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Pentraxins in Innate Immunity: From C-Reactive Protein to the Long Pentraxin PTX3

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Abstract Pentraxins are a family of multimeric pattern-recognition proteins highly conserved in evolution. Based on the primary structure of the subunit, the pentraxins are divided into two groups: short pentraxins and long pentraxins. C-reactive protein and serum amyloid P-component are classic short pentraxins produced in the liver, whereas the prototype of the long pentraxin family is PTX3. Innate immunity cells and vascular cells produce PTX3 in response to proinflammatory signals and Toll-like receptor engagement. PTX3 interacts with several ligands, including growth factors, extracellular matrix components, and selected pathogens, playing a role in complement activation, facilitating pathogen recognition, and acting as a predecessor of antibodies. In addition, PTX3 is essential in female fertility acting on the assembly of the cumulus oophorus extracellular matrix. Thus, PTX3 is a multifunctional soluble pattern recognition receptor acting as a nonredundant component of the humoral arm of innate immunity and involved in tuning inflammation, in matrix deposition and female fertility. Evidence suggests that PTX3 is a useful new serological marker, rapidly reflecting tissue inflammation and damage under diverse clinical conditions.

Keywords Pentraxin · innate immunity · inflammation · fertility · extracellular matrix · cardiovascular disease

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Introduction

Similarly to adaptive immunity, components of humoral immunity include members of the complement cascade and soluble pattern recognition receptors (PRR), such as collectins [surfactant protein–A, (SP–A), and SP–D], ficolins, and pentraxins [1–4]. Fluid phase PRR are therefore a heterogeneous group of molecular families, which represent functional ancestors of antibodies. They play a key role as effectors and modulators of innate resistance in animals and man. There is evidence that this heterogeneous set of soluble PRR interacts with cellular innate immunity.

Pentraxins are prototypic components of the humoral arm of innate immunity. Indeed, C-reactive protein was the first PRR to be identified [4]. Moreover, they have represented invaluable diagnostic tools. In this paper, we will review the properties of pentraxins focusing in particular on discovery, function, and possible clinical relevance of the long pentraxin PTX3.

The Pentraxin Superfamily

Pentraxins are a superfamily of proteins, phylogenetically conserved from arachnids to mammals and characterized by the presence in their carboxy-terminal of a 200 amino acid pentraxin domain [4–8]. The term pentraxin was first assigned to C-reactive protein (CRP) for its ultrastructural appearance of five subunits. Based on the primary structure of the subunits, the pentraxins are divided in short and long pentraxins. Pentraxins recognize a wide range of exogenous pathogenic substances and altered self molecules and, in species-specific manner, behave as acute phase proteins.

CRP and serum amyloid P component (SAP) are the prototype of the short pentraxin family: they are mainly

produced in the liver in response to inflammatory signals, most prominently IL-6, and are acute phase proteins in man and mouse, respectively. PTX3 is the prototype of the long pentraxin family, whose members were identified in the 1990s as cytokine-inducible genes or molecules expressed in specific tissues: guinea pig apexin in spermatozoa [9, 10], neuronal pentraxin (NP) 1 or NPTX1 [11, 12], NP2, also called Narp or NPTX2 [13, 14], and neuronal pentraxin receptor (NPR), a transmembrane molecule in neurons [15, 16]. PTX3 is a 45 kDa protein that assembles to form high molecular weight multimers linked by interchain disulfide bounds [17]. The C-terminal domain (203 amino acids) of PTX3 shares homology with the classic short pentraxins, whereas the N-terminal domain (178 amino acids) does not show any significant homology with other known proteins. PTX3 differs from CRP and SAP also for gene organization, cellular source and ligand-binding properties [4]. Unlike the classic short pentraxins CRP and SAP, whose sequence and regulation have diverged from mouse to man, PTX3 is highly conserved in evolution. Thus, results obtained using genetic approaches in the mouse are likely to be informative for the function of PTX3 in man. Actually, structural analysis and gene-modified mice have provided a new level of understanding of the role of pentraxins in immunity and homeostasis. In particular, PTX3 plays a complex, non-redundant role in vivo, recognizing a diverse range of pathogens, modulating complement activity by binding C1q and facilitating pathogen recognition by macrophages and dendritic cells (DC). Moreover, PTX3 is a multifunctional protein at the crossroads between immunity and inflammation, extracellular matrix construction and female fertility [3, 4].

Ligand Specificity

The multifunctional properties exerted by PTX3 can be at least in part explained by its capacity to interact with a number of different ligands, a characteristic shared with the classical short pentraxins CRP and SAP. A list of selected ligands recognized by PTX3, CRP, and SAP is reported in Table I.

The complement component C1q is the first and best characterized ligand described for PTX3 [17, 18]. PTX3 binds to plastic immobilized C1q, interacting with C1q globular head (gC1q), in particular with charged residues localized on the apex of the molecule and involving all the three C1q chains (gC1qA, gC1qB, and gC1qC) [18, 19]. In the same experimental conditions, PTX3 fails to interact with other components of the complement system, such as C3, C4, and C4bP (B. Bottazzi and L. Deban, unpublished observations). While CRP and SAP show optimal interaction with C1q only after chemical cross-linking [20], PTX3

Table I Ligand Specificity of CRP, SAP and PTX3

Ligand	CRP	SAP	PTX3	Calcium requirement
Complement components				
C1q	+	+	+	−
Factor H	+	NT	+	+
C4b-binding protein	NT	+	−	
Extracellular matrix proteins				
TSG-6	NT	NT	+	+
Inter-α-trypsin inhibitor	−	NT	+	+
Hyaluronan	NT	NT	−	
Laminin	+	+	−	
Collagen IV	NT	+	−	
Fibronectin	+	+	−	
Growth factors				
FGF2	+/−	NT	+	+
FGF1	NT	NT	−	
FGF4	NT	NT	−	
Membrane moieties				
PC	+	−	−	
PE	−	+	−	
LPS	−	+	−	
KpOmpA	NT	NT	+	+
Pathogens				
Bacteria	+	+	+	NT
Fungi (*A. fumigatus*)	+	NT	+	−
Yeast	+	+	+	NT
Viruses	−	+	+	−

Calcium requirement is referred to PTX3 only
NT, not tested

does not require a previous aggregation, probably as a consequence of its stable multimeric structure.

Interaction of PTX3 with surface immobilized C1q results in the activation of the classical complement cascade, measured as C3 and C4 deposition. On the other hand, fluid-phase binding of PTX3 to C1q inhibits complement activation by blocking relevant interaction sites [18]. These data indicate that PTX3 may exert a dual role in complement activation, depending on the way C1q is presented.

The extent of PTX3 glycosylation affects PTX3 interaction with C1q and subsequent complement activation, as demonstrated by the observation that removal of sialic acid or complete deglycosylation of the protein significantly increases its binding to C1q [21]. In accordance, PTX3 desialylation increases complement activation, as assessed by C3 and C4 deposition.

In 1999, H. Jarva showed that CRP can modulate the alternative pathway of complement activation through interaction with Factor H, the main soluble regulator of the alternative pathway [22]. In accordance, preliminary data show that PTX3 can also interact with Factor H (L. Deban and S. Meri, unpublished observation), suggesting a more general and complex role of PTX3 in the control of complement functions.

Similarly to CRP and SAP, PTX3 binds to apoptotic cells during late phases of apoptosis inhibiting their removal by DC [23]. Confocal analysis shows that PTX3 binds to discrete membrane domains of late apoptotic cells, but the structures recognized have not been identified so far, even if competition experiments with both CRP and SAP suggest that all the three pentraxins may to some extent interact with a common site [23]. Small nuclear ribonucleoproteins and chromatin/nucleolar components recognized, respectively, by CRP and SAP [24], redistribute to the plasma membrane during late apoptosis. PTX3 can bind histones raising the possibility that interaction with nuclear components could actually occur.

