

Pentraxins in innate immunity: lessons from PTX3

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Abstract The innate immune system constitutes the first line of defence against microorganisms and plays a primordial role in the activation and regulation of adaptive immunity. The innate immune system is composed of a cellular arm and a humoral arm. Components of the humoral arm include members of the complement cascade and soluble pattern recognition molecules (PRMs). These fluid-phase PRMs represent the functional ancestors of antibodies and play a crucial role in the discrimination between self, non-self and modified-self. Moreover, evidence has been presented that these soluble PRMs participate in the regulation of inflammatory responses and interact with the cellular arm of the innate immune system. Pentraxins consist of a set of multimeric soluble proteins and represent the prototypic components of humoral innate immunity. Based on the primary structure of the protomer, pentraxins are divided

into two groups: short pentraxins and long pentraxins. The short pentraxins C-reactive protein and serum amyloid P-component are produced by the liver and represent the main acute phase proteins in human and mouse, respectively. The long pentraxin PTX3 is produced by innate immunity cells (e.g. PMN, macrophages, dendritic cells), interacts with several ligands and plays an essential role in innate immunity, tuning inflammation and matrix deposition. PTX3 provides a paradigm for the mode of action of humoral innate immunity.

Keywords Innate immunity · Inflammation · Pattern recognition · Pentraxins · PTX3

Introduction

The mammalian immune system is organized around two components: innate and adaptive immunity. The innate immune system is older in terms of evolution and constitutes the first line of defence against microorganisms. In vertebrates, this system is complemented by adaptive immunity, a more recent feature in terms of evolution; adaptive immunity provides the basis of immunological memory and involves the use of specific receptors.

Organisms are constantly in contact with microbes and our ability to induce a protective immune response resides in our competence to identify potential pathogens. The molecules involved in this recognition are germline-encoded receptors called pattern recognition molecules (PRMs). In essence, these receptors specifically recognize highly conserved motifs called pathogen-associated molecular patterns (PAMPs) expressed by microorganisms (Iwasaki and Medzhitov 2010). Based on their localization, PRMs are divided into the cell-associated receptors, such as the scavenger receptors (Murphy et al. 2005) and the toll-

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like receptors (TLR; Akira et al. 2006), and the fluid-phase molecules (Bottazzi et al. 2010). Fluid-phase molecules (collectins, ficolins and pentraxins) constitute the humoral arm of the immune system and represent the functional ancestors of antibodies (Bottazzi et al. 2010; Endo et al. 2007; Holmskov et al. 2003).

Pentraxins are a pivotal component of the innate immune system (Fig. 1). During the 1930s, C-reactive protein (CRP) was the first PRM to be identified and purified from the serum of infected patients (Abernethy and Avery 1941; Tillett and Francis 1930). Here, we review the key interactions and functions of pentraxins in innate immunity. In particular, we focus our attention on the prototypic long pentraxin 3 (PTX3), whose regulation has been conserved in evolution.

