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Interplay between Myeloid Cells and Humoral Innate Immunity

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ABSTRACT The innate immune system represents the first line of defense against pathogens and comprises both a cellular and a humoral arm. Fluid-phase pattern recognition molecules (PRMs), which include collectins, ficolins, and pentraxins, are key components of the humoral arm of innate immunity and are expressed by a variety of cells, including myeloid, epithelial, and endothelial cells, mainly in response to infectious and inflammatory conditions. Soluble PRMs share basic multifunctional properties including activation and regulation of the complement cascade, opsonization of pathogens and apoptotic cells, regulation of leukocyte extravasation, and fine-tuning of inflammation. Therefore, soluble PRMs are part of the immune response and retain antibody-like effector functions. Here, we will review the expression and general function of soluble PRMs, focusing our attention on the long pentraxin PTX3.

INTRODUCTION

The immune system of mammalians is organized around two components: the innate immunity and the adaptive immunity. Older in terms of evolution, the innate immune system constitutes the first line of defense against microorganisms. This system is supplemented by the adaptive immunity, which is more recent in terms of evolution and provides the basis of immunological memory. Both the adaptive and innate immune systems are composed of a cellular and a humoral arm acting in a complementary and coordinated manner to regulate the innate response.

The induction of a protective immune response against pathogens resides in the capacity to identify them. Adaptive immunity uses specific receptors encoded by gene rearrangements. In contrast, innate immune molecules involved in the recognition of pathogens and in the initiation of the immune response are germ line-encoded receptors. These receptors are called pattern recognition molecules (PRMs) and recognize highly conserved motifs expressed by microorganisms, called pathogen-associated molecular patterns (PAMPs) (1).

Based on their localization, PRMs are divided into cell-associated receptors and soluble molecules. Cell-associated receptors include endocytic receptors (e.g., scavenger receptors), signaling receptors (e.g., Toll-like receptors [TLRs]), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and RNA helicases such as melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I). Fluid-phase molecules belonging to the humoral arm of innate immunity represent the functional ancestor of antibodies (2).

Soluble PRMs form a heterogeneous group of molecules, comprising collectins, ficolins, and pentraxins, and share basic functions, including pathogen opsonization, recognition of modified self, and regulation of complement activation. These molecules are expressed by a variety of cells, including myeloid, epithelial, and

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endothelial cells (2). This diversification supports the production of key molecules over time and in specific sites (Fig. 1).

Here, we will review the key soluble PRMs, their production by phagocytes, and their roles in innate immunity and inflammation (Table 1). In particular, we will focus our attention on the prototypic long pentraxin 3 (PTX3), whose regulation and function have been conserved in evolution.

COMPONENTS OF HUMORAL INNATE IMMUNITY

Collectins

Collectins are a family of multimeric proteins belonging to the superfamily of Ca^{2+}-dependent lectins (C-type lectins) (3). Nine members have been identified: mannose-binding lectin (MBL), conglutinin (only in bovidae), surfactant protein (SP)-A, SP-D, collectin (CL)-43 (only in bovidae), CL-46 (only in bovidae), CL-P1 (in placenta), CL-L1 (in liver), and CL-K1 (in kidney) (4). In addition, heteromeric complexes formed by CL-L1 and CL-K1 have been detected in the circulation and called CL-LK (5). This heterocomplex binds mannan-binding lectin-associated serine proteases (MASPs) and can mediate complement activation (5). Whereas all collectins are soluble and secreted, CL-P1 is a type II transmembrane protein (6, 7). However, shedding of this transmembrane molecule generates a soluble form of CL-P1 with effector activities (i.e., recognition of pathogens and activation of the complement cascade) (8). In addition to these molecules, the complement component C1q is related to this family, based on structure and function similarities. For instance, the classical and lectin pathways of complement activation are induced by interaction between C1q and MBL, respectively, with their ligands. Phylogenetic analysis revealed that components of the classical and lectin pathways of complement can

FIGURE 1 Humoral innate immunity. Soluble PRMs are expressed and secreted by a variety of cells, including in particular myeloid cells, allowing the production of PRMs over time. Some PRMs (e.g., PTX3, PGRP-S, and ficolin-1) are stored in neutrophil granules for rapid release in minutes. PRMs such as PTX3 and PGRP-S are also found among NET-associated molecules (NAMs). Production of PRMs by mononuclear phagocytes, dendritic cells, and endothelium in a gene expression-dependent manner sustains the presence of these molecules over time. Finally, epithelial tissues (e.g., liver) sustain systemic mass production.

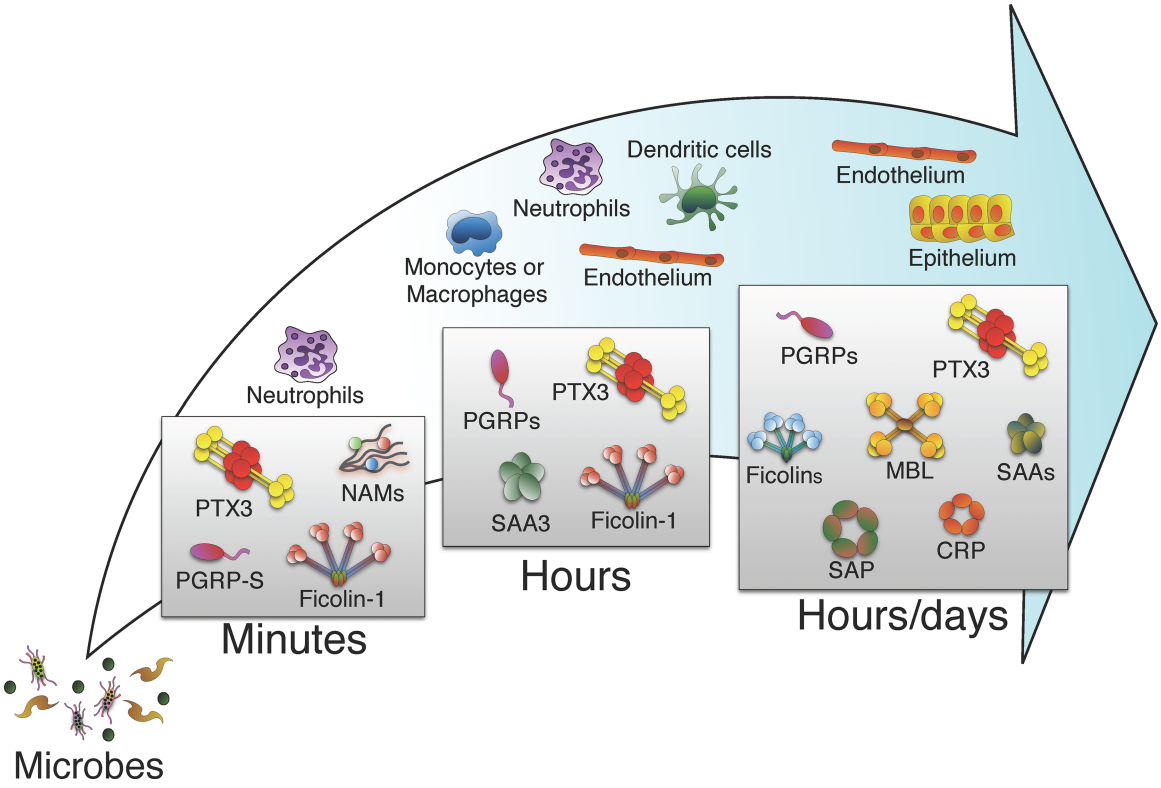

TABLE 1 Expression sites, ligands, and activities of soluble PRMs[a]

