Long pentraxin 3 in pulmonary infection and acute lung injury

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He X, Han B, Liu M. Long pentraxin 3 in pulmonary infection and acute lung injury. Am J Physiol Lung Cell Mol Physiol 292: L1039–L1049, 2007. First published February 2, 2007; doi:10.1152/ajplung.00490.2006.—Long pentraxin 3 (PTX3) is a newly discovered acute phase protein produced at the sites of infection and inflammation by tissue cells, macrophages, monocytes, and dendritic cells. PTX3 plays an important role in preventing infection of certain fungi, bacteria, and viruses in the lung. Recombinant PTX3 has been proposed as a potential antifungal molecule for therapy. However, under certain experimental conditions, such as intestinal ischemia-reperfusion, high volume mechanical ventilation, or severe bacterial infection, increased expression of PTX3 is associated with more severe lung injury. Therefore, it is necessary to further explore the sources of PTX3 in the lung and the regulatory mechanisms of its expression. It is also essential to further determine how PTX3 binds to pathogens, complement, and apoptotic cells, and to determine whether PTX3 has a specific receptor in targeted cells. These studies will provide insight into the pathological processes of pulmonary infection and acute lung injury and provide potential novel therapeutic strategies to control pulmonary infections without severe lung injury.

host defense; acute respiratory distress syndrome; acute phase protein; innate immunity

BIOLOGY OF LONG PTX3

PTX3, a “new” member of an old family. Pentraxins are a superfamily of conserved proteins, characterized by a cyclic multimeric structure and a conserved carboxy-terminal domain. The classic short pentraxins, such as C-reactive protein (CRP) and serum amyloid P component (SAP), share a high amino acid sequence homology and similar annular disc-like pentameric structure. The plasma levels of CRP in healthy individuals are less than 3 mg/l, and that of SAP are ~30–50 mg/l. Both could increase significantly during acute phase reaction, mainly due to increased production in the liver. They both play a key role in innate immunity by regulating resistance to microbes and scavenging cellular debris and components of extracellular matrix (20).

PTX3 was the first long pentraxin discovered, through a differentiation screening study, as one of the TNF-stimulated genes (TSG) in human diploid FS-4 fibroblasts (37). It was initially named TSG-14 and later was identified as an IL-1 inducible gene in human umbilical vein endothelial cells, sharing similarity with pentraxin family members (12). In addition to PTX3, other long pentraxins identified in human are PTX4, neuronal pentraxin 1, neuronal pentraxin 2, and neuronal pentraxin receptor (20). PTX3 gene is localized in the human chromosome 3q24-28 (12). Pentraxins are evolutionarily conserved from insects to mammals (20). Human PTX3 is highly conserved and shares 82% of the identity and 92% of the similarity in primary sequence with murine PTX3 (12, 28).

The long pentraxins share the pentraxin signature sequence and high homologies with short pentraxins in their carboxy-pentraxin domain. In addition, long pentraxin members have longer amino termini and therefore possess longer sequences and higher-molecular-weights (Fig. 1A). The PTX3
carboxy-terminal domain contains the canonical pentraxin signature of eight-amino acid sequence (HxCxS/TWxS) (Fig. 1A). The PTX3 gene is organized in three exons encoding for 381 amino acids (Fig. 1B) (28). The human and murine ptx3 proximal promoters contain AP-1, NF-κB, Sp1, and NF-IL-6 binding sites (Fig. 1B).

Although both short and long pentraxins are acute phase proteins involved in the innate immunity and inflammation, they are encoded by different genes, each with different cellular sources under different regulatory mechanisms. The comparisons of their structures, ligands, and functions have been done by Garlanda and colleagues (20). How these molecules interact with each other in immune responses has been speculated (20) and merits further investigation.

**Regulation of PTX3 expression by cytokines.** Unlike the short pentraxins (e.g., CRP and SAP) that are produced in the liver under the stimulatory influence of IL-6, PTX3 gene and protein expression is induced by IL-1β or TNF-α in multiple human tissue cells, including fibroblasts, endothelial cells, epithelial cells, and hepatic cells; in contrast, IL-6 usually is not a good inducer for PTX3 production (Table 1). Similar results are also seen in murine fibroblasts, myoblasts, and endothelial cells (28). Production of PTX3 has also been found in other tissue cells, such as adipocytes (1), human vascular smooth muscle cells (33), and in selected brain tissues (60, 65) under various inflammatory stimulations.