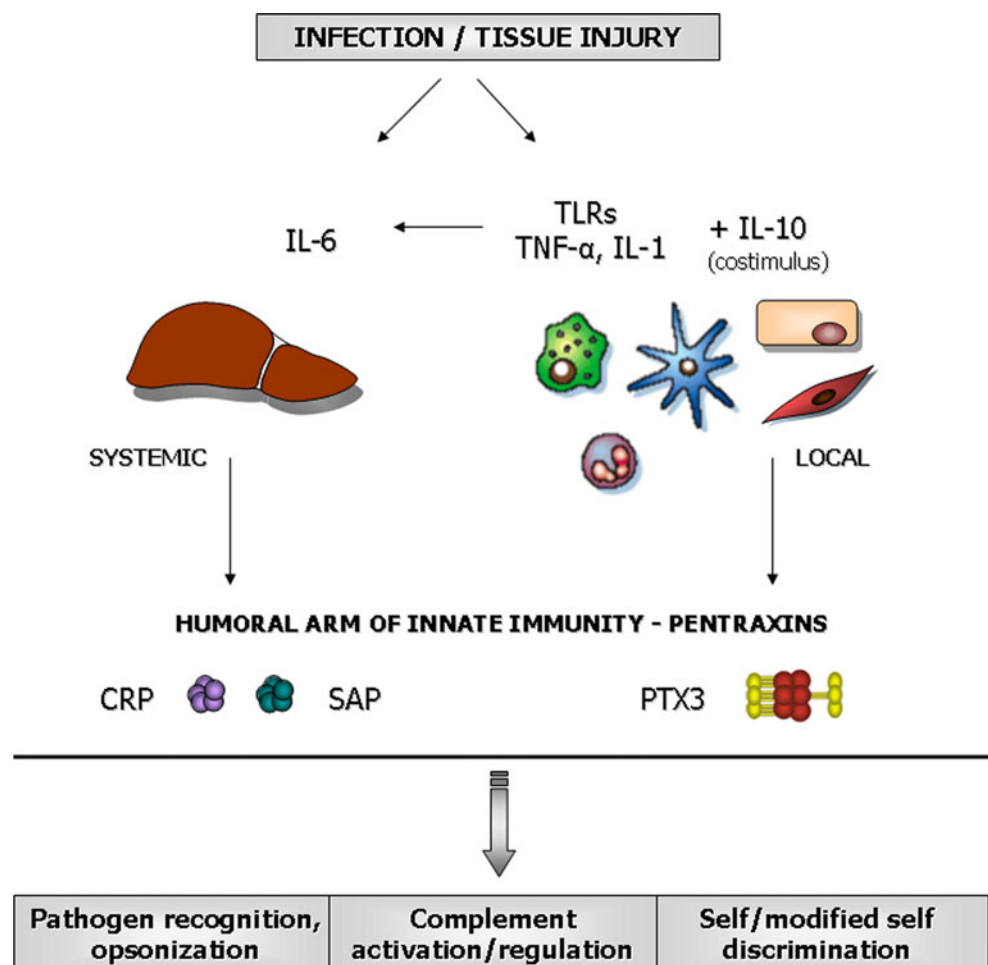
The pentraxin superfamily

Pentraxins constitute a superfamily of multifunctional multimeric proteins that are phylogenetically conserved from arachnids to mammals. All members of the family contain, in their carboxy-terminal, the “pentraxin domain”,

which is characterized by a conserved 8-amino-acid long sequence (HxCxS/TWxS, where x is any amino acid) called the “pentraxin signature” (Garlanda et al. 2005). Based on the primary structure of the protomer, pentraxins are divided into two groups: short pentraxins and long pentraxins.

CRP and serum amyloid P-component (SAP) are prototypic short pentraxins. Following the identification of CRP, human SAP was identified as its relative on the basis of amino acid sequence identity (51%). Short pentraxins are 25-kDa proteins characterized by a common structural organization in five or ten identical subunits arranged with pentameric radial symmetry (Emsley et al. 1994; Rubio et al. 1993). CRP and SAP orthologues in various mammal species share substantial sequence similarity but present notable differences in serum basal levels and changes during the acute phase response (e.g. CRP and SAP are the main acute-phase reactants in human and mouse, respectively). Circulating CRP is produced by hepatocytes, mainly in response to the proinflammatory cytokine, interleukin-6 (IL-6). IL-1 might also contribute as an additional CRP-inducing signal acting synergistically with IL-6 (Pepys and Hirschfield 2003). SAP is produced exclusively by hepatocytes and is the main

Fig. 1 Schematic overview of pentraxins in innate immunity (*IL* interleukin, *TLR* toll-like receptor, *TNF* tumour necrosis factor, *CRP* C-reactive protein, *SAP* serum amyloid P-component, *PTX* pentraxin)



acute-phase protein in mice, whereas in human serum, it is constitutively present at 30–50 mg/l (Emsley et al. 1994; Fig. 1).

During the early 1990s, PTX3, a new pentraxin-domain-containing secreted protein, was identified and classified as a prototypic long pentraxin (Breviario et al. 1992; Lee et al. 1993). Components of the subfamily of long pentraxins differ from short pentraxins in their gene organization, chromosomal localization, cellular sources, inducing stimuli and ligand-recognition ability. The long pentraxins identified after PTX3, as inducible genes or molecules expressed in specific tissues (e.g. neurons, spermatozoa), include guinea pig apexin, neuronal pentraxin (NPTX) 1 or NP1, NPTX2 (also called Narp or NP2) and neuronal pentraxin receptor (NPTXR), a transmembrane molecule (for a review, see Bottazzi et al. 2010). Orthologue molecules of long pentraxins have also been found in lower vertebrates such as zebrafish and puffer-fish (Martinez de la Torre et al. 2010). Long pentraxins have additionally been identified in *Xenopus laevis* (XL-PXN1; Seery et al. 1993). In an attempt to find new pentraxin-domain-containing proteins, we have recently identified a new long pentraxin, which we have named PTX4. As described for the long pentraxins, PTX4 is characterized by an unrelated N-terminal domain coupled to a C-terminal pentraxin domain. Analysis of PTX4 *in silico* and by transcript expression shows that the gene is well conserved from mammals to lower vertebrates. Moreover, PTX4 has a unique pattern of mRNA expression, distinct from that of other members of the family (Martinez de la Torre et al. 2010).

Short pentraxins in innate immunity

The first ligand described for CRP was the C-polysaccharide of *Streptococcus pneumoniae*: this interaction is attributable to the direct binding of CRP to phosphorylcholine, a major constituent of C-type capsule polysaccharides (Tillett and Francis 1930). Moreover, CRP binds various microbes, including fungi, yeasts, bacteria and parasites through phosphorylcholine and carbohydrate structures, promoting phagocytosis and resistance to infections (Szalai 2002). However, binding to bacteria is not always necessary for protection, because CRP also protects mice from infection with *Salmonella typhimurium*, a pathogen to which CRP does not bind (Szalai et al. 2000). Like CRP, SAP binds various pathogens but these interactions have contrasting consequences on innate immunity (Noursadeghi et al. 2000; Yuste et al. 2007). SAP has been reported to bind to phosphorylethanolamine and to lipopolysaccharide (LPS) of several bacteria and prevents LPS-mediated complement activation and LPS toxicity (de Haas et al. 2000; Schwalbe et al. 1992). In

addition, SAP binds *S. pneumoniae* and plays an important role in the complement-mediated immune response to this pathogen (Yuste et al. 2007). However, for certain organisms to which SAP binds, such as *Streptococcus pyogenes* and rough strains of *Escherichia coli*, SAP enhances virulence by protecting the bacteria against phagocytosis, whereas it is protective in infection with organisms to which it does not bind, for instance *Listeria monocytogenes* (Noursadeghi et al. 2000; Table 1). The antimicrobial activity of the short pentraxins has been thoroughly reviewed elsewhere (Garlanda et al. 2005; Szalai 2002). In the arthropod *Limulus polyphemus*, diverse forms of CRP and SAP have been identified as abundant constituents of the haemolymph involved in recognizing and destroying pathogens (Armstrong et al. 1996; Robey and Liu 1981; Shrive et al. 1999).

