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Review

PTX3 as a paradigm for the interaction of pentraxins with the Complement system

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ABSTRACT

Pentraxins are highly conserved components of the humoral arm of innate immunity. They include the short pentraxins C reactive protein (CRP) and serum amyloid P component (SAP), and the long pentraxin PTX3. These are soluble pattern-recognition molecules that are present in the blood and body fluids, and share the ability to recognize pathogens and promote their disposal. CRP and SAP are produced systemically in the liver while PTX3 is produced locally in a number of tissues, macrophages and neutrophils being major sources of this long pentraxin. Pentraxins interact with components of the classical and lectin pathways of Complement as well as with Complement regulators. In particular, PTX3 recognizes C1q, factor H, MBL and ficolins, where these interactions amplify the repertoire of microbial recognition and effector functions of the Complement system. The complex interaction of pentraxins with the Complement system at different levels has broad implications for host defence and regulation of inflammation.

1. Introduction

The innate immune system comprises a cellular and a humoral arm. The Complement system and soluble pattern recognition molecules (PRM) represent the humoral arm of innate immunity [1,2]. Neutrophils store in their specific granules some of the soluble PRMs that can be rapidly released upon neutrophil stimulation. Other PRMs are *de novo* synthesized as early gene products in mononuclear phagocytes, dendritic cells (DCs) and endothelial cells and locally released at sites of immune insults. Finally, epithelial cells, particularly in the liver and lung, act as sources of systemic PRMs [2]. Soluble PRMs belong to different molecular families, including collectins, ficolins, and pentraxins [2–5]. Despite their heterogeneity in terms of molecular structure and site or time of synthesis, soluble PRMs share basic, evolutionarily conserved functions, including Complement activation, opsonisation, agglutination and regulation of inflammation [2].

Pentraxins are a superfamily of proteins characterized by the presence of a 200 amino acid pentraxin domain in their carboxyl terminus containing a 8 amino acid-long pentraxin signature that is highly conserved in evolution from arthropods to mammals [6]. C-reactive protein (CRP) and serum amyloid P component (SAP) are the prototypes of the family and are the main acute phase reactants in humans and mice, respectively. CRP and SAP constitute the short arm of the pentraxin superfamily, the so called classical pentraxins. They are 25-kDa proteins characterized by a common structural organization of five or ten identical subunits arranged in a pentameric radial symmetry [7].

PTX3 is the first long pentraxin identified as an IL-1-inducible gene in endothelial cells (EC) and a TNF-stimulated gene in fibroblasts [6]. The main structural determinant of the long pentraxins is the presence of a long amino-terminal domain that is missing in CRP or SAP, coupled to the C-terminal pentraxin domain. The members of this family have been identified in the 1990s as cytokine-inducible genes or molecules expressed in specific tissues: guinea pig apexin in spermatozoa, neuronal pentraxin (NP) 1 or NPTX1, NP2, also called Narp or NPTX2, neuronal pentraxin receptor (NPR), a transmembrane molecule in neurons, and PTX4, a long pentraxin conserved from lower vertebrates to mammals [8,9].

Pentraxins are long known for their complex interaction with the Complement system, which takes place at different levels and has broad implications for host defence and regulation of inflammation. Here we review the available information on this crosstalk, with major focus on PTX3 as a paradigmatic member of pentraxins.

2. Short pentraxins

CRP and SAP are soluble PRMs that are found in the serum and are involved in host defence. They recognize Pathogen Associated

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Molecular Patterns (PAMPs) [6], but also membrane phospholipids and nuclear components that are present on damaged or altered tissue, the so called Danger Associated Molecular Patterns (DAMPs) [10]. Both CRP and SAP can opsonize microbial pathogens and apoptotic cells and effectively promote their clearance through Complement activation.

CRP and SAP orthologs have been identified in different mammalian species, where however they might differ, for instance, in blood levels. The classical pentraxins are synthetized by hepatocytes in response mainly to IL-6 and IL-1β typically as a reaction to microbial challenge or tissue injury [11]. As noted above, the synthesis is differentially regulated in different species: in humans CRP is a major acute phase protein, with baseline levels lower than 5 μg/ml that increase as much as 1000-fold following an acute phase stimulus. For this reason, since its first report, CRP has been extensively used as a sensitive systemic marker to monitor and diagnose diseases, with broad clinical utility in infection, inflammation, and tissue damage [12]. SAP is a major acute phase protein in the mouse, whereas it does not act as an acute phase reactant in humans [13], being constitutively present in the human serum at 30–50 μg/ml.