With respect to the cells of the immune system, IL-1β and TNF-α, but not IL-6, monocyte chemotactic protein-1 (MCP-1),

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Table 1. **Induction of PTX3 from human cells by cytokines**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IFN-γ</th>
<th>Reference No.</th>
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<tbody>
<tr>
<td>Fibroblasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(35–37)</td>
</tr>
<tr>
<td>FS-4 cells</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>(12)</td>
</tr>
<tr>
<td>Fibrosarcoma cell line 8387</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal fibroblasts</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>(42)</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>(12)</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A549 cells</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>(26)</td>
</tr>
<tr>
<td>Primary type II epithelial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAS-2B cells</td>
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<td>✓</td>
<td></td>
<td></td>
<td>(26)</td>
</tr>
<tr>
<td>Primary small airway epithelial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal renal tubular epithelial cells</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>(56)</td>
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<tr>
<td>Hepatic cells</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Hepatoma cell line (Hep3B)</td>
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<td>✓</td>
<td></td>
<td></td>
<td>(12, 35)</td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>inhibitory</td>
<td>(2, 61)</td>
</tr>
<tr>
<td>Myeloid dendritic cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(17)</td>
</tr>
</tbody>
</table>

PTX3, pentraxin 3; ✓, Stimulatory; X, no response.
macrophage colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, and IFN-γ, induced expression of PTX3 mRNA in human peripheral blood mononuclear cells (2). In resting or stimulated polymorphonuclear cells, T or B lymphocytes, and natural killer (NK) cells, PTX3 mRNA is not detectable (2). PTX3 mRNA is also inducible in monocyte-derived macrophages, in tumor-associated macrophages, and in the myelomonocytic cell lines (HL-60, U-937, and THP1). In contrast, T and B cell lines had no detectable PTX3 (2). The cellular sources of PTX3 implicate its “local” role in host defense and in the regulation of inflammation.

Table 2. Role of Toll-like receptors and microbial moieties on PTX3 expression in human monocytes, macrophages, and dendritic cells

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Monocytes</th>
<th>Macrophages</th>
<th>MoDC</th>
<th>MyDC</th>
<th>pDC</th>
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</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>OmpA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR3</td>
<td>Poly IC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Condidia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG ODN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other microorganisms</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium bovis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Reference No. (2, 17, 21) (17) (17) (17) (17) (17)

PTX3 production in human antigen-presenting cells, such as monocytes, macrophages, and dendritic cells (DCs), can be induced by a variety of microbial moieties (Table 2) (17). DCs are heterogeneous with different subpopulations. Specific ligands for TLR family members stimulated PTX3 production in myeloid DC (MyDC) and monocyte-derived DC (MoDC) but not in plasmacytoid DC (pDC) (Table 2) (17). Similarly, whole Aspergillus fumigatus conidia or Pseudomonas aeruginosa stimulated production of PTX3 in MyDC and MoDC but not in pDC (17). PTX3 production was rapidly increased in human and murine mononuclear phagocytes after being exposed to A. fumigatus conidia (21). When human monocytes were infected with conidia of A. fumigatus, a sustained upregulation of PTX3 was observed throughout 6 h of exposure (13).

Table 3. PTX3 expression induced by LPS and cytokines in vivo

<table>
<thead>
<tr>
<th>LPS</th>
<th>LPS</th>
<th>LPS</th>
<th>LPS</th>
<th>IL-1β</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μg/mouse</td>
<td>20 μg/mouse</td>
<td>1 μg/mouse</td>
<td>5 mg/kg</td>
<td>1 μg/mouse</td>
<td>1 μg/mouse</td>
</tr>
<tr>
<td>iv, 6 h</td>
<td>iv, 4 h</td>
<td>icv, 6 h</td>
<td>iv</td>
<td>icv, 6 h</td>
<td>icv, 6 h</td>
</tr>
<tr>
<td>C57BL/6 mice</td>
<td>C57BL/6 mice</td>
<td>CD1 mice</td>
<td>SD rats</td>
<td>CD1 mice</td>
<td>CD1 mice</td>
</tr>
<tr>
<td>Liver</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Spleen</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kidney</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thymus</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ovary</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Heart</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Muscle</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Skin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Brain</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Reference No.</td>
<td>(35)</td>
<td>(28)</td>
<td>(60)</td>
<td>(57)</td>
<td>(60)</td>
</tr>
</tbody>
</table>