Other ligands were described for PTX3, including growth factors, components of the extracellular matrix, and microbial moieties as well as selected pathogens, whereas no binding was observed to classical ligands of the short pentraxins CRP and SAP, such as phosphocoline, phosphoethanolamine, and high pyruvate agarose [17] as well as to different cytokines and chemokines [25]. PTX3 binds Fibroblast growth factor 2 (FGF2), but not other members of the FGF family, such as FGF1 and FGF4 [25]. FGF2 binding site has been mapped on the N-terminal domain of PTX3, as demonstrated by means of recombinant N-terminal and C-terminal PTX3 domains expressed and purified from CHO cells [26]. The two synthetic peptides PTX3 (82–110) and PTX3 (97–110), spanning in the N-terminal portion of PTX3, were able to prevent PTX3 binding to immobilized FGF2, confirming that the FGF2-binding site is located within the N-terminal domain between amino acids 97 to 110 [26].

The subfertility observed in *ptx3*-deficient mice (see below) was associated with severe abnormalities of cumulus oophorus matrix, suggesting that PTX3 may participate in the organization of this structure. Immunofluorescence studies revealed that PTX3 is localized in the cumulus matrix [27]. The major integral component of cumulus matrix is hyaluronan, a large glycosaminoglycan responsible for the viscoelastic properties of this matrix. Other proteins interact with hyaluronan and participate in the organization of cumulus matrix, such as TNF-stimulated-gene-6 (TSG-6), a multifunctional protein associated with inflammation [28, 29]. In vitro experiments demonstrate that PTX3 binds TSG-6 [27], interacting with the Link module of TSG-6. Addition of PTX3 to *ptx3*-deficient cumuli restores normal cumulus morphology, demonstrating the crucial and nonredundant role exerted by PTX3 in the organization and stabilization of the hyaluronan-rich cumulus matrix. In addition, preliminary observations indicate that PTX3 can interact with a second component of cumulus matrix, Inter-α-trypsin Inhibitor (A. Salustri, unpublished observation). Other proteins participating in the organization of extracellular matrices were investigated; however, no PTX3 binding has been observed to collagen IV, fibronectin, laminin, and hyaluronic acid (HA) [17, 27].

As CRP and SAP, PTX3 can interact with a number of different pathogens: bacteria as well as fungi and virus. A specific binding has been observed for some selected gram positive and gram negative bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Neisseria meningitides* [30, 31]. PTX3 binds zymosan, *Paracoccidioides brasiliensis* and conidia from *Aspergillus fumigatus* [31, 32] and recognizes both human and murine cytomegalovirus as well as H3N2 influenza virus but not H1N1 [33]. Binding to conidia is competed by galactomannan but a direct interaction of PTX3 with this glucan has not been demonstrated.

Pentraxins are lectin-like molecules and interaction of CRP and SAP with sugars has been well characterized. The similarities between PTX3 and classical pentraxins prompted us to investigate whether PTX3 can interact with sugar moieties. A preliminary glycoarray screening has been performed by Core H of the Consortium for Functional Glycomics and results indicate a modest interaction with LacNAc gangliosides, but further experiments are necessary to better characterize PTX3 interactions with glucans.

In an attempt to identify the molecular structures recognized by PTX3 on the surface of pathogens, the direct interaction of PTX3 with different bacterial moieties localized on the microbial cell wall was analyzed [30]. PTX3 does not bind lipopolysaccharide (LPS), lipotheicoic

acid (LTA), enterotoxin A and B, exotoxin A and *N*-acethylmuramyl-L-alanyl-D-isoglutamine (MDP), whereas it binds outer membrane protein A from *Klebsiella pneumoniae* (KpOmpA), a major component of the outer membrane of gram negative bacteria highly conserved among the *enterobacteriaceae* family [30].

The interaction of short pentraxins CRP and SAP with their ligands is generally dependent on the presence of calcium [34]. On the contrary, inductive coupled plasma/atomic emission spectroscopy shows that PTX3 does not have a specific coordination site for calcium ions [17]. Accordingly, calcium is not required for PTX3 interaction with C1q, the first PTX3 ligand identified [17]. However, with the discovery of other PTX3 ligands, it appears that this is not a general rule. As summarized in Table I, the presence of calcium is required for PTX3 interaction with some of its ligands including FGF2 and KpOmpA [30] while no calcium is necessary for interaction with apoptotic cells [23], *Aspergillus* conidia and influenza virus (P. Reading and B. Bottazzi, unpublished observations).

Cellular Sources of Pentraxins

The classic short pentraxins CRP and SAP are mainly produced in the liver in response to IL-6. Here, we will focus on "peripheral" production of PTX3.

Gene Expression Dependent Regulation

Mononuclear phagocytes and myeloid-derived DC are a major source of the long pentraxin PTX3. In addition, a variety of cell types produce PTX3 in vitro upon exposure to primary inflammatory signals, such as IL-1β, TNFα, microbial moieties such as LPS, lipoarabinomannan, outer membrane protein A (OmpA) and other agonists for different members of the TLR family [30, 35]. These cells include myeloid DCs, which are major producers of PTX3, endothelial cells, adipocytes, fibroblasts, smooth muscle cells, synovial cells and chondrocytes [35–42]. Recently, cells of epithelial origin, for instance renal and alveolar epithelial cells, have also been found to produce low amounts of PTX3 under stimulation [43, 44]. IL-6, a poor inducer of PTX3 in vitro, was found to be involved in PTX3 expression in Castelman's disease [45] and in Kaposi sarcoma [46].

IFNγ and IL-10 have different effects on PTX3 production. IFNγ, which generally has a synergistic effect with LPS [47], inhibits LPS-induced PTX3 expression and production in different cellular contexts [40, 48, 49], whereas IL-10 weakly induces PTX3 expression in DCs and monocytes and significantly synergizes with LPS, other TLR agonists and IL-1β [50]. IL-10 induces a set of genes (e.g., type I collagen, fibronectin, versican, α1-antitrypsin) related to tissue remodeling [50, 51] and is involved in the chronic and resolution phase of inflammation [52]. Given its role in matrix organization [27], PTX3 expression in M2 mononuclear phagocytes and IL-10-treated DCs and fibroblasts is likely to be related to the orchestration of matrix deposition, tissue repair and remodeling [53]. Moreover, it is interesting that, in addition to the stimulation of B cell differentiation and antibody production [52] (the humoral arm of adaptive antibody mediated immunity), IL-10 also stimulates the humoral arm of innate immunity (PTX3).

Vascular endothelial and smooth muscle cells produce PTX3 in response to inflammatory signals including oxidized low density lipoproteins (ox-LDLs) [41] and accordingly, PTX3 was observed in human atherosclerotic lesions [54]. In addition, it was recently described a significant correlation between LDL plasma levels and PTX3 mRNA expression in subcutaneous adipose specimens and in white blood cells [55].

A peculiar tissue is the cumulus oophorus, in which PTX3 mRNA expression is orchestrated by hormonal ovulatory stimuli (FSH or hCG), by oocyte-derived soluble factors and in particular by a member of the TGFβ family, growth differentiation factor-9 (GDF-9) [27, 56]. In this tissue, PTX3 expression is restricted to the preovulatory period, showing close temporal correlation to matrix deposition by cumulus cells. Western blot and immunofluorescence analysis indicate that PTX3 is associated with the extracellular matrix of the cumulus oophorus.

Recent analysis of PTX3 gene regulation has yielded unexpected results. First, two independent studies have shown that PTX3 is a major responsive gene downstream of the FUSS-CHOP translocation involved in the pathogenesis of a subset of soft tissue sarcomas [57]. The pathophysiological significance of this finding and its value for monitoring of disease remains to be elucidated. In addition, recent results have shown that glucocorticoid hormones (GC) have divergent effects on PTX3 expression and production in mononuclear phagocytes and in nonhaematopoietic cells (A. Doni et al. unpublished observations). In myeloid DC, GC inhibited the PTX3 production. In contrast, in fibroblasts and EC, GC alone induced and, under inflammatory conditions, enhanced and extended PTX3 production. In vivo administration of GC augmented the blood levels of PTX3 in mice and humans. Moreover, patients with Cushing's syndrome had increased levels of circulating PTX3 whereas PTX3 levels were decreased in subjects affected by iatrogenic hypocortisolism. In nonhematopoietic cells, GC receptor functioned as a ligand-dependent transcription factor (dimerization-dependent) to induce PTX3 gene expression. In contrast, in hematopoietic cells, GC receptor repressed PTX3 gene transcription by interfering (dimerization-independent) with the action of other signaling pathways, likely NFkB and AP-1. Thus, divergent effects of GC were found to be due to different GC receptor mechanisms.