PRMs	Expression sites	Ligands	Activities
Collectins (MBL, SP-A, SP-D)	Liver (hepatocytes) Lung (type II alveolar cells)	Microorganisms (bacteria, fungi, viruses) and microbial moieties (LPS, LTA, LOS, PDG) Carbohydrates and lipids exposed on pathogens (gp55, viral glycoprotein envelopes)	Activation and regulation of the complement system Opsonic activity
Ficolins	Liver (hepatocytes) Lung (type II alveolar cells) Myeloid cells (neutrophils, monocytes, macrophages)	Microorganisms (bacteria, fungi, viruses) and microbial moieties [LPS, LTA, β-(1, 3)-D-glucan] Carbohydrates	Activation and regulation of the complement system Opsonic activity Inhibition of viral infectivity
Short pentraxins (CRP, SAP)	Liver (hepatocytes)	Microorganisms (bacteria, fungi, viruses) and microbial moieties (phosphorylcholine [CRP], LPS [SAP]) Complement components Apoptotic cells Phosphorylcholine, carbohydrates Extracellular matrix protein (fibronectin, collagen IV, laminin, proteoglycan) Amyloid fibrils	Activation and regulation of the complement system Opsonic activity (controversial data) Elimination of apoptotic cells
Long pentraxin PTX3	Myeloid cells (neutrophils, monocytes, macrophages, dendritic cells) Epithelial cells Endothelial cells Fibroblasts Adipocytes	Microorganisms (bacteria, fungi, viruses) and microbial moieties (OmpA) Complement components Extracellular matrix protein (IαI, TSG-6, fibrin) Plasminogen	Activation and regulation of the complement system Opsonic activity Inhibition of viral infectivity Elimination of apoptotic cells Matrix remodeling Fibrinolysis Regulation of P-selectin-dependent leukocyte recruitment
SAA	Liver (hepatocytes) Myeloid cells (monocytes, macrophages) Synovial cells Adipocytes	Microorganisms (bacteria, viruses) and microbial moieties (Omp)	Opsonic activity Inhibition of viral infectivity
PGLYRPs	Epithelial cells Liver Neutrophils	Bacteria PDG	Bactericidal activity

[a]Abbreviations: LOS, lipooligosaccharide; PDG, peptidoglycan; IαI, inter-α-trypsin inhibitor; TSG-6, TNF-α-induced protein 6.

be traced back to lamprey and ascidians, respectively (9, 10). For instance, an MBL-like lectin was isolated from the plasma of a urochordate, and an ortholog of mammalian C1q was found in lamprey (10, 11). Therefore, before the appearance of adaptive immunity and immunoglobulins in jawed vertebrates, C1q may have functioned as a PRM in innate immunity. Subsequently, the emergence of adaptive immunity has directed the specificity of C1q for the Fc region of immunoglobulins.

Collectins are formed by subunits composed of three identical polypeptide chains, with the exceptions of human SP-A, formed by two polypeptide chains, SP-A1 and SP-A2; and CL-LK (see above). It is thought that two SP-A1 molecules combine with one SP-A2 molecule to form a heterotrimer (4, 12, 13). The number of trimeric units required per molecule differs among collectins, and the degree of multimerization of collectins can affect their functions. Each chain of collectins is formed by a globular C-terminal carbohydrate recognition domain (CRD), which mediates the lectin activity; a short α-helical hydrophobic neck region composed of 24 to 28 amino acids; a collagen-like region consisting of *n* repetitions of the triplet Gly-Xaa-Yaa, where Xaa and Yaa are usually proline or hydroxyproline; and an N-terminal region composed of 7 to 28 amino acids (14). The collagen-like region is involved in the oligomerization and stability of the molecule, and the multimeric organization is stabilized by hydrophobic interactions and interchain disulfide bonds. SP-A is an octadecamer formed by six trimeric subunits, giving rise to a bouquet-like structure, whereas SP-D and conglutinin are dodecamers formed by four trimeric subunits, giving rise to a cruciform-like structure (15–17). A dodecameric structure was also proposed for CL-46 (18). Different oligomers of the homotrimeric structural unit of MBL were found in serum, ranging from dimers to octamers (19–21). The two major forms, termed MBL-I and MBL-II, are trimers and tetramers of the structural unit, respectively. Among them, MBL-II is the most prominent oligomer (20–22). The tertiary structure

of C1q resembles that of collectins, but three different chains compose the basic building block of C1q.

Collectins interact with bacterial PAMPs, such as lipopolysaccharide (LPS) from Gram-negative bacteria, lipoteichoic acid (LTA) from *Bacillus subtilis*, and peptidoglycan from *Staphylococcus aureus* (4, 23). The CRD of collectins recognizes neutral sugar on the surface of microorganisms. In particular, collectins interact with 3- and 4-hydroxyl groups. All collectins have a mannose-type CRD with the exception of CL-P1, which has a galactose-type CRD (6). The recognition of mannose- and galactose-type ligands depends on the orientation of hydroxyl groups on sugars presented. Mannose-type CRDs interact with hydroxyls in horizontal position, while galactose-type CRDs interact with hydroxyls in vertical orientation. The selectivity may be changed from horizontal 4-OH (i.e., mannose) to vertical 4-OH (i.e., galactose) by site-directed mutagenesis introducing the residues found in galactose-type CRD (24). Glycosylated proteins found on surface of fungi, such as gp55 and gp45 from *Aspergillus fumigatus* or mannan and β-glucan from *Saccharomyces cerevisiae*, are recognized by collectins (4, 25, 26). Collectins also bind to viruses, such as HIV, influenza A virus (IAV), severe acute respiratory syndrome-associated coronavirus, and herpes simplex virus, via recognition of viral envelope glycoproteins; and to parasites, including *Leishmania major*, *Leishmania mexicana*, *Plasmodium falciparum*, and *Trypanosoma cruzi* (4).

Collectins can also bind to and regulate the activity of immune cells. For instance, SP-D can interact with T lymphocytes via its CRD, inducing the expression of cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) in T lymphocytes (27). Therefore, administration of a fragment of SP-D containing the C-terminal lectin domain had the capacity to decrease lymphoproliferation and production of interleukin-2 (IL-2) from activated splenocytes *in vitro* as well as allergen-induced inflammation *in vivo* (27). In addition, SP-A was involved in protection against the development of gastrointestinal graft-versus-host disease (28). Therefore, mice deficient in SP-A that had received allogeneic bone marrow transplantation showed increased incidence of gastrointestinal graft-versus-host disease associated with increased Th17 cells and decreased regulatory T cells (28).

The recognition of pathogens by collectins was shown to be protective for the host and to be associated with the induction of an appropriate immune response. For instance, collectins have opsonic activity, enhancing phagocytosis of pathogens, and the capacity to activate the lectin pathway of the complement system, leading to the formation of the membrane attack complex on microbial surfaces (3). However, coating of protozoa with MBL was shown to increase infectivity of cells, and MBL levels were associated with increased risk of developing visceral leishmaniasis, suggesting that MBL can act as a "double-edged sword" (4). Collectins were shown to have antiviral activity. For instance, SP-D had a protective role against IAV, reducing hemagglutination activity and causing aggregation of the virus (29). In addition, the preincubation of IAV with dodecameric SP-D had an opsonic effect, increasing the uptake of IAV by neutrophils and the respiratory burst (29, 30). However, the preincubation of neutrophils with SP-D increased neutrophil uptake of IAV but reduced the respiratory burst activity (30, 31). The neutrophil oxidative response was also reduced in the presence of other innate immune proteins, such as salivary scavenger and agglutinin (SALSA; also known as gp340) (30). These innate immune proteins may regulate the neutrophil response to IAV by reducing an excessive and potentially harmful burst response (30, 31).

