis, and mice deficient in both TNF-α or TNFR1 and RelA (as opposed to those deficient in RelA alone) are born in the expected ratios based on Mendelian genetics.[84–86] Although extremely prone to infections and with typical lifespans of only several weeks, these mice provide a window of opportunity to study innate immune responses in the lungs in the absence of RelA.

The intranasal insufflation of *E. coli* LPS results in the accumulation of neutrophils in the alveolar air spaces of WT mice which are 3–5 days old.[86] This LPS-elicited neutrophil emigration is not significantly affected by TNFR1 deficiency, but it is significantly reduced in mice deficient in both TNFR1 and RelA when compared to either WT or TNFR1-deficient mice.[86] Thus, RelA is essential to LPS-induced neutrophil recruitment in the lungs.

Pulmonary expression of the chemokines KC and MIP-2 and the adhesion molecule ICAM-1 is also dependent on RelA. The LPS-induced expression of KC and MIP-2 in the lungs is not significantly affected by TNFR1-deficiency, but expression of both chemokines is almost completely inhibited by the combined deficiency of TNFR1 and RelA.[86] ICAM-1 transcript levels in the lungs do not differ across these 3 genotypes prior to LPS insufflation.[86] The LPS-induced increase in pulmonary ICAM-1 expression is reduced by the deficiency of TNFR1 alone, and yet further reduced by the combined deficiency of TNFR1 and RelA.[86] Therefore, RelA promotes the coordinated expression of adhesion molecules and chemokines essential to neutrophil emigration in response to bacterial LPS in the lungs.

RelA contains a transactivation domain which promotes gene expression by engaging *trans* activators which remodel chromatin and recruit RNA polymerase activity.[87,88] In contrast, p50 does not contain a transactivation domain. Despite this lack, interactions with other nuclear proteins allow p50 to engage *trans* activators and promote gene expression under some circumstances.[89,90] However, p50 can also repress gene expression,[91–94] by mechanisms which remain largely speculative. While it is clear that p50 translocates to the nucleus in response to LPS or bacteria in the lungs, its functions in promoting and/or repressing the local expression of genes that regulate innate immunity and neutrophil recruitment remain to be demonstrated.

Conclusions

Lung infections are common and important causes of morbidity and mortality.[95,96] Innate immune functions, including the recruitment and activation of neutrophils, determine the outcome of interactions with microbes in the lungs. Insights into the functional roles of adhesion molecules, cytokines, and regulatory factors in mediating pulmonary immune responses may contribute to rationally designing and appropriately using therapeutic and prophylactic agents. The specificity of distinct molecular responses to diverse physiological settings will need to be considered for strategies aimed at altering leukocyte recruitment in order to improve host defense (e.g. against antibiotic-resistant organisms) or to prevent inflammatory injury (e.g. respiratory distress or cardiovascular collapse).

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