2.1. Protein structure

The CRP gene is located on the short arm of chromosome 1 and is organized in two exons, the first coding for the leader peptide and the initial two amino acids of the mature protein, and the second coding for the remaining 204 amino acids. The human CRP is composed of five identical non-glycosylated protomers arranged in a cyclic pentameric structure with a total molecular weight of 115 kDa. Two cysteine residues in position 36 and 97 are conserved in all members of the pentraxin superfamily and are involved in intra-chain disulfide bonds [14].

Human SAP was identified as a close relative of CRP with an amino acid sequence identity of 51% [7]. The human gene is located in close physical and genetic linkage with the CRP gene on chromosome 1, and shares with CRP the same organization in two exons, the first coding for the signal peptide and the second for the mature protein. SAP is a highly conserved plasma glycoprotein composed of 5 or 10 identical subunits of 23 kDa, non-covalently associated into pentameric rings, where these interact face to face in the 10-mer molecule [7]. A single glycosylation site has been described at Asn32 occupied by a single N-linked biantennary oligosaccharide [38].

X-ray diffraction has been used to resolve the three-dimensional structure of human SAP and CRP in association with their ligands [7,15–17] or in complex with Fcγ receptor (FcγR) [18]. The pentameric disc-like configuration described for the two human pentraxins has a diameter of 100 Å and is 35 Å deep. Each protomer has a conserved flattened jelly-roll topology similar to that of lectins. The crystal structure of SAP in complex with FcγRIIa has provided a structural frame for the interaction of pentraxins with FcγRs [19–21] and highlighted some similarities to antibody recognition by these receptors, thus bridging functions of the innate and adaptive immune systems [18,21].

2.2. Interaction with Complement

CRP and SAP are endowed with the ability to interact in a calcium-dependent manner with a broad number of ligands and effector molecules, and analysis of these interactions has provided insights into to biological properties of this family of proteins. Amongst the different ligands, both CRP and SAP can recognize Complement components. The capacity to activate Complement is a crucial feature of pentraxins and represents one of the possible mechanisms underlying the defensive role of these PRMs against pathogens. CRP and SAP, aggregated or attached to most of their ligands, interact with C1q, the recognition subunit of the classical Complement pathway [22]. Complexes of ligand-bound CRP and C1q lead to formation of the C3 convertase, thus activating the classical Complement cascade. Structural studies suggest that CRP recognizes and binds the globular head modules of C1q [23,24]. In addition, CRP interacts with ficolins. This interaction stabilizes CRP binding to bacteria and activates the lectin-mediated Complement pathway amplifying Complement-mediated killing of pathogens, such as *Salmonella enterica* [25].

Complement activation by short pentraxins is also one of the mechanisms that contribute to removal of the cellular debris [22]. When CRP is bound to self surfaces (e.g. apoptotic cells, damaged tissue), it retains the capacity to interact with C1q and activate the classical Complement pathway, however this activation is restricted to the initial stages of the Complement cascade and little consumption of C5–C9 is observed [26].

Surface bound CRP has been shown to inhibit the alternative pathway amplification loop through a specific interaction with factor H (FH), the main soluble regulator of this pathway [27,28]. In addition, CRP and SAP bind C4b binding protein (C4BP), a soluble regulator of the classical and lectin pathways [29,30].

Complement-mediated inflammation plays a key role in the pathogenesis of tissue injury [31]. Due to the interplay with the Complement system, pentraxins are main actors of this process, whose exhaustive discussion is beyond the scope of this review. As an example, it has been well described that CRP bound to damaged cells may lead to Complement-mediated exacerbation of tissue damage in the ischaemic necrosis following heart attacks and stroke [12,32]. On the same line, a lectin pathway-CRP cross-activation has been recently reported in the ischaemia reperfusion injury, where the interaction of CRP with ficolin-2 is associated with infarct size and left ventricular (LV) remodelling [33]. The interaction of CRP with the Complement regulators FH and C4BP could represent a mechanism to limit the Complement-mediated damage of the host tissue, where impaired regulatory as well as ligand and cell recognition functions of FH, caused by mutations or autoantibodies, are associated with kidney diseases (e.g., atypical hemolytic uraemic syndrome, aHUS) and eye disorders (e.g., age-related macular degeneration) [34].