The divergent effects of GC on PTX3 production are likely to reflect the different functions of this multifunctional molecule in innate immunity and in the construction of the extracellular matrix.

Neutrophils as a Source of Preformed PTX3

In an unexpected twist, we recently found that PTX3 is stored in specific granules and undergoes release in response to microbial recognition and inflammatory signals [58]. Released PTX3 can partially localize in neutrophil extracellular traps (NETs) formed by extruded DNA. Eosinophils and basophils do not contain preformed PTX3. *ptx*3-deficient neutrophils have defective microbial recognition and phagocytosis, and PTX3 is nonredundant for neutrophil-mediated resistance against *Aspergillus fumigatus*. Thus, neutrophils serve as a reservoir, ready for rapid release, of the long pentraxin PTX3, a key component of humoral innate immunity with opsonic activity. Myeloid, but not plasmacytoid, DC and macrophages are major producers of PTX3 [36]. Over a period of 24 h, DC release approximately 50 ng of PTX3 per 10^6 cells [36]. Neutrophils contain 24.9±3.8 ng of this PRR per 10^6 cells (n=5). Upon stimulation, they release approximately 25% of stored PTX3, a part of it remaining cell-associated, presumably with NETs. Given the abundance of neutrophils in the circulation and in the early phases of inflammatory reactions in tissues, these cells represent a major source of PTX3 covering a temporal window preceding gene expression-dependent production. Under conditions of tissue damage (e.g., myocardial infarction) or infection (e.g., sepsis), PTX3 levels increase rapidly. For instance, in acute myocardial infarction with ST elevation, PTX3 reaches a peak in 6–8 h, compared to 36–48 h for CRP [59]. Under these conditions, high PTX3 is an independent marker associated with death [60].

The results reported here shed new light on PTX3 elevations in pathological conditions and on their pathophysiological implications. It is likely that rapid release of stored PTX3 by activated neutrophils plays a role in the early phases of its elevation in pathology, preceding gene expression-dependent production. PTX3 expressed by neutrophils is essential to control fungal growth in vitro and in vivo. Innate and adaptive immunity are both essential for the development of a protective antifungal immune response. Generation of a Th1-oriented *A. fumigatus*-specific immune response is associated to protection [61, 62]. Injection of PTX3 in *ptx3*-deficient mice favors the generation of a protective Th1 anti-*Aspergillus* immune response [31]. Neutrophil-derived PTX3, in addition to DC-derived PTX3, may be involved in the orientation of the immune response toward a protective Th1 phenotype. Neutrophils, an innate cell type without professional antigen presenting functions, may participate, via the release of this preformed soluble PRR to the activation and orientation of adaptive immunity.

Functions

While CRP and SAP are produced at a systemic level by hepatocytes, PTX3 is produced by a wide range of different cell types and exerts its functions locally. As outlined above, PTX3 is expressed in response to a variety of inflammatory or infectious stimuli and interacts with different ligands. These observations suggest that, compared to classical short pentraxins CRP and SAP, PTX3 could play different roles. The evolutionary divergence of CRP and SAP has hampered unequivocal agreement of their in vivo function using genetic approaches. Data available so far indicate that PTX3 is a soluble PRR playing crucial nonredundant roles in innate immunity, inflammation, matrix deposition, and female fertility.

Innate Resistance and Inflammation

The hypothesis that PTX3 could play a crucial role in the defense mechanisms is supported by at least two series of observations: (1) innate immune cells such as macrophages and DC produce high levels of PTX3 upon stimulation with proinflammatory signals or TLR engagement [36]; (2) neutrophils represent a reservoir of “ready to use” protein promptly released in response to microbial recognition and inflammatory signals [58]. In addition, macrophages from PTX3-overexpressing mice show an increased phagocytic activity of zymosan and *Paracoccidioides brasiliensis* [32], whereas macrophages and PMN from *ptx*3-deficient mice are characterized by a defective phagocytosis of conidia from *Aspergillus fumigatus* [31, 58], all pathogens recognized in vitro by PTX3. Thus, PTX3 secreted by neutrophils or produced by macrophages and DC can facilitate pathogen recognition and removal, playing important nonredundant functions in the defense against selected pathogens. This data support the idea that cells of the monocytic lineage can express a receptor for PTX3 on their surface. In accordance, a specific, dose-dependent, and saturable binding of PTX3 to murine macrophages, as well as human monocytes and DC, has been observed [31].

Consistent with its binding and opsonic properties, PTX3 is nonredundant in selected fungal and bacterial infections (*A. fumigatus*, *P. brasiliensis*, *P. aeruginosa*, *S. typhymurium*) and irrelevant in others (*L. monocytogenes*, *S. aureus*, polymicrobic intraabdominal sepsis) [31, 32]. The results obtained in these studies suggest that PTX3 deficiency does not cause a generalized impairment of host resistance to microbial pathogens, and that PTX3 is involved in recognition and resistance against specific

micro-organisms. In particular, *ptx*3-deficient mice were extremely susceptible to invasive pulmonary aspergillosis, and the specificity of the defect and the therapeutic potential of PTX3 could be demonstrated by the complete protective effect of treatment with recombinant PTX3 [31, 63]. Moreover, in this model, the defective recognition of *A. fumigatus* conidia by *ptx*3-deficient mice was associated to the lack of development of appropriate and protective Th1 antifungal responses and to an unbalanced cytokine profile skewed toward a Th2 response [31].

Recently, Bozza et al. studied the role of PTX3 in viral infections and found that PTX3 binds both human and murine cytomegalovirus, reducing viral entry and infectivity in DC in vitro [33]. Consistently, *ptx*3-deficient mice were more susceptible to murine cytomegalovirus (MCMV) infection than PTX3 wild-type mice, and PTX3 administration protected susceptible BALB/c mice from MCMV primary infection and reactivation in vivo, as well as *Aspergillus* superinfection. This occured through the activation of Interferon regulatory factor 3 (IRF3) in DC via the TLR9/MyD88-independent viral recognition sensing and the promotion of the IL-12/IFN-γ-dependent effector pathway [33].

As PTX3 binds to C1q and modulates the activation of the classical pathway of complement cascade, besides a direct opsonic effect of PTX3, an indirect complement-mediated immune response could be activated by PTX3. The in vivo relevance of complement activation by PTX3 in aspergillosis has been studied by evaluating the therapeutic potential of PTX3 in C1q-deficient mice. Results suggest that PTX3 can mediate resistance, at least against *A. fumigatus*, independently of C1q [31].

The role played by PTX3 in innate resistance to pathogens could also be exerted in an opsonizing-independent manner: in the case of *K. pneumoniae* infection, A. C. Soares et al. could not demonstrate binding of PTX3 to *K. pneumoniae*; however, overexpression of PTX3 by transgenic mice during infection was associated with an enhanced ability to produce proinflammatory mediators, including NO and TNFα, and, as a consequence, with protection or faster lethality, depending on the dimension of inocula [64]. Thus, according to studies with transgenic mice, PTX3 overexpression under the control of its own promoter is associated to enhanced inflammatory responses, which, depending on the model studied, can be beneficial or detrimental for the host (see below).

In conclusion, PTX3, released by PMN and produced by DC, neighboring macrophages, and other cell types upon TLRs engagement or pathogen recognition, recognizes microbial moieties, opsonizes fungi, selected Gram positive and Gram negative bacteria and viruses and activates complement. Opsonization results in facilitated pathogen recognition (increased phagocytosis and killing), and in innate immune cell activation (increased cytokine and nitric oxide production); moreover, opsonization by PTX3 is likely involved in the activation of an appropriate adaptive immune response (DC maturation and polarization). All these properties suggest that this long pentraxin behaves as a bona fide ante-antibody.

PTX3 behaves as an acute phase response protein since its blood levels, low in 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) during endotoxic shock, sepsis and other inflammatory and infectious conditions, correlating with the severity of the disease. The in vivo role of PTX3 in inflammatory conditions has been investigated using PTX3 overexpressing and deficient mice. In a model of LPS toxicity and in cecal ligation and puncture, PTX3 overexpression resulted in increased resistance [65], whereas its deficiency was irrelevant [31]. After intestinal ischemia reperfusion injury, PTX3 overexpressing mice showed exacerbated inflammatory response and reduced survival rate, due to enhanced production of proinflammatory mediators (TNFα in particular) [66].