CRP and SAP, aggregated or attached to most of their ligands, interact with the globular head modules of complement component C1q (Roumenina et al. 2006). Complement activation by short pentraxins may be one of the mechanisms leading to the removal of cellular debris (Nauta et al. 2003b). When bound to self surfaces (e.g. apoptotic cells, damaged tissue), CRP activates the classical pathway of complement through interaction with C1q but this activation is restricted to the initial stages with only little consumption of C5–C9 (Gershov et al. 2000). Surface-bound CRP has been shown to decrease the alternative pathway C3-convertase activity, to inhibit the alternative pathway amplification loop and to reduce the deposition of C3b and lysis by the lectin pathway (Mold and Gewurz 1981; Suankratay et al. 1998). The inhibition of the alternative pathway amplification loop is, at least in part, a consequence of the interaction of CRP with Factor H (Jarva et al. 1999; Mihlan et al. 2009; Okemefuna et al. 2010). In addition to its roles in the activation of classical and alternative complement pathways, CRP has been shown to interact with Ficolin-2 (L-ficolin). Immobilized CRP recruits Ficolin-2, leading to MASP-2 activation and deposition of C4. Likewise, immobilized Ficolin-2 can recruit CRP, suggesting that Ficolin-2 widens the range of bacteria opsonized by CRP (Ng et al. 2007). Indeed, a recent study has shown that infection-induced local inflammatory conditions trigger a strong interaction between CRP and Ficolin-2; this elicits complement amplification and enhances antimicrobial activity of the classical and lectin pathways (Zhang et al. 2009; Table 1). The interaction of CRP with Ficolin-1 (M-ficolin) has been recently reported but the functional significance of this interaction has not been investigated (Tanio et al. 2009).

A specific and saturable binding to all three classes of Fc γ receptors (Fc γ R) has been demonstrated for both CRP and SAP, the interaction with Fc γ R being able to mediate

Table 1 Ligands and functions of C-reactive protein (CRP), serum amyloid P-component (SAP) and long pentraxin 3 (PTX3). Other abbreviations: C1q complement component 1q, FH complement Factor H, FcγR Fcγ receptor, KpOmpA outer membrane protein A of *Klebsiella pneumoniae*, LPS lipopolysaccharide, MASP mannan-binding lectin-associated serine protease)

Pentraxin type	Ligand	Consequence of interaction	References
CRP	Microorganisms (bacteria, fungi, yeasts)/membrane moieties (e.g. phosphocoline, phosphoetanolamine)	Complement activation Opsonization Phagocytosis enhancement Resistance to infections	Garlanda et al. 2005; Szalai 2002
	C1q	Classical pathway modulation Removal of cellular debris	Giershov et al. 2000; Mold and Gewurz 1981; Nauta et al. 2003b; Roumenina et al. 2006
	FH	Inhibition of alternative pathway amplification loop	Jlarva et al. 1999; Mihlan et al. 2009; Okemefuna et al. 2010
	Ficolin-2	MASP-2 activation C4 deposition	Ng et al. 2007; Zhang et al. 2009
	FcγR	Inhibition of immune-complex-mediated phagocytosis Activation of FcγR-mediated phagocytosis	Biharadwaj et al. 1999; Lu et al. 2008; Mold et al. 2001
	Apoptotic cells	Enhancement of clearance	Nauta et al. 2003b; Pepys and Hirschfield 2003
	SAP	Microorganisms/membrane moieties (LPS, phosphoetanolamine)	Contrasting outcomes: Aiding complement-mediated immunity, contributing to survival (<i>Streptococcus pneumoniae</i> , smooth <i>Escherichia coli</i>) Inhibition of phagocytosis, promotion of bacterial survival, pathogenicity and virulence (<i>Streptococcus pyogenes</i> , rough <i>Escherichia coli</i>)
DNA		Stabilization Protection from degradation Modulation of chromatin autoantigen handling and prevention of tolerance loss	Bickerstaff et al. 1999; Botto et al. 1997
Amyloid fibrils		Stabilization Contribution to pathogenesis of amyloidosis	Botto et al. 1997; Tennent et al. 1995
C1q		Classical pathway modulation	Nauta et al. 2003b
FcγR		Inhibition of immune-complex-mediated phagocytosis Activation of FcγR-mediated phagocytosis and cytokine secretion	Lu et al. 2008; Mold et al. 2001
Apoptotic cells		Enhancement of clearance	Gillmore et al. 2004; Nauta et al. 2003b
PTX3		Microorganisms (bacteria, fungi, yeasts, viruses)/membrane moieties (e.g. KpOmpA)	Increased opsonization Phagocytosis enhancement Complement activation/regulation Amplification of inflammatory responses
	C1q	Classical pathway activation/inhibition (depending on PTX3 presentation)	Bottazzi et al. 1997; Nauta et al. 2003a
	FH	Localization of FH activity Inhibition of alternative pathway amplification loop	Deban et al. 2008)
	Ficolin-2	Amplification of microbial recognition Amplification of complement-mediated immune responses	Ma et al. 2009