In humans, three polymorphisms were reported in the *MBL2* gene and were associated with decreased systemic concentrations of MBL and increased susceptibility to infections (32). MBL is primarily expressed in the liver (32). Hormones (e.g., growth hormone and thyroid hormones) and IL-6 can induce the expression of MBL in hepatocytes in a gene expression-dependent fashion, and early reports have suggested that MBL can act as an acute-phase reactant in different scenarios (e.g., malaria and patients with major surgery) (33–36). However, other studies showed discordant results, and in patients with sepsis and septic shock, the levels of MBL were variable and not associated with the acute-phase response (37, 38). In addition to the activity related to pathogen recognition, MBL can recognize endogenous oligosaccharides (e.g., fucosylated type 1 Lewis glycans) expressed by human colorectal carcinoma cells but not present in nonmalignant tissues (39). The expression of MBL ligands was detected more frequently in transverse, descending, and sigmoid colon and was associated with an infiltration of HLA-DR$^+$ cells and a favorable prognosis in patients with colorectal cancer (39).

Ficolins

Ficolins are lectin proteins with a general structure resembling that of collectins. Ficolins constitute a family of several members identified in vertebrates (40). Three members have been identified in humans: M-ficolin (also called ficolin-1), L-ficolin (also called ficolin-2), and H-ficolin (also called Hakata antigen or ficolin-3)

(40). L-ficolin and H-ficolin are serum proteins expressed mainly in the liver (L-ficolin) and in the liver and lung (H-ficolin). M-ficolin was initially identified on the cell surface of circulating monocytes and granulocytes (41). Subsequently, M-ficolin was identified in serum and characterized as a secreted protein expressed in neutrophils, monocytes, macrophages, and type II alveolar epithelial cells (42). Two ficolins have been identified in mouse (ficolin-A and ficolin-B) and pig (ficolin-A and ficolin-B), and one ficolin has been identified in the horseshoe crab *Tachypleus tridentatus* (42).

Ficolins are oligomeric proteins assembled from an N-terminal region with two functionally important cysteine residues, a collagen-like domain, and a C-terminal fibrinogen-like domain organized in a globular structure and involved in the recognition of pathogens (43). The proposed structure for L-ficolin is a dodecamer supported by the cross-linking of subunits via disulfide bonds (44, 45). H-ficolin and M-ficolin are oligomers consisting of octadecamers with similar structures (44, 45).

The fibrinogen-like domain of ficolins is functionally similar to the CRD of collectins and is responsible for the recognition of carbohydrate by ficolins. L-ficolin, H-ficolin, and M-ficolin can interact with GlcNAc, and M-ficolin also recognizes sialic acid. In addition, L-ficolin and H-ficolin were shown to interact with microbial moieties [e.g., LTA and β-(1,3)-D-glucan for L-ficolin and LPS for H-ficolin] (43, 46, 47).

Ficolins are involved in defense against invading pathogens via a mechanism of opsonophagocytosis and the initiation of the lectin pathway of the complement system (42, 43). For instance, L-ficolin has the capacity to interact with Gram-negative bacteria, such as the rough type of *Salmonella enterica* serovar Typhimurium TV119 and *Pseudomonas aeruginosa*, and Gram-positive bacteria, such as *S. aureus*, and binding of L-ficolin on pathogens enhanced their clearance by phagocytes (42). M-ficolin and H-ficolin can also interact with Gram-negative bacteria (e.g., *Salmonella* Minnesota and *Escherichia* spp.) and Gram-positive bacteria (e.g., *S. aureus* for M-ficolin and *Aerococcus viridans* for H-ficolin) (42). L-ficolin also has the capacity to bind the virulent strain of *Mycobacterium tuberculosis* H37Rv, reducing the infection of human lung epithelial cells. Importantly, serum levels of L-ficolin decreased in patients infected with pulmonary tuberculosis, and administration of L-ficolin had therapeutic activity in H37Rv-infected mice (48).

In addition to bacteria, ficolins interact with viruses and can have antiviral effects. For instance, L-ficolin interacts with hepatitis C virus, leading to inhibition of cell infection and activation of the complement lectin pathway, and binding of H-ficolin to IAV inhibits viral infectivity and hemagglutination (42, 49, 50).

Pentraxins

Pentraxins constitute a superfamily of multifunctional proteins that are evolutionarily conserved and are characterized by a multimeric structure (51). All pentraxins contain a "pentraxin domain" in their carboxy terminus, which is a conserved 8-amino-acid-long sequence (HxCxS/TWxS, where x is any amino acid). Based on the primary structure of the protomer, pentraxins are divided into the short pentraxins C-reactive protein (CRP) and serum amyloid P (SAP) and the long pentraxins (51).

Short pentraxins

During the 1930s, the short pentraxin CRP was the first PRM identified and purified from the serum of infected patients. Its name derives from its capacity to recognize the C-polysaccharide of *Streptococcus pneumoniae* (51). Subsequently, human SAP was identified as closely related to CRP. Indeed, the degree of amino acid sequence identity between human SAP and human CRP is 51% (51).

CRP and SAP are ~25-kDa proteins organized in five identical subunits arranged in a pentameric radial symmetry (2, 51). CRP and SAP are produced by hepatocytes and constitute the main acute-phase proteins in human and mouse, respectively (2). The plasma level of CRP is low in healthy adults (≤3 mg/liter) and increases as much as 1,000-fold during inflammation. In human serum, SAP is constitutively present at 30 to 50 mg/liter (2, 51).

Besides their structural similarity, CRP and SAP also share functional properties, such as activation of the complement system and recognition of pathogens. CRP and SAP have the capacity to interact with Fcγ receptors (FcγRs), resulting in phagocytosis of microorganisms and cytokine secretion (52). Pentraxins share the binding site on FcγRs with IgG and have the capacity to inhibit immune complex-mediated phagocytosis, suggesting antibody-like functions for pentraxins (52).

As mentioned above, C-polysaccharide of *S. pneumoniae* was the first reported ligand for CRP. Subsequently, CRP was shown to bind to numerous pathogens including fungi, yeasts, and bacteria, leading to pathogen phagocytosis and resistance to infection. For instance, CRP binds to the cell wall carbohydrate of *S. pneumoniae*, and transgenic mice overexpressing CRP are resistant to *S. pneumoniae* infection (53).

Similarly to CRP, SAP binds various bacteria, such as *Streptococcus pyogenes* and *Neisseria meningitidis* (2). SAP interacts with LPS, leading to inhibition of LPS-mediated complement activation and LPS toxicity (54). The relationship between recognition of pathogens and the functions of CRP and SAP is still a matter of debate (2). Indeed, the host defense function of CRP and SAP may also occur against pathogens that they do not recognize (2). In addition, and in contrast to its pro-phagocytic activity, SAP has been shown to enhance virulence of *S.* Typhimurium and rough strains of *Escherichia coli* by inhibiting the phagocytosis of bacteria (54).

The short pentraxins have the capacity to regulate the activation of the three complement system pathways, by interacting with C1q, ficolins, C4b-binding protein (C4BP), and factor H (55). Complement activation by short pentraxins has been suggested to favor removal of apoptotic cells and cell debris, preventing the onset of autoimmune diseases (56). For instance, the binding of CRP to apoptotic cells promotes the activation of the classical pathway of the complement system without assembly of the terminal complement components (57). Therefore, CRP amplifies the phagocytosis of apoptotic cells by phagocytes associated with the expression of transforming growth factor β (57). In addition, CRP can also recruit factor H, which is known to inhibit the alternative pathway of complement, and C4BP, a regulator of the classical and lectin pathways of complement (55).