Limbic seizures enhance the production of proinflammatory cytokines as well as PTX3 [67]; in a model of kainate-induced seizures, *ptx3*-deficient mice had 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 [67].

These results obtained with PTX3 overexpressing animals as well as with *ptx3*-deficient mice outline the delicate role exerted by PTX3 in the inflammatory context.

Self/Non-Self Discrimination

While PTX3 promotes removal of selected pathogens by professional phagocytes, it inhibits removal of apoptotic cells. In fact, in the presence of PTX3, both immature DC and macrophages failed to internalize dying cells, thus preventing inflammatory uptake of late apoptotic cells and antigen presentation by antigen-presenting cells [23, 68]. PTX3 influences the maturation program of DC induced by LPS, inhibiting TNFα and IL-10 secretion, as well as the upregulation of membrane molecules, such as CD86, HLA-DR, and HLA-ABC [69]. On the other side, in the presence of dying cells, PTX3 enhances cytokine production but inhibits the cross-presentation of apoptotic cell-derived epitopes of self, viral or tumoral origin to autoreactive CD8+ T cells. Among the members of the pentraxin family, these effects are specific for PTX3 since CRP does not affect the expression of membrane molecules or the secretion of cytokines by DC as well as the cross-presentation of apoptotic cell-associated antigens [69]. Thus, PTX3 behaves as a flexible regulator of the function of DC, modulating the maturation program and the

secretion of soluble factors. These results have led to the speculation that PTX3 has a dual role in the protection against pathogens and in the control of autoimmunity.

Interaction with Complement

The cross-talk between PTX3 and C1q modulates complement functions at different levels. As mentioned above, activation of the classical complement cascade is observed in vitro when PTX3 interacts with surface-immobilized C1q. On the contrary, inhibition is observed when interaction occurs in the fluid-phase. Interaction of PTX3 with C1q may also play a crucial role in the clearance of apoptotic cells. Both C1q and PTX3 are produced by immature DC in response to TLR engagement [70, 71]; moreover, both the proteins bind apoptotic cells with a similar kinetic, interacting with different binding sites and remaining stably associated to the apoptotic cell membrane [71]. In addition, when PTX3 is incubated with apoptotic cells, it enhances the deposition of both C1q and C3 on the cell surface [18], suggesting a role for PTX3 in the complement-mediated removal of dying cells. On the contrary, in the fluid phase, PTX3 reduces C1q and C3 deposition on apoptotic cells as well as the C1q-mediated phagocytosis of apoptotic cells by DC [71]. These data suggest that PTX3 may play a dual role in the regulation of complement-mediated immune responses and further support accumulating evidence suggesting that complement components and pentraxins may participate in the handling of apoptotic cells [72].

Among the microbial moieties recognized by PTX3 is KpOmpA [30]. KpOmpA binds to and is internalized by DC and macrophages [73], activating both these cellular types in a TLR2-dependent way. The innate immune response to KpOmpA involves recognition by the scavenger receptors LOX-1 and SREC-I [30]. The activation program set in motion by KpOmpA involves production of PTX3, which, in turn, binds KpOmpA, amplifying the inflammatory response in vivo. The response to KpOmpA in vivo is significantly reduced in *ptx3*-deficient mice and is restored by exogenous administration of purified PTX3, indicating that PTX3 is part of a nonredundant amplification loop induced by KpOmpA. The mechanisms involved in the amplification of the response to KpOmpA have not been completely elucidated yet. However, preliminary observations suggest that complement activation by PTX3 may play a crucial role in the amplification of the innate immune response to microbial ligands (A. Cotena, personal communication).

Recently, Baruah and coworkers reported an increased presence of both C1q and PTX3 in nasal polyps compared to nasal mucosa. This observation indicates the presence of inflammation within polyp tissue and suggests that the innate factors PTX3 and C1q can be involved in the pathogenesis of nasal polyposis [74].

Role in Angiogenesis

Interaction between PTX3 and FGF2 prevents FGF2 binding to endothelial cells, leading to inhibition of FGF-dependent cell proliferation in vitro. Moreover, PTX3 overexpression in FGF2-transformed endothelial cells inhibits both their proliferation in vitro and their capacity to generate vascular lesions in vivo. FGF2 plays a key role in the induction of proliferation, migration and survival of vascular smooth muscle cells (SMC) and excessive growth of SMC is an important component in atherosclerosis and restenosis. Interaction between PTX3 and FGF2 inhibits SMC proliferation in vitro; in addition, PTX3 overexpression in transduced SMC reduces intimal hyperplasia after arterial injury as a result of direct binding to FGF2 [75]. Thus, PTX3 could act as a "FGF2 decoy" able to sequester the growth factor in an inactive form.

FGF2 plays important roles in vivo by promoting angiogenesis and neovascularization during wound healing, inflammation, atherosclerosis, and tumor growth. All these pathological conditions are also characterized by accumulation of macrophages that together with endothelial cells, may represent a major local source of both FGF2 and PTX3. The interaction between PTX3 and FGF2 may modulate angiogenesis in various physiopathological conditions, affecting the cross-talk between inflammatory cells and endothelium. In addition, the potent inhibitory effect on FGF2-mediated activation of SMC suggests that PTX3 may modulate SMC activation after arterial injury.

Role in Female Fertility

PTX3 deficiency is associated with a severe defect in female fertility [27, 31, 56]. Infertility of *ptx3*-deficient mice is associated to an abnormal cumulus oophorus characterized by an unstable extracellular matrix in which cumulus cells are uniformly dispersed instead of radiating out from a central oocyte [27]. The oocyte develops normally in the absence of PTX3, and can be fertilized in vitro, whereas the fertilization failure observed in vivo is due to the defective cumulus expansion [27]. Cumulus cells express *ptx3* mRNA under ovulatory stimuli [27, 76]. PTX3 produced by cumulus cells localizes in the extracellular matrix and plays a crucial role in the assembly of the HA-rich matrix of the cumulus oophorus, by interacting with TSG-6, which is an HA-binding protein [27]. Thereby, PTX3 may form multimolecular complexes that can cross-link HA chains, playing a role as structural constituents of the cumulus oophorus extracellular matrix essential for female fertility.

In addition, it was recently shown that PTX3 is transiently expressed in the mouse uterus during the periimplantation period [77], and implantation and decidualization

are compromised in *ptx3*-deficient mice. These results suggest that PTX3, besides being a structural component of cumulus matrix, may affect female fertility promoting implantation and decidualization.

In the context of female fertility, it is interesting that PTX3 was one of the genes related to inflammation and angiogenesis induced in decidual stromal cells by the trophoblast, in an in vitro system which mimics the alteration of the local immune environment induced by the trophoblast in process of embryo implantation [78].

PTX3 in Human Pathology

The structural and functional similarity to the classic diagnostic CRP have given impetus to efforts aimed at assessing the usefulness of PTX3 as marker in diverse human pathological conditions. The hypothesis driving this effort is that PTX3, unlike CRP (made in the liver and induced primarily by IL-6), may represent a rapid marker for primary local activation of innate immunity and inflammation (Fig. 1). Actually, PTX3 behaves as an acute phase response protein since its blood levels, low in 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) during endotoxic shock, sepsis and other inflammatory and infectious conditions. The general characteristic emerging from studies on PTX3 blood levels in human pathology is the rapidity of its increase compared to CRP, consistent with its original identification as an immediate early gene [59], together with a lack of correlation between levels of CRP and PTX3 [59, 79].

Data collected so far in different pathologies indicate a correlation between PTX3 plasma levels and severity of disease, suggesting a possible role of PTX3 as marker of pathology. It remains to be elucidated whether the impressive correlation with outcome and severity actually reflects a role in the pathogenesis of damage, for instance by amplifying the complement and coagulation cascades [17, 18, 80, 81].

Increased levels of PTX3 have been observed in diverse infectious disorders including sepsis and septic shock, *A. fumigatus* infection, tuberculosis and dengue [31, 79, 82, 83]. In all these conditions, PTX3 levels correlated with disease severity and had a prognostic value [79].