Table 1 (continued)

Pentraxin type	Ligand	Consequence of interaction	References
	Apoptotic cells	Inhibition of clearance Inhibition of cross-presentation of apoptotic-cell-derived antigens to CD8 ⁺ T cells	Baruah et al. 2006b; Jeannin et al. 2008; Nauta et al. 2003b; van Rossum et al. 2004
	P-selectin	Attenuation of leucocyte recruitment to sites of inflammation	Deban et al. 2010
	FcγR	Not characterized	Lu et al. 2008

not only the phagocytosis of apoptotic cells and microbes, but also protective immune responses (Bharadwaj et al. 1999; Mold et al. 2001). However, the interpretation of these data has been questioned, suggesting that binding may be influenced by CRP contamination with traces of IgG (Saeland et al. 2001). The crystal structure of human SAP in complex with the extracellular domain of FcγRIIa has recently been solved. The structural mechanism for pentraxin binding to FcγRs followed by the functional activation of FcγR-mediated phagocytosis and cytokine secretion has been described; this study suggests that pentraxins and IgG share the binding site and that pentraxins inhibit immune-complex-mediated phagocytosis (Lu et al. 2008). Thus, pentraxins possess similar functions to those of antibodies that activate both the complement and FcγR pathways (Table 1).

The long pentraxin PTX3

Structure and expression

The human *PTX3* gene, which is organized in three exons and localized on the chromosome 3q25, encodes for a transcript of 1861 bp. The first two exons encode for the signal peptide and the N-terminal domain (amino acids 18–179), respectively, whereas the third exon encodes for the C-terminal domain containing the pentraxin signature (amino acids 179–381). The murine gene presents the same organization and is located on chromosome 3 (Bottazzi et al. 2010). The primary structure of PTX3 indicates the presence of a unique N-linked glycosylation site in the C-terminal domain at position Asn220. Indeed, investigations have demonstrated the presence of a heterogeneous glycosidic moiety with bi-, tri- and tetra-antennary sugar structures (Inforzato et al. 2006). PTX3 is composed of eight identical protomers associated via disulphide bonds (Inforzato et al. 2008), forming an elongated asymmetric molecule with two differently sized domains interconnected by a stalk. The N-terminal domain provides the main structural determinant underlying this quaternary organiza-

tion, supporting formation of a disulphide-linked tetramer and a dimer of dimers (a non-covalent tetramer) giving rise to the asymmetry of the molecule (Inforzato et al. 2010).

High homology is found between the human and murine PTX3 promoters in which several potential enhancer-binding elements are found, i.e. activator protein-1 (AP-1), nuclear factor kappa B (NF-κB), selective promoter factor 1 (SP1; Altmeyer et al. 1995; Basile et al. 1997). The NF-κB binding site is involved in the response to the proinflammatory cytokines tumour necrosis factor-α (TNF-α) and IL-1β, whereas AP-1 promotes the basal transcription of PTX3 (Altmeyer et al. 1995; Basile et al. 1997). Other pathways might be involved in the production of PTX3. Indeed, in lung epithelial cells, the production of PTX3 induced by TNF-α does not require NF-κB but relies on the c-Jun N-terminal kinase pathway (Han et al. 2005).

A recent study reports that glucocorticoid hormones (GC) can modulate PTX3 expression in a cell-dependent manner. Indeed, whereas GC induce and amplify the production of PTX3 in fibroblasts and endothelial cells, GC treatment suppresses its production by haematopoietic cells (dendritic cells and macrophages). Moreover, the in vivo administration of GC increases the blood level of PTX3 and patients with Cushing's syndrome exhibit increased levels of circulating PTX3 (Doni et al. 2008).

PTX3 was originally identified as a cytokine-inducible gene in vascular endothelial cells and fibroblasts (Breviario et al. 1992; Lee et al. 1993). A variety of cell types express PTX3 upon exposure to inflammatory signals, such as cytokines (e.g. IL-1β, TNF-α), TLR agonists, microbial moieties (e.g. LPS, OmpA, lipoarabinomannans) or microorganisms (Bottazzi et al. 2009; Fig. 1). Whereas myeloid dendritic cells are a major source of PTX3, this molecule is also expressed by fibroblasts, endothelial cells, monocytes, macrophages, smooth muscle cells, kidney epithelial cells, synovial cells, chondrocytes, adipocytes and alveolar epithelial cells (Abderrahim-Ferkoune et al. 2003; Breviario et al. 1992; Doni et al. 2003; dos Santos et al. 2004; Goodman et al. 2000; Han et al. 2005; Introna et al. 1996; Nauta et al. 2005). In contrast, the expression of PTX3 is undetectable in T and B lymphocytes, natural killer cells or plasmacytoid dendritic