Besides the interaction with Complement, both CRP and SAP share with antibodies the capacity to interact with FcR. As discussed above, the crystal structure of the complex formed by SAP with FcγRIIa has been recently generated [18] that structurally explains the interaction of pentraxins with Fcγ receptors. SAP and IgG interact with similar affinity to FcγR, share the same binding sites and compete for FcγR binding; in line with this, pentraxins inhibit immune-complex-mediated phagocytosis. Thus, pentraxins possess functions similar to those of antibodies, which activate both Complement and FcγR pathways.

3. The long pentraxin PTX3

PTX3 is the prototypic long pentraxin that was first identified in the early 1990s as a cytokine-inducible gene in endothelial cells and fibroblasts. Long pentraxins have an unrelated amino-terminal region coupled to a carboxy-terminal pentraxin-like domain and differ from short pentraxins in gene organization, chromosomal localization, cellular source, inducing stimuli and recognized ligands [6]. Experimental evidences indicated that PTX3, originally identified as early induced protein in response to proinflammatory stimuli, is a soluble PRM involved in the innate immune response.

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3.1. Gene and protein organization

The human and murine PTX3 genes have been localized on chromosome 3 and are organized in three exons separated by two introns. The first two exons code for the leader peptide and the N-terminal domain of the protein, respectively, and the third exon encodes the pentraxin domain. The proximal promoters of both human and murine PTX3 genes share numerous potential binding sites for transcription factors, including Pu1, AP-1, NF-κB, SP1, and NF-IL-6 sites. The NF-κB binding site is essential for the transcriptional response to the proinflammatory cytokines TNF-α and IL-1β, whereas AP-1 controls the basal transcription of PTX3 [35–37].

PTX3 is a multimeric glycoprotein whose composing subunits are made of 381 amino acids, including a 17-residue signal peptide [35]. PTX3 primary sequence is highly conserved among animal species (human and murine PTX3 sharing 92% of conserved amino acid residues), suggesting a strong evolutionary pressure to maintain its structure/function relationships. Like other members of the long-pentraxin family, PTX3 is composed of a unique N-terminal region and a 203 amino acid C-terminal domain homologous to the short pentraxins CRP and SAP [38].

The N-terminal region is unrelated to any known protein structure. Nevertheless, secondary structure predictions indicate that this part of the protein is likely to form four α-helices, three of which (amino acids 78–97, 109–135, and 144–170) are probably involved in the formation of coiled-coil structures [39,40]. The C-terminal domain of PTX3 (residues 179–381 of the preprotein) is homologous to the short-pentraxins CRP and SAP, with up to 57% similarity [38]. The three-dimensional model of this domain generated based on the crystallographic structures of CRP and SAP [8,41,42] shows that the pentraxin-like domain of PTX3 adopts a β-jelly roll topology containing both intra- and inter-chain disulfide bonds that are important determinants of the PTX3 quaternary structure [43]. The amino acids residues that form the calcium-binding pocket in CRP and SAP are missing in the pentraxin domain of PTX3, which might explain some binding properties of this long pentraxin (i.e., PTX3 binding to C1q is calcium-independent, as opposed to the short pentraxins) [8].

A single *N*-glycosylation site has been identified in the C-terminal domain of PTX3 at Asn220, which is fully occupied by complex type oligosaccharides, mainly fucosylated and sialylated biantennary sugars with a minor fraction of tri- and tetrantennary glycans [42]. The relative content of bi-, tri-, and tetrantennary oligosaccharides and subsequently the degree of sialylation vary amongst PTX3 isolates from different cellular sources, thus suggesting that PTX3 glycosylation might change depending on cell type and inducing stimuli. The glycosidic moiety of PTX3 has been shown to modulate the interaction with components of the Complement system, such as C1q [42], factor H [44] and ficolin-1 [45]. In addition, the terminal residues of sialic acid are necessary for PTX3 recognition of influenza virus [46], and the interaction of PTX3 with P-selectin is glycosylation-dependent [47] (see below). All together, these data suggest that changes in the glycosylation status might have important functional implications and represent a strategy to tune the biological activity of PTX3.