In a recent study, polymorphisms at the PTX3 locus were associated to *Mycobacterial tuberculosis* infection [84]. The study, performed on 321 patients with pulmonary tubercolosis and 347 healthy controls from Guinea–Bissau, analyzed five single nucleotide polymorphisms and showed a significant protective effect toward tubercolosis associated to a specific haplotype.

Several evidences link PTX3 to ischemic heart disorders. PTX3 is induced in vascular smooth muscle cells by

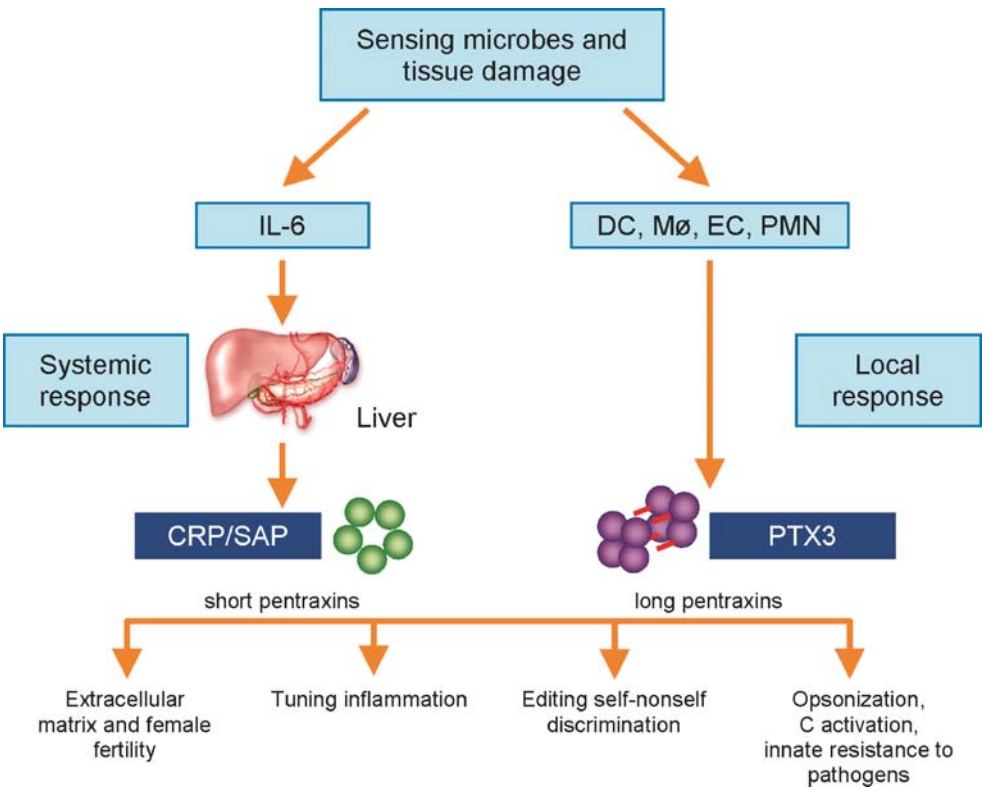

Fig. 1 Pentraxins in innate immunity: a general view. Liver-derived short pentraxins (e.g., CRP and SAP) and tissue-expressed long pentraxins (e.g., PTX3) are produced in response to microbial sensing and inflammatory cytokines and are likely to fulfill complementary functions in innate resistance to pathogens, tuning of inflammation, editing self–non-self discrimination and participating in extracellular matrix architecture and female fertility.

atherogenic modified LDL and is present in human atherosclerotic lesions [41, 54]. PTX3 levels increase rapidly in Acute myocardial infarction (AMI), reaching a peak at around 7 h after the onset of symptoms [59]. In a series of 748 patients with ST elevation AMI, PTX3, measured along with established markers including CRP, emerged as the only independent predictor of mortality [60]. Patients with arterial inflammation, eligible for coronary intervention, exhibited high concentrations of plasma PTX3; in particular, patients with unstable angina pectoris, exhibited PTX3 levels three times higher than the normal range [85]. Thus, PTX3 is a candidate new prognostic marker in ischemic heart disorders including AMI.

A role for PTX3 has been recently proposed in patients with coronary artery disease after percutaneous coronary intervention [86]: in a group of 20 patients undergoing coronary stenting, PTX3 levels increase both in peripheral blood and in the coronary sinus and correlate with Mac-1 expression on neutrophil surface. Furthermore, the relative PTX3 increase observed at 24 h is the most powerful predictor of late lumen loss, suggesting that PTX3 may be a useful marker for the evaluation of an inflammatory response and neointimal thickening after vascular injury [86].

Increased levels of PTX3 have been observed in a restricted set of autoimmune disorders (e.g., in the blood in small vessel vasculitis, in the synovial fluid in rheumatoid arthritis), but not in others (e.g., systemic lupus erythematosus) [87, 88]. In small vessel vasculitis, PTX3 levels correlate with clinical activity of the disease and represent a candidate marker for monitoring the disease [87]. Immunohistochemistry performed on skin sections at sites of vasculitis shows that endothelial cells are responsible for PTX3 production [89]. Moreover, in these patients, PTX3 is abundantly present at sites of leukocytoclastic infiltration; the finding that PTX3, in contrast to the short pentraxin SAP, inhibits the uptake of apoptotic PMNs by macrophages [68], suggests that PTX3 is a key factor in the incomplete clearance of apoptotic and secondary necrotic PMNs observed in small-vessels vasculitis [89].

Patients with chronic kidney disease (CKD) also show increase in PTX3 plasma levels [90], with the highest concentrations observed in the group of patients with a more severe disease. In a parallel study, it has been observed that patients undergoing chronic haemodyalisis have higher plasma PTX3 levels compared to those undergoing peritoneal haemodyalisis [91]; in addition, the presence of peripheral or coronary artery disease results in significantly higher levels of PTX3. Finally, patients with high PTX3 levels had higher all-cause mortality and cardiovascular mortality, suggesting that PTX3 could have a predictive value of mortality in CKD patients [90]. The latter finding is reminiscent of AMI data [60].

Expression of PTX3 mRNA and protein by human cumulus cells [27, 76, 92] suggests that this molecule might have the same role in murine and human female fertility. Studies on PTX3 mRNA in cumulus cells from fertilized oocytes compared with cumulus cells from unfertilized oocytes indicated that PTX3 might be a possible marker for oocyte quality and success in fertilization [76]. PTX3 protein is abundantly present in the follicular fluid, where its concentrations are sixfold higher than in plasma, but we could not find a correlation between its levels in follicular fluid at the time of oocyte retrieval and oocyte quality, possibly because PTX3 shedding from the cumulus matrix to the follicular fluid is not a finely regulated phenomenon [92].

Recent results show that pregnancy itself, a condition associated with relevant involvement of inflammatory molecules at the implantation site [93], is associated with slight increase in maternal circulating PTX3 levels compared to the nonpregnant condition. Higher maternal PTX3 levels were observed in pregnancies complicated by preeclampsia [94, 95], which represents the clinical manifestation of an endothelial dysfunction as part of an excessive maternal inflammatory response to pregnancy [93, 96, 97].

Finally, PTX3 plasma and vaginal levels were increased during pregnancy complicated by spontaneous preterm delivery and in particular in the cases of placenta vasculopathy [98].

Concluding Perspective

CRP was the first innate immune molecule capable of recognizing microbial moieties to be identified [4]. Yet, in spite of its widespread use as a diagnostic tool in the clinic, its in vivo function has not been unequivocally defined. Indeed, the considerable differences in sequence and, most prominently, regulation (CRP is not an acute phase protein in the mouse) have precluded the use of straightforward genetic approaches to explore its in vivo function [99]. By contrast, gene targeting of the prototypic, evolutionary-conserved, long pentraxin PTX3 has unequivocally defined the role of this molecule and, by inference, the role of the whole pentraxin family, at the crossroads of innate immunity, inflammation, matrix deposition, and female fertility [4] (Fig. 1).

Recent progress has further defined the structure, regulation, microbial recognition and in vivo function of PTX3. PTX3 (and presumably other members of the pentraxin superfamily) is a component 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. Moreover, evidence suggests that PTX3 acts as a tuner of inflammatory reactions and possibly as a component of

the decoding system, which in antigen presentation discriminates between infectious non-self and apoptotic self [32, 69]. Translational efforts suggest that PTX3 may be a new marker of innate immunity and inflammation, rapidly reflecting tissue and vascular bed involvement.