The long pentraxin PTX3

During the early 1990s, PTX3, a new secreted protein containing a C-terminal pentraxin domain, was identified as the first long pentraxin (58, 59). Subsequently to PTX3, other molecules, including guinea pig apexin, neuronal pentraxin (NP) 1, NP2, and neuronal pentraxin receptor (NPR), a transmembrane molecule, have been identified. More recently, the long pentraxin PTX4 was identified, and transcript expression analysis showed that PTX4 has a unique pattern of mRNA expression, distinct from that of other members of the family (60).

The human and murine genes coding for PTX3 are localized on chromosome 3 and organized in three exons (Fig. 2). The first two exons code for the leader signal peptide and the N-terminal domain, and the third exon codes for the C-terminal pentraxin domain (51). Many transcription factors have potential binding sites in the promoter of both the human and murine *PTX3* gene, including Pu1, AP-1, NF-κB, Sp-1, and NF-IL-6 (61). NF-κB is involved in the response to proinflammatory cytokines, and AP-1 is involved in the control of basal transcription. The production of PTX3 has been associated with different pathways, including the phosphatidylinositol 3-kinase/Akt axis and Jun N-terminal protein kinase (55). Moreover, it has been shown that the IL-1 receptor type I pathway was responsible for the production of PTX3 in the heart during acute myocardial infarction in mice and in both local and systemic sites in the model of 3-methylcholanthrene-induced carcinogenesis (62, 63). In skin wound healing, induction of PTX3 was almost completely abolished in $MyD88^{-/-}$ mice and partially reduced in $Il1r^{-/-}$, $Tlr3^{-/-}$, $Irf3^{-/-}$, and $Ticam1^{-/-}$ mice, suggesting that expression of PTX3 is downstream of TLR sensing and IL-1 amplification (64). In addition, the TLR4/MyD88 pathway controlled the production of PTX3 in uroepithelial cells during urinary tract infections mediated by uropathogenic *E. coli* (UPEC) (61).

PTX3 has a protomer of 381 amino acids composed of a signal peptide (17 amino acids), an N-terminal domain unrelated to any known protein, and a C-terminal pentraxin domain homologous to the short pentraxins CRP and SAP. The primary structure of PTX3 is highly conserved among species, with 82% identical amino acids in human and mouse, suggesting that the functional role played by PTX3 was maintained by an evolutionary pressure (51).

PTX3 is a multimeric glycoprotein with a complex quaternary structure characterized by two tetramers linked together by interchain bridges to form an octamer of 340 kDa (65). Electron microscopy and small-angle X-ray scattering (SAXS) showed that PTX3 folds into an elongated structure with a large and a small domain interconnected by a stalk region. This quaternary structure was involved in the biological function of PTX3 (Fig. 2). For instance, the N-terminal region of PTX3 forms a tetramer, which supports the recognition of several PTX3 ligands (2). In addition to the quaternary structure, the N-linked glycosidic moiety localized at Asn220 was shown to play an essential role in the interaction between PTX3 and P-selectin (66).

Inflammatory cytokines (e.g., tumor necrosis factor α [TNF-α] and IL-1β), TLR agonists (e.g., LPS), microbial moieties (e.g., outer membrane protein A of *Klebsiella pneumoniae* [KpOmpA]), or pathogens (e.g., UPEC and *A. fumigatus*) induce the expression of PTX3 in various cell types, including dendritic cells, monocytes, macrophages, epithelial cells, endothelial cells, fibroblasts, and adipocytes (55). In addition, PTX3 is also stored in neutrophil-specific granules in a ready-to-use form and rapidly released in response to microorganisms or TLR agonists (67). Upon release, a part of the molecule was found in neutrophil extracellular traps (NETs), which are known for their antimicrobial activity (67).

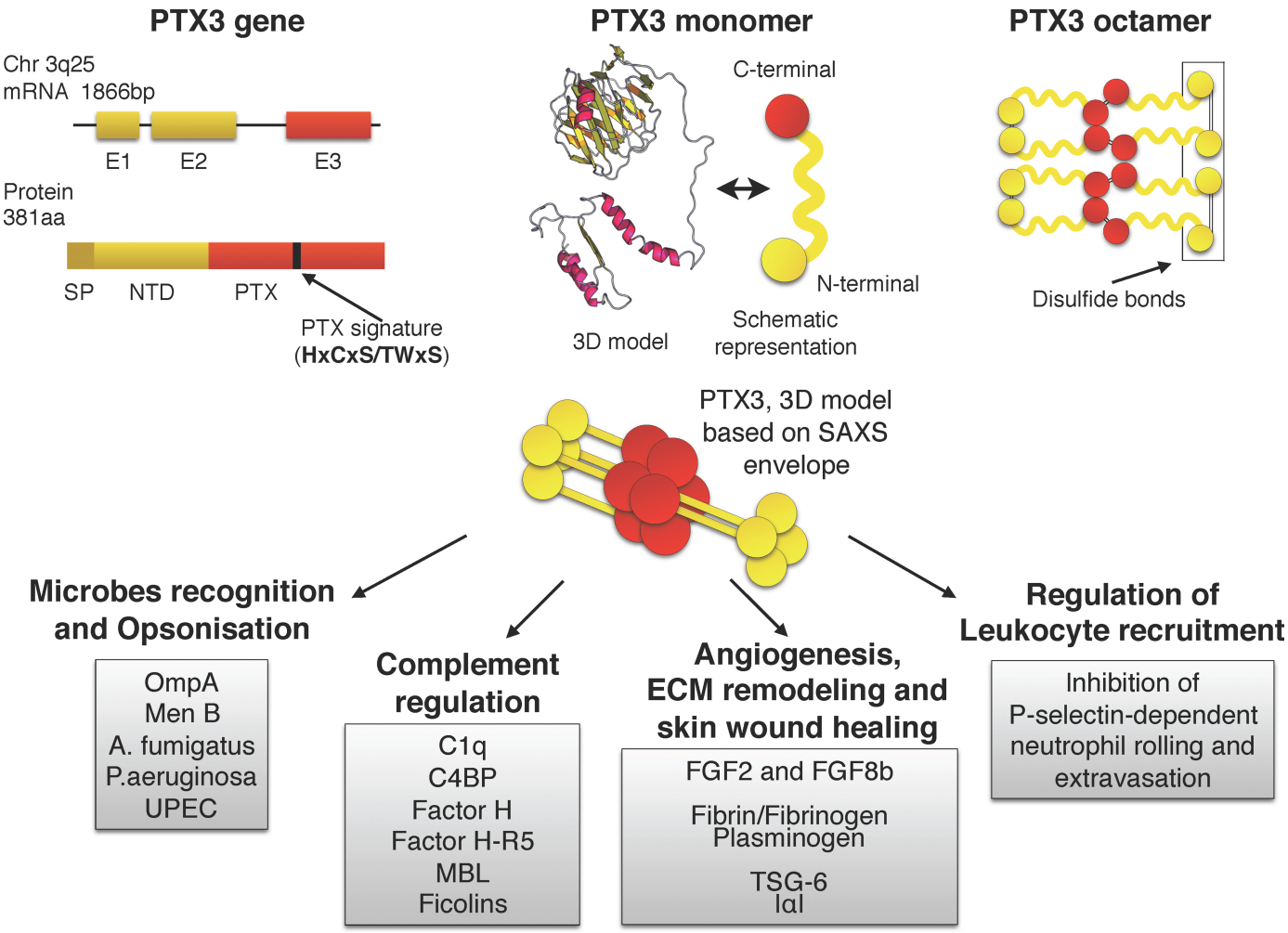

FIGURE 2 Gene organization, protein structures, and roles of PTX3. The PTX3 gene is organized in three exons. The first two exons code for the signal peptide (SP) and the N-terminal domain of the protein (NTD), respectively, and the third exon codes for the pentraxin domain (PTX). A three-dimensional model of the pentraxin domain has been generated based on the crystallographic structures of CRP and SAP, showing that the pentraxin domain of PTX3 adopts a β-jelly roll topology. PTX3 has a unique quaternary structure with eight subunits, associated together to form an octamer by disulfide bonds between cysteine residues present on both the N-terminal and C-terminal domains. Once released, PTX3 plays a role in pathogen opsonization and agglutination, complement activation, regulation of inflammation and leukocyte recruitment, angiogenesis, extracellular matrix (ECM) remodeling, and wound healing. Men B, meningococcus type B; FGF, fibroblast growth factor 2; TSG-6, tumor necrosis factor-inducible gene 6 protein; IαI, inter-alpha-trypsin inhibitor.