iv, Intravenous; icv, intracerebroventricular; –, no response; +, increased PTX3 expression.
that intratracheal instillation of LPS induced ALI in mice, which was associated with significant increase of PTX3 in the bronchoalveolar lavage fluid and in the lung tissues (unpublished data). When LPS was administered intravenously into adult rats, it also increased expression of PTX3 mRNA and protein in the alveolar wall (Table 3) (26).

These results implicate that different microorganisms (fungi, bacteria, viruses) may activate macrophages and DCs through TLRs. These cells can produce PTX3 and other cytokines. TNF-α and IL-1β produced by these inflammatory cells could further stimulate production of PTX3 by inflammatory cells and tissue cells. PTX3 could be a soluble factor for immediate innate response and also influence the adaptive immunity.

**Role of PTX3 in Pulmonary Infection**

As the major interface between the host and external environment, the lung is exposed to numerous airborne pathogens and noxious stimuli (5, 76). Host defense mechanisms have been evolutionarily developed in the lung to protect the host from inhaled potential pathogens. One of the key mechanisms for innate immunity is the recognition of pathogens and damaged tissues mediated by transmembrane, cytosolic, and secreted soluble PRRs (46). As a soluble PRR, PTX3 has demonstrated nonredundant roles in preventing pulmonary infection (Table 4).

**Role of PTX3 in fungal infection in the lung.** Generation and application of PTX3-deficient (ptx3−/−) mice have provided convincing evidence to support the importance of PTX3 in host defense. The ptx3−/− mice show a normal lifespan and no significant genetic defects, except female infertility (70). However, challenged with the fungus *A. fumigatus*, ptx3−/− mice were extremely susceptible to invasive pulmonary aspergillosis (IPA). All ptx3−/− mice died within 3 days after the *A. fumigatus* challenge, with significantly higher brain and lung colonization and severe inflammatory injury in the lung (21). Administration of recombinant PTX3 completely protected the ptx3−/− mice from IPA. Recombinant PTX3 bound conidia of *A. fumigatus* selectively, which facilitated the internalization and inactivation of the conidia, and stimulated MCP-1 production in mononuclear phagocytes (21). Therefore, the high susceptibility of the PTX3-null mice to fungal infection may be partially due to the deficiency in recognition of *A. fumigatus* by the host, in particular, by the alveolar macrophages and DCs.

Furthermore, recombinant PTX3 has been used either alone or as an adjunctive therapy in mice with fungal infection. Invasive aspergillosis is the leading reason for nosocomial pneumonia and death in patients who received bone marrow transplantation. With the use of a murine allogeneic bone marrow transplantation model, complete resistance to infection and reinfection of *A. fumigatus* was observed in animals that received recombinant PTX3 treatment. The protective effect was associated with accelerated recovery of lung phagocytes and T helper type 1 (Th1) lymphocytes and decreased inflammatory pathology in the lung (22).

**Role of PTX3 in bacterial infection in the lung.** *P. aeruginosa* is one of the common bacterial pathogens in lung infection. It can be recognized and bound by PTX3 (21). An increase of colonization in the lung and increase in mortality was seen in ptx3−/− mice with *P. aeruginosa* pulmonary infection (21). In contrast, PTX3 knockout did not affect the susceptibility to pulmonary infection with *Listeria monocyogenes*, a bacterial strain not recognized by PTX3 for binding (21).