cells (Alles et al. 1994; Doni et al. 2003). Interestingly, interferon- γ (IFN γ) reduces the production of PTX3 by dendritic cells, monocytes and macrophages through the inhibition of its transcription and the reduction of the transcript stability (Doni et al. 2006; Goodman et al. 2000; Polentarutti et al. 1998). On the other hand, IL-10 amplifies LPS-induced PTX3 production (Doni et al. 2006). Given the role that IL-10 plays in the resolution of inflammation, this result suggests that PTX3 participates in tissue repair and remodelling (the function of PTX3 in extracellular matrix has been reviewed elsewhere; Deban et al. 2009). Although the expression of PTX3 mRNA is absent in neutrophils (Alles et al. 1994), we have found that PTX3 is stored in specific granules in a ready-to-use form and undergoes release in response to microorganisms or TLR agonists (Jaillon et al. 2007). PTX3 mRNA expression is confined to immature myeloid cells. Moreover, PTX3 is found associated with neutrophil extracellular traps generated upon neutrophil stimulation and neutrophil-associated-PTX3 promotes the in vivo control of *Aspergillus fumigatus* infection (Jaillon et al. 2007).

Pathogen recognition by PTX3

Among the first functions reported for PTX3 was its ability to bind certain pathogens. Indeed, PTX3 binds conidia of *A. fumigatus*, selected gram-positive and gram-negative bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *S. typhimurium*), *Paracoccidioides brasiliensis* and several viral strains (Bozza et al. 2006; Diniz et al. 2004; Garlanda et al. 2002; Jeannin et al. 2005; Reading et al. 2008). In contrast, PTX3 does not bind *Candida albicans* or *Burkholderia cepacia* (Garlanda et al. 2002). Genetically modified mice have allowed the exploration of the in vivo functions of PTX3. *Ptx3*^{-/-} mice are highly susceptible to invasive pulmonary aspergillosis (Garlanda et al. 2002). This susceptibility is associated with a low protective T helper cell type 1 (Th1) antifungal response coupled with an inappropriate Th2 response. Treatment with recombinant PTX3 restores the protective Th1 response demonstrating that PTX3 can participate in the tuning of immune responses (Garlanda et al. 2002; Gaziano et al. 2004). PTX3-deficient neutrophils, dendritic cells and alveolar macrophages exhibit defective recognition and killing of conidia. Treatment with recombinant PTX3 or neutrophil-associated PTX3 reverses this phenotype (Garlanda et al. 2002; Jaillon et al. 2007). Moreover, in an experimental model of chronic granulomatous disease (p47^{phox}^{-/-} mice), PTX3 limits the Th17 response and the pathogenic inflammation induced by *A. fumigatus* infection. In both p47^{phox}^{-/-} and p47^{phox}^{+/+} mice, PTX3 reduces neutrophil counts and mononuclear cell recruitment to the lung parenchyma and bronchoalveolar lavage fluid. Furthermore,

in p47^{phox}^{-/-} mice infected by *A. fumigatus*, PTX3 modulates cytokine production and adaptive immunity through the enhancement of a Th1/Treg response associated with the restriction of a Th2/Th17 response (D'Angelo et al. 2009). Recent investigations strongly suggest that PTX3 acts as an opsonin, enhancing the recognition and phagocytosis of conidia by neutrophils through a Fc γ receptor II (Fc γ R2)- and complement-dependent mechanism. Indeed, through its binding to Fc γ R2/CD32, PTX3 promotes the activation of CD11b and thus the phagocytosis of C3b-opsonized *A. fumigatus* conidia (F. Moalli, unpublished data).

Ptx3-overexpressing macrophages exhibit greater phagocytosis of zymosan and *P. brasiliensis* than macrophages from wild-type mice (Diniz et al. 2004). Interestingly, in response to zymosan, *ptx3*-transgenic macrophages have an enhanced expression of dectin-1, the cellular receptor primarily involved in the interaction between macrophages and zymosan. Moreover, since zymosan induces PTX3 expression, dectin-1 up-regulation promotes a positive feedback for this phagocytosis (Diniz et al. 2004).

The recognition of a microbial component by PTX3 can also amplify the inflammatory response, as reported for the interaction between PTX3 and the outer membrane protein A of *K. pneumoniae* (KpOmpA). KpOmpA is recognized by scavenger receptors lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and scavenger receptor expressed by endothelial cell-1 (SREC-1) and induces a pro-inflammatory response through the collaboration between these receptors and TLR2. KpOmpA induces the expression of PTX3 by dendritic cells and monocytes, which, in turn, bind this microbial component (Jeannin et al. 2005). In contrast to zymosan, PTX3 does not enhance the in vitro recognition of KpOmpA or the cell activation induced by KpOmpA. However, in vivo PTX3 improves the local inflammation induced by KpOmpA, in terms of cell recruitment and proinflammatory cytokine production (Jeannin et al. 2005; Table 1). This effect is complement-dependent and is abrogated after treatment with complement inhibitors. Furthermore, this amplification loop is not a general mechanism, since PTX3 does not modify the inflammatory response induced by LPS, a microbial moiety not recognized by PTX3 (Cotena et al. 2007).