PTX3 binds to a wide range of microorganisms, including fungi (e.g., *A. fumigatus* conidia and *Paracoccidioides brasiliensis*), bacteria (e.g., *P. aeruginosa*, *K. pneumoniae*, UPEC, and *N. meningitidis*), and viruses (e.g., human and murine cytomegalovirus [CMV] and selected strains of influenza virus) (55). The role played by PTX3 in host defense and inflammation was revealed by the generation of PTX3-deficient mice. For instance, *Ptx3*$^{-/-}$ mice showed increased susceptibility to invasive pulmonary aspergillosis associated with defective phagocytosis and clearance of *A. fumigatus* conidia by PTX3-deficient phagocytes and a low protective Th1 response, which were restored by treatment with recombinant PTX3 (67–69). Neutrophil-associated PTX3 was essential for resistance against this pathogen (67–69). Similarly, PTX3 was involved in

defense against *P. aeruginosa*, some viruses, and UPEC (55). These protective activities and the molecular mechanisms involved will be discussed below. More recently, it has been shown that PTX3 binds to fibrin/fibrinogen and plasminogen, increasing plasmin-mediated fibrinolysis (64). Therefore, PTX3 deficiency was associated with defective wound healing of skin (64).

PTX3 participates in the fine-tuning of the inflammatory response. For instance, PTX3 binds selectively to P-selectin and competes with the interaction of P-selectin with P-selectin glycoprotein ligand-1 (PSGL-1) (66). Therefore, PTX3 can prevent exacerbated P-selectin-dependent recruitment of neutrophils in mouse models of inflammation (66, 70, 71). In contrast, PTX3 can positively regulate the inflammatory response, as demonstrated upon the recognition of the KpOmpA by PTX3 (72). Despite the fact that PTX3 does not affect the recognition of KpOmpA by cellular receptors, PTX3 amplifies the inflammation induced by KpOmpA *in vivo*, through a complement-dependent mechanism (72, 73). Accordingly, PTX3-overexpressing mice infected by *K. pneumoniae* present an increased production of proinflammatory mediators, including nitric oxide and TNF-α (74).

As reported for CRP and SAP, PTX3 has the capacity to regulate the activation of the complement system. The complement component C1q, which is the main activator of the classical pathway of the complement system, was the first ligand identified for PTX3. This interaction occurs between the globular head of C1q and PTX3 and can activate or inhibit the complement cascade, depending on the way in which the binding occurs. Plastic-immobilized PTX3 recruits C1q and induces the activation of the classical cascade of complement, whereas the interaction between PTX3 and C1q in the fluid phase inhibits complement activation by blocking the interaction of C1q with immunoglobulins (55). In addition, PTX3 has the capacity to regulate the lectin pathway of the complement system via direct interaction with ficolin-1, ficolin-2, and MBL. For instance, the formation of heterocomplexes PTX3/ficolin-2 and PTX3/MBL can promote the deposition of complement, as observed on the surface of *A. fumigatus* and *Candida albicans*, respectively (75, 76). In addition, the deposition of ficolin-1 on late apoptotic cells is amplified in the presence of PTX3, leading to increased phagocytosis of apoptotic cells by macrophages (77).

PTX3 also interacts with factor H-related protein 5 and negative complement regulators, such as factor H and C4BP, favoring their deposition on PTX3-coated surfaces (78–80). For instance, PTX3 binds to apoptotic cells and induces the recruitment of C4BP, limiting complement activation and an exacerbated inflammatory response (78). In a murine model of myocardial infarction, PTX3-deficient mice showed higher myocardial damage associated with increased neutrophil infiltration and C3 deposition, likely due to the missing deposition of factor H (62, 79).

Recently, PTX3 deficiency was found to be associated with increased susceptibility to mesenchymal and epithelial carcinogenesis and increased cancer-related inflammation (63). Tumors developed in a PTX3-deficient context were characterized by exacerbated inflammation, amplification of complement activation, recruitment of tumor-promoting macrophages, higher frequency of *Trp53* mutations, increased DNA oxidative damage, and higher expression of DNA damage response markers (63). It has been shown that PTX3 acts as an oncosuppressor through recruitment of the negative regulator factor H and that exacerbated inflammation observed in PTX3-deficient mice contributes to cancer genetic instability (63).

Other Soluble PRMs

Peptidoglycan recognition proteins

Peptidoglycan recognition proteins (PGRPs) are innate immune molecules highly conserved from insects to humans and playing a key part in defense against bacteria (81, 82). PGRPs constitute a family of four proteins named PGRP-S, PGRP-L, PGRP-Iα, and PGRP-Iβ. The Human Genome Organization Gene Nomenclature Committee modified their names to PGLYRP (peptidoglycan recognition protein)-1, -2, -3, and -4, respectively (81).

The short peptidoglycan recognition protein, PGLYRP-1, is 196 and 182 amino acids long in humans and mice, respectively, and composed of a signal peptide and a type 2 amidase domain. This domain is called the PGRP domain and is homologous to bacteriophage and bacterial type 2 amidases (81). Long (i.e., PGLYRP-3 and PGLYRP-4) and intermediate-sized (i.e., PGLYRP-2) molecules present a C-terminal PGRP domain and a unique amino-terminal sequence not conserved and with variable length among PGRPs (81).

Mammalian PGRPs are expressed in various cell types and involved in amidase activity and antibacterial activity. PGLYRP-1 is expressed in bone marrow and stored in neutrophil granules, and PGLYRP-3 and PGLYRP-4 are expressed by epithelial cells from tissues that come in contact with the external environment (e.g., tongue, eyes, skin, salivary gland, throat, and esopha-

gus) (83). PGLYRP-2 is an *N*-acetylmuramoyl-L-alanine amidase produced by the liver and secreted into the circulation. PGLYRP-2 hydrolyzes the lactyl bond between the MurNAc and the L-Ala in peptidoglycan, reducing the proinflammatory activity of polymeric peptidoglycan (83).

PGLYRP-1 (PGRP-S), PGLYRP-3 (PGRP-Iα), and PGLYRP-4 (PGRP-Iβ) bind bacterial peptidoglycan and have bactericidal activities against nonpathogenic and pathogenic bacteria (83). PGRPs kill bacteria by mechanisms similar to those employed by antibiotics. Briefly, PGRPs bind to the cell wall of Gram-positive bacteria and to the outer cell membrane of Gram-negative bacteria, inducing a bacterial stress response that leads to membrane depolarization, arrest of macromolecule synthesis, accumulation of toxic hydroxyl radicals, and bacterial death (82, 84).