Mass spectrometry, site directed mutagenesis, Electron Microscopy (EM) and Small Angle X-ray Scattering (SAXS) analyses of the recombinant human protein indicate that PTX3 is made of covalent octamers with a molecular mass of 340 kDa [43] and has an elongated structure with a large and a small domain interconnected by a stalk region [40]. The structural determinants of the PTX3 quaternary organization are mainly localized in the protein N-terminal domain, where this region mediates the association of protomers into tetramers via both covalent (i.e., disulfide bonds) and non-covalent (i.e., inter-chain coiled coils) interactions; Cys residues in the C-terminal domain form additional disulfide bonds that stabilize two tetramers into the functional octamer. Quaternary structure and glycosylation of PTX3 are fundamental in dictating the protein binding properties and, ultimately, its biological functions.

3.2. Cellular source and transcriptional regulation

PTX3 expression is rapidly induced by several stimuli, such as cytokines (e.g. IL-1β, TNF-α), TLR agonists, microbial moieties (e.g. LPS, OmpA, lipoarabinomannans) or intact microorganisms [48] in several cell types, including myeloid dendritic cells (DC), macrophages, endothelial cells, fibroblasts, kidney epithelial cells, synovial cells, chondrocytes, adipocytes, alveolar epithelial cells, glial cells, mesangial cells and granulosa cells [48]. In addition, oxidized and enzymatically modified low density lipoproteins (ox-LDL) as well as anti-inflammatory high-density lipoproteins (HDL) promote PTX3 production in ECs and human primary vascular smooth muscle cells (SMCs) [39,49]; in contrast, both human and murine lymphatic endothelial cells constitutively express the protein [50]. Expression of the PTX3 mRNA is temporally confined to immature myeloid cells, but the protein is stored in neutrophil specific granules and is released in response to TLR engagement by microorganisms or TLR agonists [51], partially localizing in neutrophil extracellular traps (NETs). Resting T and B lymphocytes and natural killer cells do not express PTX3 mRNA.

IFN-γ inhibits PTX3 production in dendritic cells, monocytes and macrophages by reducing both gene transcription and transcript stability, while IL-10 amplifies LPS-induced PTX3 expression [52,53]. IL-4, dexamethasone, 1α,25-dihydroxivitamin D3 and prostaglandin E2 also inhibit LPS-induced PTX3 in myeloid dendritic cells [53]. Depending on the mode of action of the glucocorticoid (GC) receptor in hematopoietic or stromal cells, GC hormones inhibit PTX3 production in hematopoietic cells (e.g., dendritic cells and macrophages) but induce PTX3 production in non-hematopoietic cells (e.g., fibroblasts and endothelial cells) [54].

Different signalling pathways can affect PTX3 production, depending on cell type and/or stimuli. PTX3 expression in a model of acute myocardial ischaemia is controlled by the NF-kB pathway [55], induction of the protein by TNF-α in lung epithelial cells involves the c-Jun N-terminal Kinase (JNK) pathway [56], whereas HDL-induced PTX3 in endothelial cells requires the activation of the PI3 K/Akt pathway through G-coupled lysosphingolipid receptors [49].

3.3. Interaction with the Complement system

As mentioned above, modulation of Complement activation is a common feature of pentraxins and also PTX3 can affect this essential pathway of the innate resistance (Fig. 1) [22,38,44,57].

C1q was the first ligand identified for PTX3 [38]. Unlike short pentraxins, binding of PTX3 to C1q does not require previous aggregation of the protein or the presence of calcium ions [22,26,38,79]. The interaction occurs with the globular head of C1q [24,79]; Lys170 in the C1q globular head (gh) chain C (ghC) or Tyr175 in the ghB are essential for the interaction since the substitution of these aminoacids leads to a 40–50% reduction of binding [24]. Through the binding with C1q, PTX3 may exert a dual role in Complement activation, depending on the way it is presented. When immobilized on plastic wells, PTX3 induces activation of the classical Complement pathway, resulting in increased deposition of C3 and C4. In contrast, the Complement cascade is inhibited when binding occurs in the fluid-phase, likely via the competitive blocking of relevant interaction sites [22].

PTX3 interacts with members of the lectin pathway, namely ficolin-1, ficolin-2 and mannose-binding lectin (MBL)[45,57,58].