A dual effect of PTX3 has been recently shown in the PTX3 transgenic mice with *Klebsiella pneumoniae* pulmonary infection (66). To investigate the in vivo role of PTX3 in inflammation, transgenic mice carrying multiple copies of PTX3 gene under the control of its own promoter were generated (14). In these PTX3 “knockin” mice, a high inoculum of *K. pneumoniae* was associated with higher lethality, increased nitrate in plasma, inability of neutrophils to migrate to lung tissue, and greater dissemination of bacteria into the blood (66). In contrast, transgenic PTX3 expression conferred protection to mice given lower pulmonary inocula, with an enhanced TNF-α production, greater neutrophil influx into the lung, and phagocytosis of bacteria by migrated neutrophils (66). These experiments highlight that PTX3 is important for the host to control bacterial infection; the outcome, however, may depend on the bacterial strains and the severity of the infection.

**Role of PTX3 in viral infection.** Human cytomegalovirus (HCMV) is a ubiquitous opportunistic pathogen existing as a latent form after the clearance of initial infection. Reactivation of HCMV occurs in patients with immunosuppression or under critical conditions. Murine CMV (MCMV) infection has been used as a model to determine the mechanisms of CMV infection. It has been demonstrated that PTX3 bound both HCMV and MCMV and reduced viral entry and infectivity in cultured cells. Recombinant PTX3 protected mice from MCMV primary infection and reactivation as well as invasive pulmonary *Aspergillus* superinfection (11). Virus titers were found to be significantly reduced in the lung and the spleen in mice treated with recombinant PTX3. MCMV-induced cell infiltration in the lung, associated with parenchymal destruction, peribronchial fibrosis, and goblet cell hyperplasia, were also ameliorated by PTX3 treatment (11).

**PTX3 and Acute Inflammatory Response and Lung Injury**

The optimal defensive strategy in the lung would include not only preventive control of microbial proliferation and clearance but also the execution of a finely tuned inflammatory response, sufficient to contain the infection without inducing a harmful degree of alveolar infiltration and exudation. Excessive local and systemic inflammatory responses may lead to ALI and mortality. Increased expression of PTX3 may contribute to the pathogenesis of ALI (Table 5).

**PTX3 serum levels and clinical outcome.** The levels of PTX3 in the sera are very low in normal human subjects (<2 ng/ml), which are rapidly and dramatically increased in patients with inflammatory and infectious conditions (4, 34, 43, 48). The increase of PTX3 levels in critically ill patients was correlated with the severity of the diseases from systemic inflammatory response syndrome to septic shock and sepsis (48). Measuring PTX3 levels in the blood has been proposed to be a marker for early diagnosis and prognosis of certain diseases (7, 27, 34).

**PTX3 in endotoxemia.** PTX3 knockin transgenic mice were protected from endotoxic shock induced by LPS administration or by polymicrobial sepsis resulting from cecal ligation and puncture (CLP). A constitutively higher expression of IL-10 and a stronger early response of TNF-α were found in PTX3 transgenic mice. Moreover, the macrophages from transgenic
mice showed a profound increase of nitric oxide production in response to IFN-γ/TNF-α or IFN-γ/LPS costimulation (14). In contrast, PTX3 knockout mice did not show different susceptibility to LPS or CLP in terms of mortality, cytokine profiles, and neutrophil recruitment in the lungs (21). The lack of cohesive results in PTX3 knockout and knockout animals may reflect the complexity of cytokine network in the regulation of infection and inflammation.

**PTX3 in intestinal ischemia-reperfusion-induced ALI.** In contrast to results from endotoxic/sepsis studies, PTX3 overexpression increased the mortality in the transgenic mice exposed to intestinal ischemia-reperfusion (IIR) (67). The decreased survival rate appeared secondary to an exacerbated inflammatory response with much severer tissue damage in local (duodenum and ileum) and remote (lung) organs, with increased vascular permeability, hemorrhage, and neutrophil accumulation. Severe lung injury occurred rapidly, together with significantly augmented production of cytokines (TNF-α, IL-1β, MCP-1, and KC) in the duodenum, serum, and the lung. TNF-α appeared to be the most important factor contributing to the higher mortality, because soluble TNF receptor rescued animals from IIR-induced death within the first hour of reperfusion (67).