PTX3 is also involved in resistance against some viral infections. Indeed, PTX3 binds both human and murine cytomegalovirus (HCMV and MCMV, respectively) and reduces the viral infection of dendritic cells in vitro. Accordingly, *ptx3*^{-/-} mice present a higher susceptibility to infections than wild-type mice and the viral titre is reduced upon treatment with recombinant PTX3. Moreover, PTX3 protects MCMV-infected mice from an *A. fumigatus* superinfection and enhances the production of IL-12 and IFN- γ by dendritic cells and T cells, respectively. The

protective effect of PTX3 is lost in TLR3^{-/-}, TLR2^{-/-} or TLR4^{-/-} mice but is still efficient in TLR9^{-/-} and MyD88^{-/-} mice and reflects the activation of interferon regulatory factor 3 (IRF3) mediated by TLR9/MyD88-independent viral recognition sensing (Bozza et al. 2006).

Finally, human and murine PTX3 binds influenza virus (H3N2) through the interaction between the viral haemagglutinin glycoprotein and the sialic acid residue present on PTX3. PTX3 inhibits virus-induced haemagglutination and viral neuramidase activity and neutralizes the virus infectivity. Consistently, *ptx3*^{-/-} mice are more susceptible than wild-type mice to influenza virus infection. Treatment with recombinant PTX3 reduces mortality and the viral load (Reading et al. 2008).

Interaction of PTX3 with complement components

The first described and best characterized ligand of PTX3 is the complement component C1q. Unlike classical pentraxins, PTX3 interacts with C1q in a calcium-independent manner, without previous aggregation of the protein. Interaction of PTX3 with plastic-immobilized C1q induces the activation of the classical complement pathway, as demonstrated by an increased deposition of C3 and C4. In contrast, fluid-phase PTX3 binding to C1q inhibits complement activation via competitive blocking of relevant interaction sites. These data indicate that PTX3 exerts a dual role in classical pathway activation, depending on the way that C1q is presented (Bottazzi et al. 1997; Nauta et al. 2003a). This hypothesis is supported by the observation that PTX3 enhances the deposition of both C1q and C3 on the cell surface when incubated with apoptotic cells. On the contrary, in the fluid phase, PTX3 reduces C1q and C3 deposition on apoptotic cells and C1q-mediated phagocytosis of apoptotic cells by dendritic cells (Baruah et al. 2006a; Table 1). Furthermore, PTX3 desialylation enhances the activation of the classical complement pathway, suggesting the involvement of the PTX3 sugar moiety in C1q recognition and complement activation (Inforzato et al. 2006).

Recently, PTX3 has been shown to interact with Ficolin-2, similarly to CRP. Indeed, Ficolin-2 could be affinity-isolated from human plasma on immobilized PTX3. In binding studies, Ficolin-1 and particularly Ficolin-2 interact with PTX3 in a calcium-independent manner. Ficolin-2, but not Ficolin-1 and Ficolin-3, binds *A. fumigatus* directly and this binding is enhanced by PTX3 and vice versa. Likewise, the Ficolin-2-dependent deposition of complement components on the surface of *A. fumigatus* is enhanced by PTX3. This effect is alleviated by a common amino acid change in the fibrinogen-like domain of Ficolin-2. Moreover, a polymorphism in the *FCN2* gene causing a T236M amino acid change in the fibrinogen-like binding domain, which affects

binding to GlcNAc, reduces Ficolin-2 binding to PTX3 (Ma et al. 2009). PTX3 and Ficolin-2 may recruit each other to the surface of recognized microbes and amplify synergistically complement-mediated innate response (Table 1). Thus, two distinct components of the humoral innate immune system (pentraxins and ficolins), which activate different complement pathways, cooperate and amplify microbial recognition and antimicrobial effector functions.

In addition to the interaction with the classical and lectin complement cascades, PTX3 has recently been observed to be able to interact with Factor H, the main soluble regulator of the alternative pathway of complement activation. PTX3-bound Factor H remains functionally active and PTX3 enhances Factor H and iC3b deposition on apoptotic cells. These observations suggest that the interaction of PTX3 with Factor H modulates alternative pathway activation by promoting Factor H deposition on PTX3-coated surfaces and preventing exaggerated complement activation (Deban et al. 2008; Table 1). Together, the above observations suggest a dual role for PTX3 in the regulation of complement-mediated immune responses. Interactions with key components of all three complement pathways (C1q, Ficolin-2, Factor H) identify PTX3 as an important component of the complex network controlling complement functions.

Self and modified-self discrimination

In addition to PAMP recognition, PRMs bind apoptotic cells and modulate their clearance by phagocytes (Jeannin et al. 2008). Both short pentraxins and PTX3 are involved in this process but promote two opposite effects. Indeed, whereas CRP and SAP recognize dying cells and enhance their elimination (Gillmore et al. 2004; Pepys and Hirschfield 2003), the opsonization of apoptotic cells by PTX3 inhibits their clearance (Gershov et al. 2000; van Rossum et al. 2004). Moreover, PTX3 inhibits the cross-presentation of apoptotic-cell-derived antigens to CD8⁺ T cells (Baruah et al. 2006a; Table 1).