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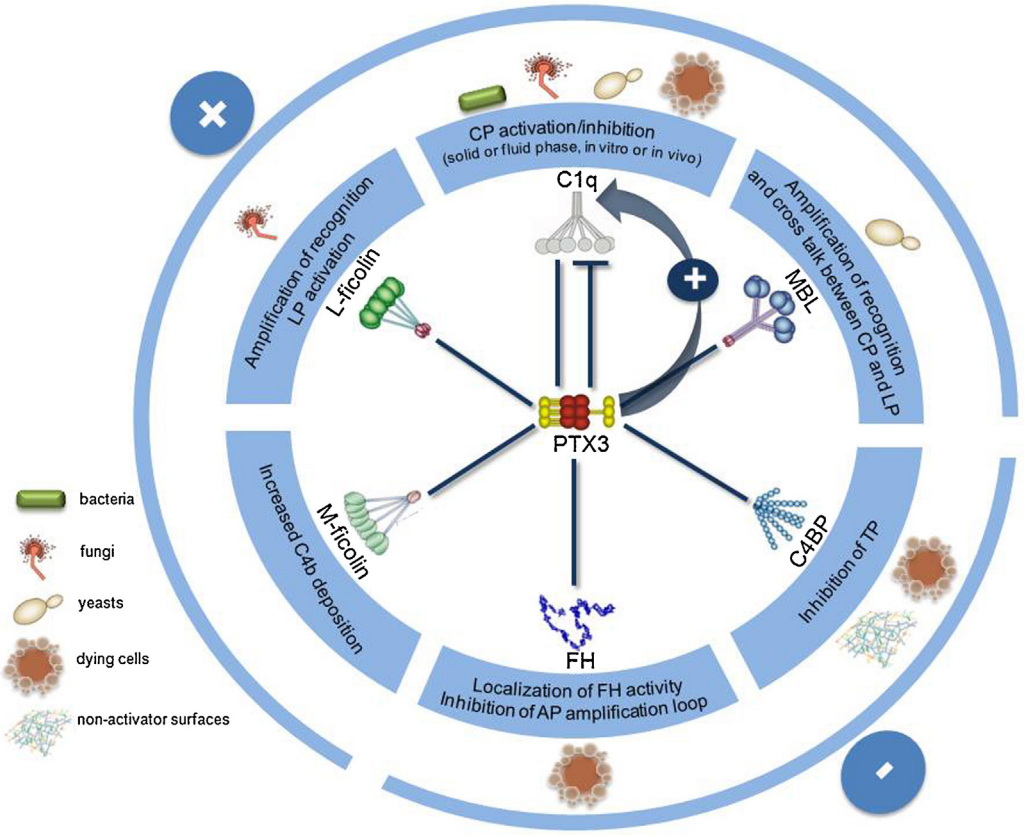

Fig. 1. Roles of PTX3 in the initiation and regulation of the Complement cascade. PTX3 participates in the activation and regulation of Complement activation through the three pathways, by interacting with C1q, lectins, and with factor H or C4BP. PTX3 plays a complex role in removal of pathogens or dying cells via classical Complement activation. The classical and the lectin pathways are initiated when PTX3 binds to the activation structure (e.g., antigens or microbial moieties). Upon this interaction, downstream Complement components are activated. On the handling of apoptotic cells, the effect of PTX3 on Complement could be divergent depending on the experimental setting (fluid phase or solid phase, *in vitro* or *in vivo*). PTX3 interacts also with soluble Complement regulators factor H and C4BP, limiting the activation of the terminal pathway and hence Complement mediated excessive inflammation.

The binding occurs via the fibrinogen-binding domain (FBG) of ficolins or via the collagen like domain of MBL, and involves the terminal sialic acid of the glycosidic moiety of PTX3. The physical interaction of PTX3 and ficolin-2 or MBL is accompanied by a functional cooperation that promotes recruitment of the molecules onto the surface of the recognized microbes (i.e., *Aspergillus fumigatus, Candida albicans*) and synergistically amplifies the Complement-mediated innate response [2,57,58].

In addition to the interaction with components of the classical and lectin Complement pathways, PTX3 interacts with regulators of the Complement system, namely FH and C4BP [44,59]. The interaction of PTX3 with FH modulates the alternative pathway activation by promoting FH deposition on PTX3-coated surfaces and preventing exaggerated Complement activation [44]. Two PTX3-binding sites have been identified in the Short Consensus Repeats (SCR) 7 and SCR 19–20 of FH, which are targeted by residues of the C- and N-terminal domains, respectively [43]. In this regard, it is worth mentioning that SCR 7 contains the site of interaction with the short pentraxin CRP, which is homologous to the C-terminal region of PTX3. In a recent study residues of SCR 20 of FH have been identified that are relevant to PTX3 binding. aHUS-associated FH mutations within this site caused a reduced binding of FH to PTX3. Similarly, anti-factor H autoantibodies isolated from aHUS patients inhibited the FH/PTX3 interaction. Thus, recognition of FH by PTX3 might be impaired by FH mutations and autoantibodies, which might result into enhanced local Complement-mediated inflammation in aHUS [34].