LPS has been commonly used as a reagent to induce inflammatory response, endotoxic shock, and ALI. The experimental condition can be well controlled, reproducible, and very useful for mechanistic studies. However, the inflammatory responses initiated by LPS are different from that seen after bacterial infection. The CLP model mimics clinical conditions, but the severity of bacterial infection varies from animal to animal. A self-containment of the punctured intestine occurs in some animals. In contrast, the IIR-induced damage of intestine is difficult to reverse, especially in small animals; therefore, it is often associated with immediate or chronic mortality. The detrimental effects observed in PTX3 overexpressed transgenic mice suggest that increased expression of PTX3 may contribute to excessive or persistent inflammation and jeopardize the survival of the organism.

**PTX3 and ventilator-induced lung injury.** As a life support for critically ill patients, mechanical ventilation is often indispensable. However, mechanical ventilation can induce and/or worsen ALI (25, 57). Ventilator-induced lung injury has been considered one of the most important contributing factors to the high mortality in ARDS (18, 25). To further determine the expression of PTX3 in multiple clinically related settings, adult rats were subjected to intravenous LPS or hemorrhagic shock/resuscitation, followed by mechanical ventilation with two clinically applicable regimens: low-volume ventilation [6 ml/kg, positive end-expiratory pressure (PEEP) 5 cmH2O] or relatively higher-volume ventilation (12 ml/kg, no PEEP). PTX3 gene and protein expression in the lungs was increased by LPS or hemorrhagic shock alone, which was further increased by higher volume ventilation (57). The striking correlations between the levels of PTX3 expression and the severity of the lung injury measures suggest that PTX3 may participate in the pathogenesis of ALI (57).

In that group of studies, all animals were mechanically ventilated; thus, it is necessary to examine the direct effects of LPS, hemorrhagic shock/resuscitation, and injurious ventilation individually on the expression of PTX3. When adult rats were ventilated with much higher tidal volume (25 ml/kg, no PEEP), ALI was induced within 3 h, which was associated with a dramatic increase in expression of PTX3, much higher than that seen in LPS or hemorrhage/resuscitated animals (57). Immunohistochemical studies revealed that the increased PTX3 expression was mainly in the alveolar wall, suggesting a local response of PTX3 from lung tissue cells. Mechanical forces may induce the release of proinflammatory mediators from the lung cells (25, 47) through special signal transduction pathways (18, 25, 41). PTX3 may be one of the important mediators related to ventilator-induced lung injury. With the use of a unilateral model of mechanical ventilation in anesthetized dogs to assess regional cellular responses to local mechanical conditions, it has been found that PTX3 gene expression was increased in gravitationally dependent regions compared with the nondependent regions of the base of the same lungs, suggesting that its trigger may be more related to the stresses of opening and collapse rather than overdistension (64).

**PTX3: An Open Ending of the Story.** As summarized above, PTX3 is important for the host to defend infection of certain strains of fungi, bacteria, and viruses. Recombinant PTX3 or gene delivery could be further explored as novel therapies for suppressing pulmonary and systemic infections. However, the implications of PTX3 as an inflammatory mediator in ALI warn us to proceed with caution and to continue to learn more about this rather interesting molecule. We need to understand how PTX3 expression is regulated in different cell types by cytokines and microbial moieties. The role of PTX3 in mediating cross talk between innate and adaptive immunity also requires further elaboration. How PTX3 molecule exerts its function is largely unknown. These questions are essential for us to determine whether PTX3 is a friend or a foe.

**Regulation and role of PTX3 expression in tissue cells.** One of the important features of PTX3 expression is that it is not only produced by monocytes, macrophages, and DCs, but also by different types of tissue cells (Table 1). Over the last decade, our understanding of the function of tissue cells in immune responses has been significantly advanced. Tissue cells, such as lung epithelial cells, have been considered an important source for cytokine production (39). Lung epithelial cells could be a sensor and effector to different insults, including microbes (5, 39), cytokines (39), and mechanical forces (18, 24, 25).