Interestingly, PTX3 prevents the binding of C1q to apoptotic cells and thus inhibits their clearance mediated by C1q (Baruah et al. 2006a). In addition, PTX3 interacts with Factor H, favouring its deposition on apoptotic cells and thus playing a role as the modulator of the alternative pathway of complement activation in the context of injured tissues (Deban et al. 2008). Given that the binding of Factor H to dying cells can protect them against complement-mediated lysis (Trouw et al. 2007), PTX3 might promote the clearance of apoptotic cells in an anti-inflammatory context. Notably, this mechanism has also been described for CRP (Gershov et al. 2000; Jarva et al. 1999; Mihlan et al. 2009; Okemefuna et al. 2010).

Recently, we have reported that the sequential fusion of neutrophil granules with the cell membrane occurs during the apoptotic process. This fusion leads to the translocation of PTX3 from granules to the surface of apoptotic neutrophils. PTX3 accumulation on the apoptotic blebs is a result of an active process and acts as a late “eat me” signal for the recognition of apoptotic neutrophils by macrophages (Jaillon et al. 2009). Collectively, these results underline the dichotomy between soluble PTX3, which inhibits the clearance of dying cells, and membrane-associated PTX3, which promotes the elimination of apoptotic neutrophils. This difference might have physiological significance. Indeed, soluble PTX3 secreted following a proinflammatory signal may lead to an avoidance of the capture of dying cells in an inflammatory context and thus prevent the initiation of an immune response against self-antigens (Jeannin et al. 2008). In contrast, membrane-associated PTX3 promotes the phagocytosis of late apoptotic neutrophils and thus enhances their elimination before the loss of their cell-membrane permeability and the release of self-antigens and alarmins such as high-mobility group box 1 (Jaillon et al. 2009; Poon et al. 2010).

PTX3 in inflammation and leucocyte recruitment

PTX3 behaves as an acute phase response protein since its blood levels, which are low under normal conditions (about 25 ng/ml in the mouse, <2 ng/ml in man), increase rapidly (peak at 6–8 h) and dramatically (200–800 ng/ml in human and mouse) during endotoxic shock, sepsis and other inflammatory and infectious conditions, correlating with the severity of the disease (Mantovani et al. 2008). PTX3 transgenic mice show increased resistance to LPS toxicity and to cecal ligation and puncture (Dias et al. 2001). In a model of kainate-induced seizures, PTX3-deficient mice have more widespread and severe IL-1-induced neuronal damage. In this model, PTX3 confers resistance to neurodegeneration, possibly by binding to dying neurons and rescuing them from otherwise irreversible damage (Ravizza et al. 2001). In a model of acute myocardial infarction caused by coronary artery ligation and reperfusion, *ptx3*^{-/-} mice display increased myocardial damage associated with a greater no-reflow area, an increased neutrophil infiltration, a decreased number of capillaries, and an increased number of apoptotic cardiomyocytes, suggesting that PTX3 plays a non-redundant regulatory cardioprotective role in acute myocardial infarction in mice (Salio et al. 2008). Whether PTX3 plays a role in the progression of post-infarction left ventricular dysfunction and failure remains to be assessed. Similarly, in an atherosclerosis model, Norata et al. (2009) have recently found that PTX3 has atheroprotective effects

in mice. Ptx3 expression increases in the vascular wall of apolipoprotein E (APO-E)-knockout mice from 3 to 18 months of age. Aortic lesions are significantly increased in double-knockout mice lacking both *ptx3* and APO-E and in mice heterozygous for *ptx3* compared with APO-E-deficient mice. In addition, mice lacking *ptx3* show a more pronounced inflammatory profile in the vascular wall and increased macrophage accumulation within the plaque (Norata et al. 2009). Together, the above observations suggest a cardiovascular protective function of PTX3 through the modulation of the immunoinflammatory balance in the cardiovascular system. In contrast to the protective role in cardiac ischaemia-reperfusion injury, PTX3 has divergent effects in the cascade of events leading to tissue inflammation and injury following intestinal ischaemia-reperfusion injury. Indeed, in this model, the absence of *ptx3* is accompanied by greatly diminished tissue inflammation and decreased lethality after reperfusion (Souza et al. 2009). Thus, PTX3 has different roles in mediating reperfusion injury in the heart (protective, localized response) versus the intestine (deleterious, systemic response).

Recently, our group has shown that PTX3 selectively binds P-selectin via its N-linked glycosidic moiety (Table 1). Using three *in vivo* models of P-selectin-dependent inflammation (pleurisy, acid-induced acute respiratory distress syndrome and intravital microscopy analysis of mesenteric inflammation), we have demonstrated that exogenously administered PTX3 and endogenous PTX3 released from haematopoietic cells act as a negative feedback loop, preventing excessive P-selectin-dependent recruitment of neutrophils. PTX3 released from activated leucocytes function locally to dampen neutrophil recruitment and regulate inflammation (Deban et al. 2010). This PTX3 activity is reminiscent of the recently characterized glycosylation-dependent regulatory effect of antibodies on inflammation (Kaneko et al. 2006). Therefore, PTX3, which is an essential component of humoral innate immunity, and immunoglobulins, the humoral arm of adaptive immunity, share functional outputs, including complement activation, opsonization and the glycosylation-dependent regulation of inflammation.