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References

1. Wright JR. Immunoregulatory functions of surfactant proteins. Nat Rev Immunol 2005;5:58–68.
2. Endo Y, Matsushita M, Fujita T. Role of ficolin in innate immunity and its molecular basis. Immunobiology 2007;212:371–9.
3. Bottazzi B, Garlanda C, Salvatori G, Jeannin P, Manfredi A, Mantovani A. Pentraxins as a key component of innate immunity. Curr Opin Immunol 2006;18:10–5.
4. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu Rev Immunol 2005;23:337–66.
5. Agrawal A. CRP after 2004. Mol Immunol 2005;42:927–30.
6. Gewurz H, Zhang XH, Lint TF. Structure and function of the pentraxins. Curr Opin Immunol 1995;7:54–64.
7. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189–97.
8. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805–12.
9. Reid MS, Blobel CP. Apexin, an acrosomal pentaxin. J Biol Chem 1994;269:32615–20.
10. Noland TD, Friday BB, Maulit MT, Gerton GL. The sperm acrosomal matrix contains a novel member of the pentaxin family of calcium-dependent binding proteins. J Biol Chem 1994;269:32607–14.
11. Omeis IA, Hsu YC, Perin MS. Mouse and human neuronal pentraxin 1 (NPTX1): conservation, genomic structure, and chromosomal localization. Genomics 1996;36:543–5.
12. Schlimgen AK, Helms JA, Vogel H, Perin MS. Neuronal pentraxin, a secreted protein with homology to acute phase proteins of the immune system. Neuron 1995;14:519–26.
13. Hsu YC, Perin MS. Human neuronal pentraxin II (NPTX2): conservation, genomic structure, and chromosomal localization. Genomics 1995;28:220–27.
14. Tsui CC, Copeland NG, Gilbert DJ, Jenkins NA, Barnes C, Worley PF. Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. J Neurosci 1996;16:2463–78.
15. Dodds DC, Omeis IA, Cushman SJ, Helms JA, Perin MS. Neuronal pentraxin receptor, a novel putative integral membrane pentraxin that interacts with neuronal pentraxin 1 and 2 and taipoxin-associated calcium-binding protein 49. J Biol Chem 1997;272:21488–94.
16. Kirkpatrick LL, Matzuk MM, Dodds DC, Perin MS. Biochemical interactions of the neuronal pentraxins. Neuronal pentraxin (NP) receptor binds to taipoxin and taipoxin-associated calcium-binding protein 49 via NP1 and NP2. J Biol Chem 2000;275:17786–92.
17. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, Spreafico F, Pausa M, D'ettorre C, Gianazza E, Tagliabue A, Salmona M, Tedesco F, Introna M, Mantovani A. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. J Biol Chem 1997;272:32817–23.
18. Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, Gingras AR, Tzima S, Vivanco F, Egido J, Tijsma O, Hack EC, Daha MR, Roos A. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur. J. Immunol 2003;33:465–73.
19. Roumenina LT, Ruseva MM, Zlatarova A, Ghai R, Kolev M, Olova N, Gadjeva M, Agrawal A, Bottazzi B, Mantovani A, Reid KB, Kishore U, Kojouharova MS. Interaction of C1q with IgG1, C-reactive protein and pentraxin 3: mutational studies using recombinant globular head modules of human C1q A, B, and C chains. Biochemistry 2006;45:4093–104.
20. Hicks PS, Saunero-Nava L, Du Clos TW, Mold C. Serum amyloid P component binds to histones and activates the classical complement pathway. J Immunol 1992;149:3689–94.
21. Inforzato A, Peri G, Doni A, Garlanda C, Mantovani A, Bastone A, Carpentieri A, Amoresano A, Pucci P, Roos A, Daha MR, Vincenti S, Gallo G, Carminati P, De Santis R, Salvatori G. Structure and function of the long pentraxin PTX3 glycosidic moiety: fine-tuning of the interaction with C1q and complement activation. Biochemistry 2006;45:11540–51.
22. Jarva H, Jokiranta TS, Hellwage J, Zipfel PF, Meri S. Regulation of complement activation by C-reactive protein: targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8–11. J Immunol 1999;163:3957–62.
23. Rovere P, Peri G, Fazzini F, Bottazzi B, Doni A, Bondanza A, Zimmermann VS, Garlanda C, Fascio U, Sabbadini MG, Rugarli C, Mantovani A, Manfredi AA. The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. Blood 2000;96:4300–6.
24. Pepys MB, Booth SE, Tennent GA, Butler PJ, Williams DG. Binding of pentraxins to different nuclear structures: C-reactive protein binds to small nuclear ribonucleoprotein particles, serum amyloid P component binds to chromatin and nucleoli. Clin Exp Immunol 1994;97:152–7.
25. Rusnati M, Camozzi M, Moroni E, Bottazzi B, Peri G, Indraccolo S, Amadori A, Mantovani A, Presta M. Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. Blood 2004;104:92–9.
26. Camozzi M, Rusnati M, Bugatti A, Bottazzi B, Mantovani A, Bastone A, Inforzato A, Vincenti S, Bracci L, Mastroianni D, Presta M. Identification of an antiangiogenic FGF2-binding site in the N terminus of the soluble pattern recognition receptor PTX3. J Biol Chem 2006;281:22605–13.
27. Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, Doni A, Bastone A, Mantovani G, Beck Peccoz P, Salvatori G, Mahoney DJ, Day AJ, Siracusa G, Romani L, Mantovani A. PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in in vivo fertilization. Development 2004;131:1577–86.
28. Milner CM, Day AJ. TSG-6: a multifunctional protein associated with inflammation. J Cell Sci 2003;116:1863–73.
29. Milner CM, Higman VA, Day AJ. TSG-6: a pluripotent inflammatory mediator? Biochem Soc Trans 2006;34:446–50.
30. Jeannin P, Bottazzi B, Sironi M, Doni A, Rusnati M, Presta M, Maina V, Magistrelli G, Haeuw JF, Hoeffel G, Thieblemont N, Corvaia N, Garlanda C, Delneste Y, Mantovani A. Complexity and complementarity of outer membrane protein A recognition by cellular and humoral innate immunity receptors. Immunity 2005;22:551–60.
31. Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R, Maccagno A, Riva F, Bottazzi B, Peri G, Doni A, Vago L, Botto M, De Santis R, Carminati P, Siracusa G, Altruda F, Vecchi A,