Serum amyloid A

Serum amyloid A (SAA) is a family of α-helical proteins divided into acute-phase SAAs (A-SAAs) and constitutive SAAs (C-SAAs). Four mouse SAAs (SAA1, SAA2, SAA3, and SAA4) and three human SAAs (SAA1, SAA2, and SAA4) were identified. SAA1 and SAA2 are acute-phase proteins and SAA4 is expressed constitutively (85). SAA3 is a truncated protein expressed in mice by extrahepatic cells, including adipocytes and macrophages, but is a pseudogene in humans (85).

A-SAAs are produced mainly by the hepatocytes upon proinflammatory stimuli, including proinflammatory cytokines and bacterial moieties (2). In addition to the liver, atherosclerotic plaque cells (especially macrophages and smooth muscle cells), synovial cells, chondrocytes, and epithelial cell lines can produce SAAs, and adipocytes represent the major site of SAA expression in obese individuals (85).

SAA interacts with a large variety of Gram-negative bacteria (e.g., *E. coli*, *K. pneumoniae*, *Shigella flexneri*, and *P. aeruginosa*) through binding to OmpA family members (2). SAA has opsonic activity toward bacteria, increasing their phagocytosis and the production of TNF-α and IL-10 by phagocytes (86). In addition to bacteria, SAA also recognizes hepatitis C virus and blocks viral entry into cells (87).

SAA can also interfere with the inflammatory response. For instance, SAA3 has been shown to interact with myeloid differentiation protein 2 (MD-2) and activate the MyD88-dependent TLR4/MD-2 pathway (88). Accordingly, injection of SAA3 peptide in mice increased the recruitment of wild-type but not *MD-2*$^{-/-}$ CD11b^{+}Gr-1^{+} cells in the lungs, suggesting that SAA3 serves as an endogenous signal to induce myeloid cell mobilization from the bone marrow in an MD-2-dependent manner (88). In addition, recent investigations showed that SAA3 activates the NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome, leading to the promotion of Th17 allergic asthma (89). Most recently, SAA was involved in the expression of IL-33 by monocytes and macrophages (90). TLR2, known to be a receptor for SAA, is responsible for the induction of IL-33, and interferon (IFN) regulatory factor 7 (IRF7) is a critical transcription factor for SAA-induced IL-33 expression (90).

MACROPHAGES AS A SOURCE OF PRMs

Macrophages are major components of innate immune defense, expressing a large variety of PRMs (91). Macrophages express cell surface receptors, including TLRs, scavenger receptors (e.g., SR-A, MARCO [macrophage receptor with collagenous structure], SRCL-1 [scavenger receptor C-type lectin 1], and Lox-1), C-type lectin receptors (e.g., dendritic cell (DC)-specific intercellular adhesion molecule (ICAM)-3-grabbing non-integrin (DC-SIGN), mannose receptor, Dectin-1 [dendritic cell-associated C-type lectin 1], Dectin-2, Mincle, and CLEC5A), and intracellular receptors (e.g. NOD-1, NOD-2, RIG-I, and MDA5) (91, 92). These PRMs are involved in the recognition of self, nonself, and modified self and in the subsequent responses. For instance, scavenger receptors were shown to contribute to the recognition and phagocytosis of pathogens and apoptotic cells by macrophages and to regulate the inflammatory response (91, 93). Also, the receptors of the C-type lectin family were involved in processes of pathogen recognition, cellular interactions, and regulation of the inflammatory response. For instance, Dectin-1 was involved in the recognition and uptake of β-glucans and yeast, and the immunoreceptor tyrosine-based activation motif in its intracellular tail was involved in cell activation (91).

Macrophages are also a source of fluid-phase PRMs, including PTX3, ficolins (human M-ficolin and mouse ficolin-B), and SAA3 (2). Expression of PTX3 in macrophages was induced in response to microbial components (e.g., zymosan and LPS) and proinflammatory cytokines (e.g., TNF-α and IL-1β) (55). In turn, PTX3 has the capacity to interact with zymosan, inducing aggregation and phagocytosis of a high number of particles by macrophages through a Dectin-1-dependent mechanism (94). Accordingly, macrophages from PTX3 transgenic mice showed increased phagocytosis of zymosan particles as well as the yeast form of *P. brasiliensis* (54).

Similarly, the production of PTX3 was enhanced upon stimulation by KpOmpA, and PTX3 in turn recognized this microbial moiety, enhancing the binding of this microbial moiety to the scavenger receptors SREC-1 and Lox-1 and inducing an amplification loop of the inflammatory response (72).

Macrophages also express M-ficolin and ficolin-B in humans and mice, respectively. Ficolin-B localizes in lysozymes of activated macrophages and has the capacity to interact with GalNAc and sialic acid. Mouse ficolin-B can recognize and aggregate bacteria, such as *S. aureus*, leading to increased phagocytosis of the pathogen by macrophages (43). In humans, macrophages express and secrete M-ficolin, and this expression was increased upon exposure to TLR2 or TLR4 ligands (95). Recent studies showed the formation of a ficolin-1 (M-ficolin)/PTX3 complex on the surface of apoptotic cells, facilitating the clearance of apoptotic cells and downregulating the release of IL-8 by macrophages (77).

NEUTROPHILS AS A SOURCE OF PRMs

Neutrophils express a vast repertoire of cellular-associated PRMs, including all TLRs with the exception of TLR3, C-type lectin receptors (e.g., Dectin-1, CLEC2, CLEC4E [also called Mincle], and CLEC4D [also called CLECSF8]), and cytoplasmic receptors (such as NOD-1, RIG-I, MDA5, and the DNA sensor IFN-inducible protein 16 [IFI16]) (96). These receptors are involved in the activation and regulation of neutrophil effector functions, such as phagocytosis; production of cytokines, antimicrobial peptides, and reactive oxygen species; and formation of NETs (96). For instance, phagocytosis and elimination of *C. albicans* and *A. fumigatus* by neutrophils was shown to occur through a Dectin-1-dependent mechanism (97). Interestingly, the blockage of Dectin-1-mediated phagocytosis of *C. albicans* in neutrophils can be compensated for by exogenous MBL, suggesting that a cooperation exists between cell-associated and fluid-phase PRMs in neutrophils for the development of an optimal antifungal response (98). Neutrophils also express the formyl peptide receptors (FPRs) FPR1 and FPR2, two seven-transmembrane G protein-coupled receptors with a high and low affinity, respectively, for the bacterial-derived peptide *N*-formyl-methionyl-leucyl-phenylalanine (99, 100). Activation of FPR1 or FPR2 leads to activation or inhibition, respectively, of neutrophil chemotaxis (99).

Neutrophils express a set of cytosolic DNA sensors, such as IFI16, MDA5, RIG-I, leucine-rich repeat flightless-interacting protein 1 (LRRFIP1), Asp-Glu-Ala-Asp (DEAD) box protein 41 (DDX41), and STING (stimulator of IFN genes), promoting the expression of IFN-β and CXCL10 in neutrophils upon stimulation by intracellular pathogens or transfection with plasmid DNA (101).

Neutrophils have emerged as a source of fluid-phase receptors, including PTX3, M-ficolin, and PGRP-S. These molecules are stored in neutrophil granules in a ready-to-use form. Therefore, neutrophils serve as a reservoir, ready for rapid release and covering a temporal window preceding gene expression-dependent production.