To determine the direct effects of LPS, mechanical stretch, and TNF-α on lung epithelial cells, A549 cells (an alveolar type II-like cell line derived from human lung carcinoma) were exposed to these stimuli. Using microarray, PTX3 was found among other TNF-α-induced genes; however, neither LPS nor mechanical stretch induced PTX3 gene expression (19). In quiescent cells, PTX3 was barely detectable. TNF-α induced a time- and dose-dependent increase in PTX3 gene expression and protein production in both A549 and BEAS-2B cells, a cell line derived from human normal bronchial epithelium. This was confirmed in primary cultured human alveolar type II epithelial cells and small airway epithelial cells. Pretreatment with either actinomycin D or cycloheximide abolished TNF-α-induced PTX3 expression, indicating the requirement of a de novo regulation at both transcriptional and translational levels (26). IL-1β also induced PTX3 expression in human lung...
epithelial cells. A549 cells produced similar levels of PTX3 as that of U-937 cells, a human monocyte cell line, in response to TNF-α challenge (26). Since epithelial cells are one of the major tissue cells and stand in the front line of host defense in the lung, rapid increase of PTX3 production in this type of cell may play an important role in the lung in regulation of innate immunity and inflammatory responses.

As mentioned above, increased PTX3 expression was observed in rats after LPS, hemorrhagic shock/resuscitation, and mechanical ventilation (57). The lack of responses of lung epithelial cells to LPS or mechanical stretch in culture suggests that these factors may stimulate PTX3 expression indirectly, via proinflammatory mediators (such as TNF-α and IL-1β). Indeed, the levels of PTX3 in the lung were highly correlated with that of TNF-α and IL-1β (57).

During acute inflammatory responses, multiple intracellular signal transduction pathways can be activated in different cell types (50, 58). Although TNF-α activated NF-κB in human lung epithelial cells, blocking this pathway with specific inhibitors did not reduce PTX3 expression. Inhibition of ERKs and p38 MAP kinase also had no significant influence on the PTX3 expression. A significant blockade of TNF-α-induced PTX3 expression was observed by inhibiting JNK activity with specific chemical inhibitors (26). Knocking down JNK1 or JNK2

Fig. 2. Potential role of PTX3 in mediating interactions between innate and adaptive immune systems. Multiple microbial moieties may activate dendritic cells and other antigen-presenting cells via related Toll-like receptors (TLRs). Signals through MyD88-dependent and MyD88-independent pathways may induce production of PTX3 from these cells. PTX3 as a soluble mediator may promote T lymphocytes toward T helper type 1 (Th1), rather than Th2. Th1 cytokines, such as IL-12 and IFN-γ, are important for PTX3-related functions. However, IFN-γ may inhibit further PTX3 production from dendritic cells, as a negative feedback signal. In contrast, IL-10, a Th2 type cytokine, may enhance production of PTX3 from dendritic cells.

Fig. 3. Local production and function of PTX3 in host defense and inflammation. PTX3 can be produced by multiple tissue cells, including epithelial cells, endothelial cells, fibroblasts, smooth muscle cells (SMCs), macrophages (Mφ), and monocyte-derived dendritic cells (DCs). PTX3 can selectively bind to certain pathogens (fungi, bacteria, and viruses) and binds to C1q and apoptotic cells. It may enhance nitric oxide and cytokine production from macrophages, induced by proinflammatory cytokines and LPS, via an unidentified receptor (PTX3-R).
with small interfering RNA selectively reduced TNF-α-induced PTX3 expression, suggesting both JNK isozymes are involved in the regulation of PTX3 expression in human lung epithelial cells (26).

The JNK-dependent, NF-κB-independent expression of PTX3 in lung epithelial cells is interesting because NF-κB has been considered the major pathway for TNF-α and IL-1β signaling. The JNK-dependent expression of PTX3 suggests signal transduction pathways could be cell type specific. Indeed, it has been shown that primary cultured rat lung alveolar epithelial cells produced TNF-α (31, 45, 75) and macrophage inflammatory protein-2 (a CXC chemokine) (30, 31, 75), but the regulatory mechanisms of cytokine production in these cells appear different and sometimes opposite from that in macrophages (29–31). Knowing the detailed signal transduction mechanisms in different cell types may help to develop cell type-specific regulation of proinflammatory mediators, depending on the clinical scenario. For instance, under conditions of pulmonary infection, PTX3 expression should be upregulated to enhance host defense, whereas it should be downregulated when excessive inflammatory response already exists. Therefore, it is critical to determine the production and regulation of PTX3 in lung tissue cells.