Similarly, PTX3 recruits C4BP onto apoptotic cells or extracellular matrix (ECM), increasing the rate of C4b inactivation and reducing deposition of the lytic C5b-9 terminal complex [59]. Thus PTX3 can recruit both Complement regulators FH and C4BP to limit excessive Complement activation on apoptotic cells.

3.4. Functional relevance in host defence

As a fluid phase PRM, PTX3 recognizes and binds a wide range of microbes, including bacteria, fungi and viruses. A specific binding has been observed to conidia of *A. fumigatus* [60], *Paracoccidoides brasiliensis* (*P. brasiliensis*) and zymosan [61]; to selected Gram-positive and Gram-negative bacteria (e.g. *Pseudomonas aeruginosa; Klebsiella pneumoniae*) [60,62,63] and to virus, such as selected strains of influenza virus type A (IAV), and human or murine cytomegalovirus (HCMV and MCMV) [46,64]. PTX3 recognizes membrane moieties on the surface of pathogens, such as member of the Outer Membrane Protein (Omp) family (i.e. Omp-A from *K. pneumonia* Kp-OmpA) and viral haemoagglutinin [46,62]. PTX3 amplifies the inflammatory response in terms of leucocyte recruitment and pro-inflammatory cytokine production induced by a microbial moiety such as Kp-OmpA, an effect mediated by Complement activation [65].

In vitro PTX3 amplifies neutrophil phagocytosis of *Aspergillus* conidia [66]. In an effort to define the molecular mechanisms underlying the opsonic activity of PTX3, it has been demonstrated that the molecule promotes neutrophils phagocytosis through a

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mechanism dependent on FcγRII, CD11b and Complement. In models of acute infections, the pro-phagocytic effect exerted by PTX3 was maintained in C1q-deficient mice but was lost in C3- and FcγR-deficient mice, suggesting that recognition and phagocytosis of conidia mediated by PTX3 involves the interplay between Complement, in particular the alternative pathway, and FcγRs [63].

PTX3 also modulates inflammation in sterile conditions. In a murine model of experimental myocardial infarction, PTX3 peaked in serum of wild-type animals after ischaemia followed by reperfusion, with a kinetic similar to humans [55,67]. Infarct area in *PTX3*-deficient mice was larger and associated with higher C3 deposition, suggesting that modulation of Complement activation by PTX3 could have a possible protective role in limiting tissue damage. In addition, the interaction between PTX3 and FH, resulting in promotion of FH deposition on PTX3-coated surfaces, could represent a mechanism for preventing tissue damage associated with exaggerated Complement activation [44].

The surprisingly observation that PTX3 binds P-selectin via its N-linked glycosidic moiety [47], prompted us to investigate the role of this interaction in the regulation of leucocyte recruitment during the inflammatory response. We found that PTX3-P-selectin interaction reduced leucocyte recruitment in experimental models of acute lung injury (ALI), pleurisy, mesenteric inflammation and acute kidney injury models [47,68]. Exogenously administered PTX3 and endogenous PTX3 released from hematopoietic cells provided a negative feedback loop that prevented excessive P-selectin-dependent recruitment of neutrophils. This observation suggests that PTX3 produced by activated leukocytes might locally dampen neutrophil recruitment and regulate inflammation. In keeping with this, Liu and colleagues suggested that PTX3 plays a protective role in the pathogenesis of ALI and that lack of PTX3 may enhance neutrophil recruitment, cell death, and inflammatory responses in the LPS instillation-induced ALI [69].