NETs are an extracellular fibrillary network formed by activated neutrophils during the process of "NETosis" (102). NETs are decorated by nuclear components and a set of proteins from primary, secondary, and tertiary granules, comprising the PRMs PTX3 and PGRP-S (96). NETs trap bacteria (e.g., *E. coli*, *S. flexneri*, and *S. aureus*) and fungi (e.g., *A. fumigatus* and *C. albicans*), promoting their interaction with effector molecules and their elimination (102). More recently, NETs were involved in the host defense against viruses, such as HIV-1 and myxoma virus (103, 104). In addition to neutrophil-associated molecules, SP-D can simultaneously recognize NETs and carbohydrate ligands *in vivo*. Therefore, agglutination of bacteria by SP-D allows an efficient bacterial trapping in NETs (105).

The expression of PTX3 mRNA is restricted to immature cells, and mature neutrophils stored the protein into the secondary granules (also known as specific granules) (67). Released PTX3 has opsonic activity against pathogens (e.g., *A. fumigatus*, *P. aeruginosa*, and UPEC), and part of the molecule is localized into NETs. Molecular mechanisms involved in the protective effects of PTX3 during bacterial, fungal, and viral infections are discussed below. PTX3 accumulates in blebs at the surface of late apoptotic neutrophils, resulting from its active translocation from granules to the membrane, and acts as a late "eat me" molecule involved in the recognition and elimination of apoptotic neutrophils by macrophages (106).

PGRP-S and M-ficolin are stored in secondary and tertiary granules (107, 108). PGRP-S was identified in bovine, murine, and human neutrophils, and similarly to PTX3, PGRP-S was found in NETs, where it can exert bacteriostatic and bactericidal activities against selected microorganisms (e.g., *Micrococcus luteus*, *S. aureus*, and *B. subtilis*) (84, 107). PGRP-S deficiency in mice was associated with increased susceptibility to intraperitoneal infection with *M. luteus* and *B. subtilis* (109). Neutrophils isolated from PGRP-S-deficient mice were able to recognize and phagocytose bacteria but failed to generate an oxidative burst (109). In addition, both PGRP-S and lysozyme recognize peptidoglycan and

showed a synergistic antimicrobial effect against *E. coli* (containing mesodiaminopimelic acid-type peptidoglycan) (107).

M-ficolin released from neutrophil granules can bind to the neutrophil surface through a direct interaction with CD43, leading to cell adhesion and aggregation and activation of the lectin complement pathway on neutrophils (108, 110).

REGULATION OF PHAGOCYTE FUNCTION BY FLUID-PHASE PRMs: PTX3 AS A PARADIGM

PTX3 in Defense Against Viruses

PTX3 recognizes both human and murine CMV, coronavirus murine hepatitis virus strain 1, and specific strains of influenza virus (H3N2). PTX3-deficient mice showed increased susceptibility to infections by these viruses, and PTX3 had therapeutic activity (111–113).

PTX3 interacts with H3N2 via the recognition of the hemagglutinin glycoprotein found on the surface of viruses and the glycosidic moiety of PTX3 (113). This interaction was shown to have antiviral activities, inhibiting viral hemagglutination and neuraminidase activity and neutralizing the virus infectivity (113). In contrast, both seasonal and pandemic H1N1 influenza virus and other subtypes of H3N2 viruses were not recognized by PTX3 and were resistant to the antiviral activity of PTX3 (114, 115).

PTX3 also has the capacity to bind to human and murine CMV, inhibiting the entry of virus into dendritic cells (112). In addition, it has been proposed that PTX3 can protect mice from murine CMV primary infection and reactivation *in vivo*, as well as *Aspergillus* superinfection, through the activation of IRF3 in dendritic cells and the promotion of the IL-12/IFN-γ-dependent effector pathway (112).

PTX3 in Defense Against Fungi

Zymosan was shown to induce the expression of PTX3 in macrophages, and, in turn, PTX3 can bind to zymosan and the yeast form of *P. brasiliensis* (94). As previously mentioned, PTX3 has opsonic activity, and phagocytosis of *P. brasiliensis* was increased in PTX3-overexpressing macrophages. Mechanistically, opsonization of zymosan by PTX3 induced the aggregation of zymosan and the phagocytosis of a high number of particles by macrophages via a mechanism dependent on Dectin-1 (94).

As previously mentioned, PTX3 has the capacity to bind to *A. fumigatus* conidia, and $PTX3^{-/-}$ mice are highly susceptible to invasive pulmonary aspergillosis (69). Interestingly, PTX3-deficient neutrophils and macrophages have defective phagocytosis of conidia, and opsonization of spores by recombinant PTX3 or neutrophil-associated PTX3 reverses this phenotype (67, 69). Accordingly, PTX3 expressed by neutrophils is essential to control fungal growth *in vitro* and *in vivo* (67).

Investigations showed that the opsonic activity of PTX3 against fungi occurred through an FcγRII- and complement-dependent mechanism (68). Indeed, PTX3 can increase the phagocytosis of *A. fumigatus* conidia by neutrophils by interacting with FcγRII, which has been proposed as pentraxin receptor (52). Briefly, the binding of PTX3-opsonized conidia to FcγRII was shown to induce activation of the complement receptor 3 (CD11b/CD18), with subsequent increased phagocytosis of C3b-opsonized conidia by neutrophils (Fig. 3) (68). Recently, it has been proposed that PTX3 binds to MD-2, inducing protective antifungal activity through TLR4/MD-2-mediated signaling (116).

PTX3 can also interact with ficolin-2, and the binding of PTX3 on conidia was amplified by ficolin-2 and vice versa (75). Subsequently, ficolin-2-dependent complement deposition on the surface of *A. fumigatus* was enhanced by PTX3, suggesting that PTX3 and ficolin-2 can cooperate to amplify microbial recognition and effector functions (75). Similarly, PTX3 can interact with MBL on the surface of *C. albicans*, and the formation of this heterocomplex triggered complement deposition and phagocytosis of *C. albicans* by neutrophils (117) (Fig. 3).

In humans, single-nucleotide polymorphisms within the *PTX3* gene were associated with reduction of the intracellular stock of PTX3 in neutrophils and PTX3 plasma levels and increased susceptibility to *A. fumigatus* infection in patients undergoing bone marrow transplantation (118, 119).

PTX3 in Defense Against Bacteria

PTX3 interacts with selected bacteria, including *P. aeruginosa*, *N. meningitidis*, *K. pneumoniae*, and UPEC (55, 120).

PTX3 displays opsonic activity against *P. aeruginosa*, facilitating the phagocytosis of this pathogen by neutrophils. As observed for *A. fumigatus*, PTX3 amplifies the phagocytosis of *P. aeruginosa* through an interplay between complement and FcγRs (121). Importantly, PTX3 showed therapeutic activity in a mouse model of chronic *P. aeruginosa* lung infection, reducing bacterial load and preventing excessive activation of the inflammatory response (121). Moreover, orally administered