**Role of PTX3 in mediation of cross talk between innate and adaptive immunity.** Macrophages and DCs are known as antigen-presenting and -presenting cells that play a critical role in initiating adaptive immune responses (11). These cells produce a large amount of PTX3 on a per-cell basis. The increased PTX3 may in turn facilitate the pathogen recognition by macrophages and DCs (16, 17, 21).

An inefficient Th1 cytokine profile and unbalanced Th2 responses were observed in *ptx3*−/− mice after *A. fumigatus* infection (21). In the allogeneic bone marrow transplantation model, PTX3 treatment, either before, in concomitance with, or after the infection with *A. fumigatus* conidia, demonstrated significant antifungal effects, associated with accelerated recovery of lung phagocytic cells and lymphocytes, increased frequency of Th1 and decreased frequency of Th2 cells in the spleens, and increased IFN-γ and reduced IL-4 expression in CD4+ splenocytes (22).

In the MCMV infection model, recombinant PTX3 treatment recovered DC and NK cell reactivity, activated T cells,
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and increased production of IL-12, IFN-α, IFN-γ, and IL-10 from splenocytes (11). With the use of transgenic mice, it was further found that the antiviral efficacy of PTX3 was dependent on the IL-12/IFN-γ pathway (11). More interestingly, the protective effects of PTX3 were mediated through the TLR9/MyD88-independent but TLR2/TLR4-dependent mechanisms, as determined by administration of recombinant PTX3 into several strains of transgenic mice, each with deficiency of a specific TLR (11).

The beneficial effect of PTX3 indicates that PTX3 may be an important soluble factor to link innate and adaptive immunity. As shown in Table 2, multiple microbial moieties, of which many are recognized as TLR ligands, can induce PTX3 production in DCs. TLRs may promote expression of PTX3 via MyD88-dependent or -independent pathways in DCs or other antigen-presenting cells. PTX3 as a soluble factor may participate in the presentation of signals from DCs to lymphocytes, especially in favor of Th1 type of response. Th1 type cytokines, IL-12 and IFN-γ, are important for the antifungal and antiviral functions of PTX3. IFN-γ and IL-10 have divergent effects on LPS-induced PTX3 production in DCs. IFN-γ inhibited LPS-induced PTX3 expression and production in different cellular contexts (23, 61), whereas IL-10 enhanced LPS-induced PTX3 expression in DCs and monocytes (16). IFN-γ, but not IL-10 or IL-13, inhibited mycobacterial cell wall component lipoarabinomannan-induced PTX3 production in human peripheral blood mononuclear cells (71). Therefore, IFN-γ may be a negative feedback signal to reduce production of PTX3 from monocytes and DCs. In contrast, IL-10, a Th2 cytokine, may enhance the production of PTX3 to maintain the balance between these two subtypes of lymphocytes (Fig. 2). This hypothesis should be further studied.

Molecular mechanisms of PTX3-mediated functions. Although the studies summarized in this article demonstrated the importance of PTX3 in innate immunity and acute inflammation in the lung, the exact roles that PTX3 plays are largely unknown. On the basis of our current knowledge, PTX3 may exert its function through several mechanisms (Fig. 3).

Direct binding to pathogens. PTX3 binds selectively to certain fungal strains, including A. fumigatus, Aspergillus flavus, and Aspergillus niger, but not to Candida albicans (21). The binding of PTX3 to A. fumigatus is specific to conidia but not to the hyphae of A. fumigatus, and the binding facilitates the clearance of conidia of A. fumigatus by the resident and recruited phagocytes (21). It has been shown that macrophages from PTX3 knockin transgenic mice showed better opsonin-independent phagocytosis of zymosan particles and the yeast form of the fungus Paracoccidioides brasiliensis, with enhanced microbialicidal activity and nitric oxide production (15). Furthermore, recombinant PTX3 protein binds to zymosan particles and yeast cells of P. brasiliensis and enhanced phagoctytic activity of macrophages from wild-type mice, demonstrating the opsonin-like activity of PTX3 (15).

PTX3 also binds P. aeruginosa, Salmonella typhimurium, and P. brasiliensis, but not Escherichia coli, Burkholderia cepacia, and L. monocytogenes (21). Binding of PTX3 to human and mouse CMVs has also been reported (11).