Romani L, Mantovani A. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. Nature 2002;420:182–6.
32. Diniz SN, Nomizo R, Cisalpino PS, Teixeira MM, Brown GD, Mantovani A, Gordon S, Reis LF, Dias AA. PTX3 function as an opsonin for the dectin-1-dependent internalization of zymosan by macrophages. J Leukoc Biol 2004;75:649–56.
33. Bozza S, Bistoni F, Gaziano R, Pitzurra L, Zelante T, Bonifazi P, Perruccio K, Bellocchio S, Neri M, Iorio AM, Salvatori G, De Santis R, Calvitti M, Doni A, Garlanda C, Mantovani A, Romani L. Pentraxin 3 protects from MCMV infection and reactivation through TLR sensing pathways leading to IRF3 activation. Blood 2006;108:3387–96.
34. Szalai AJ, Agrawal A, Greenhough TJ, Volanakis JE. C-reactive protein: structural biology and host defense function. Clin Chem Lab Med 1999;37:265–70.
35. Breviario F, D'aniello EM, Golay J, Peri G, Bottazzi B, Bairoch A, Saccone S, Marzella R, Predazzi V, Rocchi M, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. J Biol Chem 1992;267:22190–7.
36. Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, Romani L, Garlanda C, Mantovani A. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. Eur J Immunol 2003;33:2886–93.
37. Lee GW, Lee TH, Vilcek J. TSG-14, a tumor necrosis factor- and IL-1-inducible protein, is a novel member of the pentaxin family of acute phase proteins. J Immunol 1993;150:1804–12.
38. Introna M, Alles VV, Castellano M, Picardi G, De Gioia L, Bottazzai B, Peri G, Breviario F, Salmona M, De Gregorio L, Dragani TA, Srinivasan N, Blundell TL, Hamilton TA, Mantovani A. Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites. Blood 1996;87:1862–72.
39. Abderrahim-Ferkoune A, Bezy O, Chiellini C, Maffei M, Grimaldi P, Bonino F, Moustaid-Moussa N, Pasqualini F, Mantovani A, Ailhaud G, Amri EZ. Characterization of the long pentraxin PTX3 as a TNFalpha-induced secreted protein of adipose cells. J Lipid Res 2003;44:994–1000.
40. Goodman AR, Levy DE, Reis LF, Vilcek J. Differential regulation of TSG-14 expression in murine fibroblasts and peritoneal macrophages. J Leukoc Biol 2000;67:387–95.
41. Klouche M, Peri G, Knabbe C, Eckstein HH, Schmid FX, Schmitz G, Mantovani A. Modified atherogenic lipoproteins induce expression of pentraxin-3 by human vascular smooth muscle cells. Atherosclerosis 2004;175:221–8.
42. Agnello D, Carvelli L, Muzio V, Villa P, Bottazzi B, Polentarutti N, Mennini T, Mantovani A, Ghezzi P. Increased peripheral benzodiazepine binding sites and pentraxin 3 expression in the spinal cord during EAE: relation to inflammatory cytokines and modulation by dexamethasone and rolipram. J Neuroimmunol 2000;109:105–11.
43. Nauta AJ, De Haij S, Bottazzi B, Mantovani A, Borrias MC, Aten J, Rastaldi MP, Daha MR, Van Kooten C, Roos A. Human renal epithelial cells produce the long pentraxin PTX3. Kidney Int 2005;67:543–53.
44. Dos Santos CC, Han B, Andrade CF, Bai X, Uhlig S, Hubmayr R, Tsang M, Lodyga M, Keshavjee S, Slutsky AS, Liu M. DNA microarray analysis of gene expression in alveolar epithelial cells in response to TNFalpha, LPS, and cyclic stretch. Physiol Genomics 2004;19:331–42.
45. Malaguarnera L, Pilastro MR, Vicari L, Di Marco R, Malaguarnera M, Messina A. PTX3 gene expression in Castleman's disease. Eur J Haematol 2000;64:132–4.
46. Klouche M, Brockmeyer N, Knabbe C, Rose-John S. Human herpesvirus 8-derived viral IL-6 induces PTX3 expression in Kaposi's sarcoma cells. Aids 2002;16:F9–18.
47. Ehrt S, Schnappinger D, Bekiranov S, Drenkow J, Shi S, Gingeras TR, Gaasterland T, Schoolnik G, Nathan C. Reprogramming of the macrophage transcriptome in response to interferon-gamma and Mycobacterium tuberculosis: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. J Exp Med 2001;194:1123–40.
48. Goodman AR, Cardozo T, Abagyan R, Altmeyer A, Wisniewski HG, Vilcek J. Long pentraxins: an emerging group of proteins with diverse functions. Cytokine Growth Factor Rev 1996;7:191–202.
49. Polentarutti N, Picardi G, Basile A, Cenzuales S, Rivolta A, Matteucci C, Peri G, Mantovani A, Introna M. Interferon-gamma inhibits expression of the long pentraxin PTX3 in human monocytes. Eur J Immunol 1998;28:496–501.
50. Perrier P, Martinez FO, Locati M, Bianchi G, Nebuloni M, Vago G, Bazzoni F, Sozzani S, Allavena P, Mantovani A. Distinct transcriptional programs activated by interleukin-10 with or without lipopolysaccharide in dendritic cells: induction of the B cell-activating chemokine, CXC chemokine ligand 13. J Immunol 2004;172:7031–42.
51. Lang R, Patel D, Morris JJ, Rutschman RL, Murray PJ. Shaping gene expression in activated and resting primary macrophages by IL-10. J Immunol 2002;169:2253–63.
52. Moore KW, De Waal Malefyt R, Coffman RL, O'garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
53. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004;25:677–86.
54. Rolph MS, Zimmer S, Bottazzi B, Garlanda C, Mantovani A, Hansson GK. Production of the long pentraxin PTX3 in advanced atherosclerotic plaques. Arterioscler. Thromb Vasc Biol 2002;22:e10–4.
55. Bosutti A, Grassi G, Zanetti M, Aleksova A, Zecchin M, Sinagra G, Biolo G, Guarnieri G. Relation between the plasma levels of LDL-cholesterol and the expression of the early marker of inflammation long pentraxin PTX3 and the stress response gene p66ShcA in pacemaker-implanted patients. Clin Exp Med 2007;7:16–23.
56. Varani S, Elvin JA, Yan C, Demayo J, Demayo FJ, Horton HF, Byrne MC, Matzuk MM. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. Mol Endocrinol 2002;16:1154–67.
57. Willeke F, Assad A, Findeisen P, Schromm E, Grobholz R, Von Gerstenbergk B, Mantovani A, Peri S, Friess HH, Post S, Von Knebel Doeberitz M, Schwarzbach MH. Overexpression of a member of the pentraxin family (PTX3) in human soft tissue liposarcoma. Eur J Cancer 2006;42:2639–46.
58. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, Garlanda C, Romani L, Gascan H, Bellocchio S, Bozza S, Cassatella MA, Jeannin P, Mantovani A. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. J Exp Med 2007;204:793–804.
59. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, Pizzetti F, Maggioni AP, Moccetti T, Metra M, Cas LD, Ghezzi P, Sipe JD, Re G, Olivetti G, Mantovani A, Latini R. PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. Circulation 2000;102:636–41.
60. Latini R, Maggioni AP, Peri G, Gonzini L, Lucci D, Mocarelli P, Vago L, Pasqualini F, Signorini S, Soldateschi D, Tarli L, Schweiger C, Fresco C, Cecere R, Tognoni G, Mantovani A. Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. Circulation 2004;110:2349–54.
61. Cenci E, Perito S, Enssle K, Mosci P, Latge J, Romani L, Bistoni F. Th1 and Th2 cytokines in mice with invasive aspergillosis. Infect Immun 1997;65:564–70.