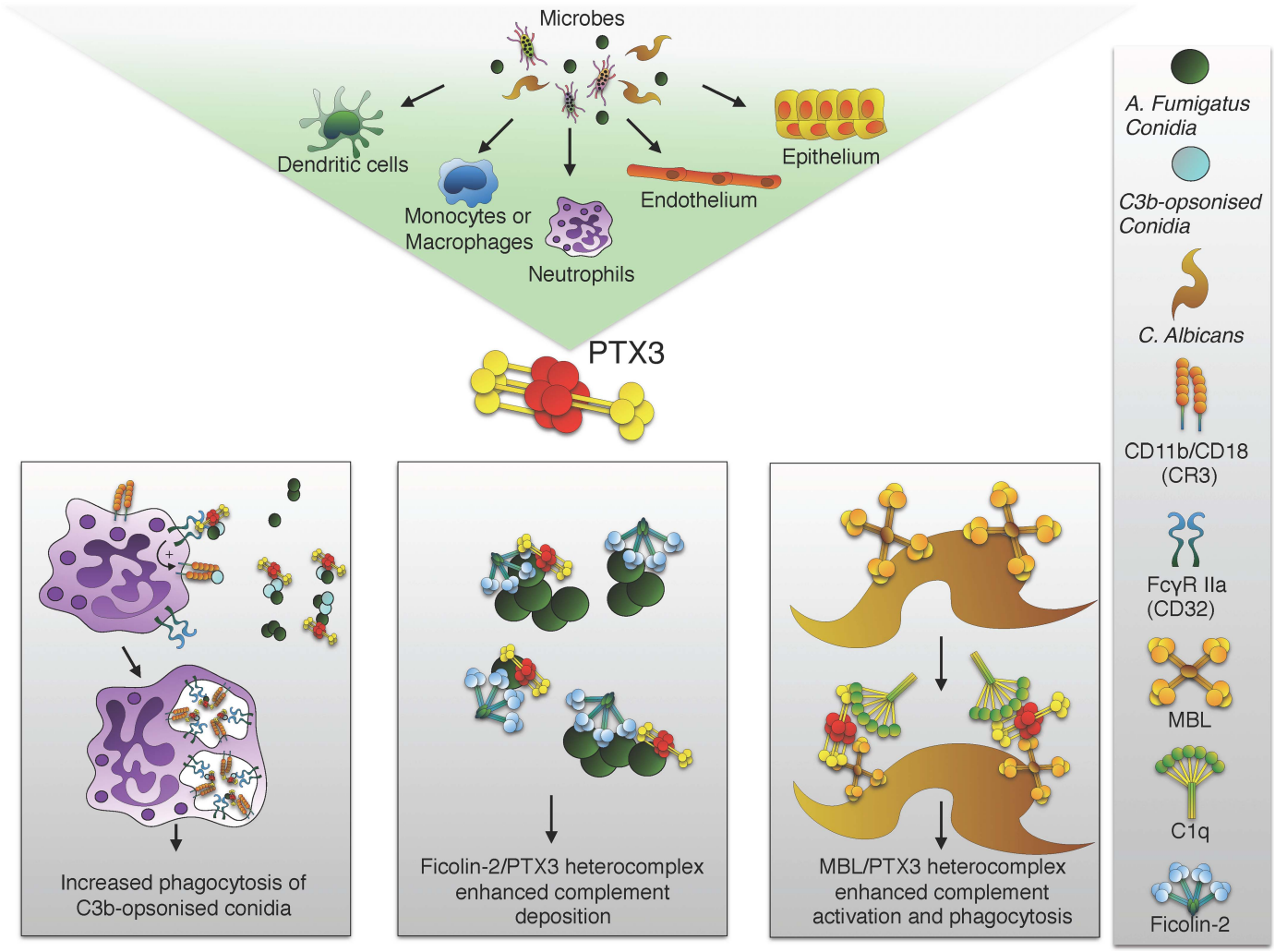

FIGURE 3 Role of PTX3 in defense against fungi. In the presence of PTX3-opsonized conidia, FcγRIIA induces inside-out CD11b/CD18 activation, recruitment to the phagocytic cup, and amplification of C3b-opsonized conidia phagocytosis (left panel). PTX3 interacts with ficolin-2 and MBL on the surface of conidia and *C. albicans*, respectively, triggering complement deposition and phagocytosis of pathogens.

PTX3 in neonate mice can diffuse rapidly in tissues and provided protection against *P. aeruginosa* lung infection (122).

Recently, PTX3 was identified as the first soluble PRM essential in defense against UPEC-induced urinary tract infection (61). UPEC induced a rapid production and secretion of PTX3 by uroepithelial cells in a TLR4- and MyD88-dependent manner. In turn, PTX3 opsonized UPEC, enhancing phagocytosis and phagosome maturation in neutrophils (61). Therefore, PTX3 deficiency in mice was associated with exacerbated inflammation and tissue damage, demonstrating a fundamental role played by PTX3 in defense against urinary tract infections (61).

In humans, genetic studies showed that *PTX3* single-nucleotide polymorphisms were associated with increased susceptibility to pulmonary tuberculosis, acute pyelonephritis, cystitis, and *P. aeruginosa* infections (61, 123, 124).

PTX3 in Clearance of Apoptotic Cells

An efficient and rapid elimination of apoptotic cells is required to maintain tissue homeostasis (93). The short pentraxins CRP and SAP interact with apoptotic cells, promoting their elimination by phagocytes. In contrast, the presence of PTX3 in fluid phase was shown to inhibit the clearance of apoptotic cells by phagocytes, likely due to the sequestration of C1q by PTX3 (125). In contrast,

when PTX3 was preincubated with apoptotic cells, the deposition of C1q and C3 on apoptotic cells was increased (126). Moreover, opsonization of apoptotic cells by PTX3 increased the deposition of factor H to their surface, suggesting that PTX3 may limit the complement-mediated lysis of apoptotic cells (79).

PTX3 was found accumulated in blebs at the surface of late apoptotic neutrophils, resulting from the translocation of PTX3 from granules to blebs mediated by activation of a caspase- and rho-associated protein kinase 1 (ROCK-1)-dependent mechanism (106). In addition to neutrophils, PTX3 was also observed at the surface of late apoptotic macrophages and membrane-associated PTX3 acted as a late "eat-me" molecule, increasing the phagocytosis of late apoptotic neutrophils and macrophages by phagocytes (106, 127). Therefore, the membrane-associated form of PTX3 may promote the elimination of apoptotic cells before loss of cell membrane integrity, whereas fluid-phase PTX3 released during inflammation can inhibit the capture of apoptotic cells.

PTX3 in Leukocyte Recruitment

The process of leukocyte extravasation is essential for the development of innate and adaptive immune responses. This process is divided in sequential cell migration events, including chemoattraction, tethering, rolling, adhesion, diapedesis, and transmigration, and is regulated by cellular adhesion molecules, including selectins (128). P-selectin is found on the surface of activated endothelial cells and interacts with PSGL-1 expressed on leukocytes, favoring tethering and rolling of leukocytes on activated endothelial cells (128).

Studies have reported that PTX3 was engaged in regulatory loops to finely tune the inflammatory response and leukocyte extravasation. Indeed, PTX3 interacted selectively with P-selectin, and not with E-selectin or L-selectin, via its N-linked glycosidic moiety and competed with the interaction of P-selectin with the leukocyte receptor PSGL-1 (66). Therefore, endogenous PTX3 from hematopoietic cells or administration of recombinant PTX3 acted as a negative feedback loop, preventing an excessive recruitment of neutrophils in inflamed tissues (66, 71).

CONCLUDING REMARKS

The humoral arm of innate immunity is composed of members of the complement cascade and soluble PRMs. These PRMs are diverse in term of structure but share fundamental mechanisms of the immune response, including activation and regulation of the classical, alternative, and lectin pathways of the complement system; opsonization of pathogens to facilitate their elimination by phagocytes; aggregation and neutralization of viral particles; and elimination of apoptotic cells.

Generation of genetically modified animals showed the importance of these PRMs in different pathological conditions, including infections, exacerbated inflammation, autoimmunity, and cancer. Importantly, genetic and epigenetic evidence supports these activities also in humans.

Therefore, PRMs are part of the immune response and participate in the fine-tuning of the inflammatory response. The expression and release of different PRMs by different cell types allow their presence at different tempos of the immune and inflammatory responses. Indeed, PRMs can be stored in neutrophil granules, ready for a rapid release, or *de novo* synthesized by mononuclear phagocytes and dendritic cells. Then epithelial tissues including the liver can sustain systemic production of soluble PRMs.

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