3.5. PTX3 in autoimmunity

PRMs and Complement components opsonize apoptotic cells and contribute to their recognition by phagocytes and are thus protective molecules in the development of autoimmune diseases [70]. In this context the interaction of PTX3 with different Complement components and regulators mentioned above plays a crucial role. It has been described that PTX3 binds to apoptotic cells [71] with a kinetic similar to C1q [72]. The interaction between PTX3 and C1q in the fluid phase prevented the binding of C1q and C3 deposition on apoptotic cells as well as the C1q-mediated phagocytosis of apoptotic cells by DC and phagocytes [26,71–73]. In contrast, when PTX3 is incubated with apoptotic cells, it enhanced the deposition of both C1q and C3 on the cell surface [22]. In late apoptotic neutrophils PTX3 translocates to the plasma membrane and accumulates in blebs, where it acts as an 'eat-me' molecule, promoting rather than inhibiting the clearance of apoptotic neutrophils by phagocytes [74]. In agreement with this, in an *in vivo* model of systemic lupus erythematosus PTX3 fostered the rapid clearance of apoptotic T cells by peritoneal macrophages. This process may keep lupus autoantigens away from dendritic cells and avoid the activation of autoreactive T cells. As a matter of fact, *PTX3*-deficiency on lupus prone genetic background (B6lpr) aggravated autoimmune lung disease (peribronchial and perivascular $CD3^+$ T cell and macrophage infiltrates), which was associated to selective expansion of CD4/CD8 double negative autoreactive T cells [75].

Altogether these results suggest the relevance of the context of PTX3 production. On one hand, elimination of apoptotic cells before loss of their cell-membrane permeability and release of self-antigens and tissue damage signals might be enhanced by cell-bound PTX3 [10,74]. On the other hand, PTX3 production and secretion during inflammation might avoid capture of apoptotic cells in a pro-inflammatory setting that is likely to trigger an immune response against self-antigens [70].

Opsonisation of apoptotic cells by FH limited the Complement-mediated lysis of these cells [76]. Through its binding to FH, PTX3 promoted recruitment of this Complement component to the surface of dying cells, therefore playing a role also as a negative modulator of the alternative pathway of Complement activation in injured tissues [44]. This would also prevent the injury of normal cells mediated by the Complement membrane attack complex.

3.6. Therapeutic role of PTX3

Given the role of PTX3 in host defence against pathogens, interest is growing on a possible application of the protein as therapeutic agent. *Ptx3*−/− mice showed higher susceptibility to infection with microbes that are recognized by the protein, such as *A. fumigatus, P. aeruginosa* and influenza virus [46,60,63]. In a model of invasive pulmonary aspergillosis in *Ptx3*-deficient mice administration of recombinant PTX3 reversed the phenotype [60]. PTX3 administration, in combination with antifungal therapy, also ameliorated respiratory function, reduced lung fungal burden, and increased survival in a rat model of pulmonary aspergillosis [77,78]. PTX3 had a therapeutic activity in chronic lung infections by *P. aeruginosa*, a major cause of morbidity and mortality in cystic fibrosis (CF) patients. Recombinant PTX3 administered to mice infected chronically with *P. aeruginosa* promoted an enhanced clearance of bacteria from the lungs, which was associated with reduced production of local pro-inflammatory cytokines and chemokines, neutrophil recruitment in the airways and histopathological lesions [63]. Furthermore, PTX3 had a protective role in mice infected with IAV strain HKx31 that it recognized *in vitro*, and was ineffective against PR8 strain, which it did not bind *in vitro* [46].

4. Concluding remarks

PTX3 is produced, although with different kinetics, by a number of cell types, in particular neutrophils, mononuclear phagocytes, DCs and epithelial tissues. This long pentraxin is an evolutionary conserved effector molecule of the humoral innate immune system that is endowed with the capacity to modulate Complement activity. Interaction with Complement is shared by the short pentraxins CRP and SAP. Differences between mouse and human in terms of ligand recognition and regulation have prevented rigorous genetic testing of their functions. PTX3 activates the Complement system via the classical and the lectin pathways and can regulate all pathways through the interaction with Complement inhibitors. Moreover, PTX3 has opsonic activity facilitating recognition by phagocytes directly or via Complement activation. It may agglutinate particles by promoting neutralizing activity and safe disposal of different classes of microorganisms. Furthermore, PTX3 has profound effects on both amplification and regulation of inflammation. In this regard, the finding of a regulatory function mediated by the glycosidic moiety is reminiscent of similar activity of immunoglobulins.

All together, these data suggest that despite the genetic and structural differences between pentraxins and immunoglobulins, the general mode of action of these two classes of molecules shares similar properties, including agglutination, Complement activation, opsonisation, and glycosylation-dependent regulation of inflammation.

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