How PTX3 binds pathogens or their components is largely unknown. Recently, it has been shown that outer membrane protein A (OmpA), a conserved major component of the outer membrane of Enterobacteriaceae and a ligand for TLR2 from K. pneumoniae (KpOmpA), activated macrophages and DCs in a TLR2-dependent way, with increased production of PTX3. PTX3, in turn, bound KpOmpA but did not affect the recognition of KpOmpA by cellular receptors (32). This study provided a new mechanism for PTX3 binding to microbial moieties.

Binding to C1q. Classic short pentraxins, CRP and SAP, can bind to complement component C1q and activate the classic complement pathway. PTX3 can also directly bind C1q through its pentraxin domain (10). The structure and function of PTX3 glycosidic moiety as a fine-tuning mechanism of the interaction with C1q and complement activation has been a subject of extensive studies (59). However, the interaction and consequence between PTX3 and C1q are less clear. C1q−/− mice showed higher susceptibility to IPA, and treatment of these mice with PTX3 reversed this process (21). Thus it appears that the antifungal function of PTX3 may be independent of C1q. Binding of C1q to immobilized PTX3 induced activation of the classic complement pathway. However, in the fluid phase, preincubation of PTX3 with C1q resulted in inhibition of complement activation by blocking the interaction of C1q with immunoglobulins (55). These results indicate that PTX3 can both inhibit and activate the classic complement pathway by binding C1q, depending on the way it is presented to C1q.

Binding to apoptotic cells. Apoptosis and other types of programmed cell death (such as oncosis) are important processes in acute inflammatory responses (49, 51, 68). It has been shown that PTX3 specifically binds to apoptotic cells, and the recognition was restricted to extranuclear membrane domains. PTX3 also binds to necrotic cells but to a lesser extent (62). Human DCs failed to internalize dying cells in the presence of PTX3 (62). PTX3 also inhibited phagocytosis of apoptotic neutrophils by macrophages (69). TLR ligands elicited production of C1q and PTX3 by immature DCs. Both can bind to dying cells with similar kinetics but recognize different domains on the cell membranes (6). C1q increased the phagocytosis of apoptotic cells by DCs and the release of IL-12 in the presence of TLR4 ligands and apoptotic cells, whereas PTX3 bound in the fluid phase to C1q decreased C1q deposition and subsequent complement activation on apoptotic cells (6). The exact binding site(s) of PTX3 to dying cells and the physiological consequence of the inhibitory effects of PTX3 on clearance of apoptotic cells need to be determined.

Function through a receptor. Garlanda et al. (20, 44) suggested that PTX3 could bind an unknown receptor to activate adaptive responses. This hypothesis is interesting; however, there is no report so far to indicate that PTX3 has chemotactic activity to inflammatory cells or can directly induce production of cytokines or other inflammatory mediators. On the other hand, it has been shown that production of nitric oxide by macrophages from PTX3 knockin mice was increased in response to IFN-γ/TNF-α or IFN-γ/LPS costimulation (14). PTX3 also enhanced tissue factor expression in human endothelial cells induced by LPS, TNF-α, or IL-1β (52) and enhanced LPS-induced tissue factor expression in monocytes (53). The cross talk between inflammatory mediators and coagulation components may cause reciprocal modulation during ALI (38, 63). We have also found that PTX3 expression is coordinated with the expression and activation of tissue factor in multimodels of ALI in rats (unpublished data). It seems that PTX3 always enhances the effects of other inflammatory mediators, such as LPS, TNF-α, or IL-1β. This implies that if a
PTX3 receptor exists, its expression and/or function is dependent on the activation of the cells by those proinflammatory mediators. We propose that microbial toxins or proinflammatory cytokines may turn on the expression of PTX3 and its putative receptor with similar kinetics.

Conclusions. Long PTX3, as a soluble pathogen PRR, plays an important role in host defense against fungal, bacterial, and viral infections. However, overexpression of PTX3 induced by severe infections, persistent tissue damage, mechanical ventilation, and other insults, may contribute to ALI/ARDS due to excessive inflammation. The precise function of PTX3 in inflammatory responses merits further investigation.

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