62. Nagai H, Guo J, Choi H, Kurup V. Interferon-gamma and tumor necrosis factor-alpha protect mice from invasive aspergillosis. J Infect Dis 1995;172:1554–60.
63. Gaziano R, Bozza S, Bellocchio S, Perruccio K, Montagnoli C, Pitzurra L, Salvatori G, De Santis R, Carminati P, Mantovani A, Romani L. Anti-Aspergillus fumigatus efficacy of pentraxin 3 alone and in combination with antifungals. Antimicrob Agents Chemother 2004;48:4414–21.
64. Soares AC, Souza DG, Pinho V, Vieira AT, Nicoli JR, Cunha FQ, Mantovani A, Reis LF, Dias AA, Teixeira MM. Dual function of the long pentraxin PTX3 in resistance against pulmonary infection with Klebsiella pneumoniae in transgenic mice. Microbes Infect 2006;8:1321–9.
65. Dias AA, Goodman AR, Dos Santos JL, Gomes RN, Altmeyer A, Bozza PT, Horta MF, Vilcek J, Reis LF. TSG-14 transgenic mice have improved survival to endotoxemia and to CLP-induced sepsis. J Leukoc Biol 2001;69:928–36.
66. Souza DG, Soares AC, Pinho V, Torloni H, Reis LF, Teixeira MM, Dias AA. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. Am J Pathol 2002;160:1755–65.
67. Ravizza T, Moneta D, Bottazzi B, Peri G, Garlanda C, Hirsch E, Richards GJ, Mantovani A, Vezzani A. Dynamic induction of the long pentraxin PTX3 in the CNS after limbic seizures: evidence for a protective role in seizure-induced neurodegeneration. Neuroscience 2001;105:43–53.
68. Van Rossum AP, Fazzini F, Limburg PC, Manfredi AA, Rovere-Querini P, Mantovani A, Kallenberg CG. The prototypic tissue pentraxin PTX3, in contrast to the short pentraxin serum amyloid P, inhibits phagocytosis of late apoptotic neutrophils by macrophages. Arthritis Rheum 2004;50:2667–74.
69. Baruah P, Propato A, Dumitriu IE, Rovere-Querini P, Russo V, Fontana R, Accapezzato D, Peri G, Mantovani A, Barnaba V, Manfredi AA. The pattern recognition receptor PTX3 is recruited at the synapse between dying and dendritic cells, and edits the cross-presentation of self, viral, and tumor antigens. Blood 2006; 107:151–8.
70. Castellano G, Woltman AM, Nauta AJ, Roos A, Trouw LA, Seelen MA, Schena FP, Daha MR, Van Kooten C. Maturation of dendritic cells abrogates C1q production in vivo and in vitro. Blood 2004;103:3813–20.
71. Baruah P, Dumitriu IE, Peri G, Russo V, Mantovani A, Manfredi AA, Rovere-Querini P. The tissue pentraxin PTX3 limits C1q-mediated complement activation and phagocytosis of apoptotic cells by dendritic cells. J Leukoc Biol 2006.
72. Nauta AJ, Daha MR, Van Kooten C, Roos A. Recognition and clearance of apoptotic cells: a role for complement and pentraxins. Trends Immunol 2003;24:148–54.
73. Jeannin P, Renno T, Goetsch L, Miconnet I, Aubry JP, Delneste Y, Herbault N, Baussant T, Magistrelli G, Soulas C, Romero P, Cerottini JC, Bonnefoy JY. OmpA targets dendritic cells, induces their maturation and delivers antigen into the MHC class I presentation pathway. Nat Immunol 2000;1:502–9.
74. Baruah P, Trimarchi M, Dumitriu IE, Dellantonio G, Doglioni C, Rovere-Querini P, Bussi M, Manfredi AA. Innate responses to Aspergillus: role of C1q and pentraxin 3 in nasal polyposis. Am J Rhinol 2007;21:224–30.
75. Camozzi M, Zacchigna S, Rusnati M, Coltrini D, Ramirez-Correa G, Bottazzi B, Mantovani A, Giacca M, Presta M. Pentraxin 3 inhibits fibroblast growth factor 2-dependent activation of smooth muscle cells in vitro and neointima formation in vivo. Arterioscler Thromb Vasc Biol 2005;25:1837–42.
76. Zhang X, Jafari N, Barnes RB, Confino E, Milad M, Kazer RR. Studies of gene expression in human cumulus cells indicate pentraxin 3 as a possible marker for oocyte quality. Fertil Steril 2005;83(Suppl 1):1169–79.
77. Tranguch S, Chakrabarty A, Guo Y, Wang H, Dey SK. Maternal Pentraxin 3 Deficiency Compromises Implantation in Mice. Biol Reprod 2007.
78. Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, Genbecev-Krtolica O, Mavrogianis P, Ferrer K, Kruessel J, Fazleabas AT, Fisher SJ, Giudice LC. Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. Biol Reprod 2007; 76:102–17.
79. Muller B, Peri G, Doni A, Torri V, Landmann R, Bottazzi B, Mantovani A. Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. Crit Care Med 2001;29:1404–7.
80. Napoleone E, Di Santo A, Peri G, Mantovani A, De Gaetano G, Donati MB, Lorenzet R. The long pentraxin PTX3 up-regulates tissue factor in activated monocytes: another link between inflammation and clotting activation. J Leukoc Biol 2004;76: 203–9.
81. Napoleone E, Di Santo A, Bastone A, Peri G, Mantovani A, De Gaetano G, Donati MB, Lorenzet R. Long pentraxin PTX3 upregulates tissue factor expression in human endothelial cells: a novel link between vascular inflammation and clotting activation. Arterioscler Thromb Vasc Biol 2002;22:782–7.
82. Mairuhu AT, Peri G, Setiati TE, Hack CE, Koraka P, Soemantri A, Osterhaus AD, Brandjes DP, Van Der Meer JW, Mantovani A, Van Gorp EC. Elevated plasma levels of the long pentraxin, pentraxin 3, in severe dengue virus infections. J Med Virol 2005;76:547–52.
83. Azzurri A, Sow OY, Amedei A, Bah B, Diallo S, Peri G, Benagiano M, D'elios MM, Mantovani A, Del Prete G. IFN-gamma-inducible protein 10 and pentraxin 3 plasma levels are tools for monitoring inflammation and disease activity in Mycobacterium tuberculosis infection. Microbes Infect 2005;7:1–8.
84. Olesen R, Wejse C, Velez DR, Bisseye C, Sodemann M, Aaby P, Rabna P, Worwui A, Chapman H, Diatta M, Adegbola RA, Hill PC, Ostergaard L, Williams SM, Sirugo G. DC-SIGN (CD209), Pentraxin 3 and Vitamin D Receptor gene variants associate with pulmonary tubercolosis risk in West-Africans. Genes and Immunity, 2007 (in press).
85. Inoue K, Sugiyama A, Reid PC, Ito Y, Miyauchi K, Mukai S, Sagara M, Miyamoto K, Satoh H, Kohno I, Kurata T, Ota H, Mantovani A, Hamakubo T, Daida H, Kodama T. Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. Arterioscler Thromb Vasc Biol 2007;27:161–7.
86. Kotooka N, Inoue T, Fujimatsu D, Morooka T, Hashimoto S, Hikichi Y, Uchida T, Sugiyama A, Node K. Pentraxin3 is a novel marker for stent-induced inflammation and neointimal thickening. Atherosclerosis, 2007.
87. Fazzini F, Peri G, Doni A, Dell'antonio G, Dal Cin E, Bozzolo E, D'auria F, Praderio L, Ciboddo G, Sabbadini MG, Manfredi AA, Mantovani A, Querini PR. PTX3 in small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. Arthritis Rheum 2001;44:2841–50.
88. Luchetti MM, Piccinini G, Mantovani A, Peri G, Matteucci C, Pomponio G, Fratini M, Fraticelli P, Sambo P, Di Loreto C, Doni A, Introna M, Gabrielli A. Expression and production of the long pentraxin PTX3 in rheumatoid arthritis (RA). Clin Exp Immunol 2000;119:196–202.
89. Van Rossum AP, Pas HH, Fazzini F, Huitema MG, Limburg PC, Jonkman MF, Kallenberg CG. Abundance of the long pentraxin PTX3 at sites of leukocytoclastic lesions in patients with small-vessel vasculitis. Arthritis Rheum 2006;54:986–91.
90. Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimburger O, Barany P, Axelsson J, Alverstrand A, Stenvinkel P, Lindholm B, Suliman M. Plasma Pentraxin 3 in chronic kidney disease

patients: association with renal function, protein-energy wasting, cardiovascular disease and mortality. Clin J Am Soc Nephrol, 2007;2:889–97.
91. Boehme M, Kaehne F, Kuehne A, Bernhardt W, Schroder M, Pommer W, Fischer C, Becker H, Muller C, Schindler R. Pentraxin 3 is elevated in haemodialysis patients and is associated with cardiovascular disease. Nephrol Dial Transplant, 2007.
92. Paffoni A, Ragni G, Doni A, Somigliana E, Pasqualini F, Restelli L, Pardi G, Mantovani A, Garlanda C. Follicular fluid levels of the long pentraxin PTX3. J Soc Gynecol Investig 2006;13:226–31.
93. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999;180:499–506.
94. Cetin I, Cozzi V, Pasqualini F, Nebuloni M, Garlanda C, Vago L, Pardi G, Mantovani A. Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction. Am J Obstet Gynecol 2006;194:1347–53.
95. Rovere-Querini P, Antonacci S, Dell'antonio G, Angeli A, Almirante G, Cin ED, Valsecchi L, Lanzani C, Sabbadini MG, Doglioni C, Manfredi AA, Castiglioni MT. Plasma and tissue expression of the long pentraxin 3 during normal pregnancy and preeclampsia. Obstet Gynecol 2006;108:148–55.
96. Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. J Clin Endocrinol Metab 2001;86:2505–12.
97. Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin JN, Jr., Bennett WA. Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. Am J Obstet Gynecol 1999;181:915–20.
98. Assi F, Fruscio R, Bonardi C, Ghidini A, Allavena P, Mantovani A, Locatelli A. Pentraxin 3 in plasma and vaginal fluid in women with preterm delivery. Bjog 2007;114:143–7.
99. Hirschfield GM, Gallimore JR, Kahan MC, Hutchinson WL, Sabin CA, Benson GM, Dhillon AP, Tennent GA, Pepys MB. Transgenic human C-reactive protein is not proatherogenic in apolipoprotein E-deficient mice. Proc Natl Acad Sci USA 2005; 102:8309–14.