PTX3 as a marker in human pathology

CRP has been extensively used clinically for over 75 years as a nonspecific systemic marker of infection, inflammation and tissue damage (Verma et al. 2005). Plasma levels of CRP are independently associated with risk of coronary heart disease, with an open question as to whether CRP is causally associated with this pathology or merely a marker of underlying atherosclerosis. Two recent genome-wide

association studies have reported data that argue against a causal association of CRP with coronary heart disease. Indeed, CRP gene polymorphisms associated with a marked increase in CRP levels are not in themselves associated with an increased risk of ischaemic vascular disease (Elliott et al. 2009; Zacho et al. 2008).

The structural and functional similarity of PTX3 to the classic diagnostic pentraxin CRP has given rise to efforts aimed at assessing the usefulness of PTX3 as a marker in diverse human pathological conditions. The hypothesis driving such work is that PTX3, unlike CRP, might represent a rapid marker for the primary local activation of innate immunity and inflammation.

Increased levels of PTX3 have been observed in diverse infectious disorders including sepsis and septic shock, meningococcal disease, tuberculosis and dengue infection (Azzurri et al. 2005; Mairuhu et al. 2005; Mauri et al. 2010; Sprong et al. 2009). In all these conditions, PTX3 levels are correlated with disease severity and have a prognostic value. Furthermore, evidence has linked PTX3 levels to ischaemic heart disorders. PTX3 levels increase rapidly in acute myocardial infarction (AMI), reaching a peak at around 7 h after the onset of symptoms (Peri et al. 2000). In a series of 748 patients with ST-elevation AMI, PTX3, measured together with established markers including CRP, has emerged as the only independent predictor of mortality (Latini et al. 2004). Patients with arterial inflammation, who are eligible for coronary intervention, exhibit high concentrations of plasma PTX3; in particular, patients with unstable angina pectoris have PTX3 levels three times higher than the normal range (Inoue et al. 2007). Thus, PTX3 is a new candidate for being a prognostic marker in ischaemic heart disorders including AMI (Brugger-Andersen et al. 2009; Jenny et al. 2009; Latini et al. 2004; Matsui et al. 2010). Likewise, PTX3 has been suggested as a useful inflammatory biomarker for predicting cognitive impairment in elderly hypertensive patients (Yano et al. 2010). Conversely, the assessment of PTX3 as a marker of metabolic syndrome characterized by increased cardiovascular risk has produced controversial data and further studies with larger number of patients are required before definitive conclusions can be made (Miyaki et al. 2010; Ogawa et al. 2010; Zanetti et al. 2009). Increased levels of PTX3 have been observed in a restricted set of autoimmune disorders (e.g. in blood in small vessel vasculitis, in synovial fluid in rheumatoid arthritis), but not in others (e.g. systemic lupus erythematosus; Fazzini et al. 2001; Luchetti et al. 2000). In small vessel vasculitis, PTX3 levels are correlated with the clinical activity of the disease and represent a candidate marker for monitoring the disease (Fazzini et al. 2001). The presence of anti-PTX3 autoantibodies has been determined in patients with systemic lupus erythematosus. Correlation of

anti-PTX3 antibody titre and disease activity remains to be confirmed (Augusto et al. 2009). Recent results show that pregnancy, a condition associated with the relevant involvement of inflammatory molecules at the implantation site, is associated with a slight increase in maternal circulating PTX3 levels compared with the non-pregnant condition. Higher maternal PTX3 levels have been observed in pregnancies complicated by preeclampsia, which represents the clinical manifestation of an endothelial dysfunction as part of an excessive maternal inflammatory response to pregnancy (Cetin et al. 2006; Rovere-Querini et al. 2006). PTX3 plasma and vaginal levels are increased during pregnancy complicated by spontaneous preterm delivery and, in particular, in the cases of placenta vasculopathy (Assi et al. 2007).

The general characteristic emerging from studies of PTX3 blood levels in human pathology is the rapidity of its increase compared with that of CRP, consistent with its original identification as an immediate early gene, together with a lack of correlation between levels of CRP and PTX3. The data collected so far in cases of diverse pathology indicate a correlation between PTX3 plasma levels and severity of disease, suggesting a possible role of PTX3 as a marker of pathology. Whether the impressive correlation with outcome and severity actually reflects a role in the pathogenesis of damage remains to be elucidated.

Concluding remarks

Pentraxins are components of the complex and complementary network of cellular and humoral pattern recognition receptors involved in the recognition and response to microbial elements and damaged tissues (Fig. 1). Whereas the regulation of expression of short pentraxins has diverged from mouse to man, the gene targeting of the prototypic, evolutionarily conserved, long pentraxin PTX3 has unequivocally defined the role of this molecule in innate immunity and inflammation. Recent progress has further defined the structure, regulation, microbial recognition and *in vivo* functions of PTX3. As a component of the humoral arm of innate immunity, PTX3 plays a role similar to that played by antibodies as the humoral arm of the adaptive immune system, including complement activation, opsonization and glycosylation-dependent regulation of inflammation. The available evidence suggests that PTX3 not only acts as a PRM, but can also function as a tuner of inflammatory reactions and may contribute to the discrimination between self, infectious non-self and modified-self. In addition, PTX3 may represent a useful marker of infectious and inflammatory (particularly cardiovascular) pathology.

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