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Three pentraxins C-reactive protein, serum amyloid p component and pentraxin 3 mediate complement activation using Collectin CL-P1

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ABSTRACT

Background: Pentraxins (PTXs) are a superfamily of multifunctional conserved proteins involved in acute-phase responses. Recently, we have shown that collectin placenta 1 (CL-P1) and C-reactive protein (CRP) mediated complement activation and failed to form terminal complement complex (TCC) in normal serum conditions because of complement factor H inhibition.
Methods: We used CL-P1 expressing CHO/ldlA7 cells to study the interaction with PTXs. Soluble type CL-P1 was used in an ELISA assay for the binding, C3 and TCC deposition experiments. Furthermore, we used our previously established CL-P1 expressing HEK293 cells for the C3 fragment and TCC deposition assay.
Results: We demonstrated that CL-P1 also bound serum amyloid p component (SAP) and pentraxin 3 (PTX3) to activate the classical pathway and the alternative pathway using factor B. CRP and PTX3 further amplified complement deposition by properdin. We found that CRP and PTX3 recruit CFH, whereas SAP recruits C4 binding protein on CL-P1 expressing cell surfaces to prevent the formation of TCC in normal serum conditions. In addition, depletion of CFH, C4BP and complement factor I (CFI) failed to prevent TCC formation both in ELISA and cell experiments. Furthermore, soluble complement receptor 1, an inhibitor of all complement pathways prevents PTX induced TCC formation.
Conclusion: Our current study hypothesizes that the interaction of pentraxins with CL-P1 is involved in complement activation.
General significance: CL-P1 might generally inhibit PTX induced complement activation and host damage to protect self-tissues.

1. Introduction

Pentraxins (PTXs) are multimeric proteins forming cyclic structures, distinguished by the presence of a C-terminal 'pentraxin domain' of 200 amino acids and a conserved 'pentraxin signature motif' of an eight amino acid-long sequence (HxCxS/TWxS, where x is any amino acid) [1]. This superfamily of proteins can be further classified into short and long PTXs. The pentraxins' C-reactive protein (CRP) and serum amyloid P-component (SAP) [2,3] are evolutionarily conserved classic short pentraxins and share a high amino acid sequence homology and similar annular disc-like pentameric structure. The CRP and SAP participate variably in the acute phase reactions in different species. For example, CRP is a major acute-phase protein in humans [4], while in mice it is a trace plasma component and only a minor acute-phase protein [5–7]. In contrast, SAP is highly inducible during the acute phase reactions in mice but only slightly (~40 μg/ml) in humans [8].

Pentraxin 3 (PTX3), the prototypic long pentraxin, which contains an additional N-terminal domain, is an octamer composed of two disulphide linked tetramers [9]. In addition to PTX3, other long pentraxins identified in humans are pentraxin 4 (PTX4), neuronal pentraxin 1 (NPTX1), neuronal pentraxin 2 (NPTX2), and a neuronal pentraxin receptor (NPTXR) [1]. The long PTXs share the pentraxin signature

Abbreviations: Pentraxins, PTXs; CRP, C-reactive protein; PTX3, pentraxin 3; SAP, serum amyloid P component; NPTX1, neuronal pentraxin 1; NPTX2, neuronal pentraxin 2; NPTXR, neuronal pentraxin receptor; APR, acute phase reaction; CL-P1, collectin placenta 1; CRD, carbohydrate recognition domain; TCC, terminal complement complex; SR, scavenger receptor; CHO, Chinese hamster ovary; HEK, human embryonic kidney; DMEM, Dulbecco's minimal essential medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; ELISA, enzyme linked immunosorbent assay; CFH, complement factor H; CFH, complement factor H; CFB, complement factor B; CFI, complement factor I.

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sequence and high homologies with short PTX in their carboxypentraxin domain. Unlike classic PTX made in the liver, PTX3 is produced locally, primarily by macrophages, neutrophils, endothelial cells, epithelial cells, and vascular smooth muscle cells [10–14]. PTX3 could increase significantly (~1 μg/ml) during acute phase reactions both in humans and mice [15,16].

The CRP, SAP and PTX3 bind to C1q and activate the classical complement pathway [17,18], resulting in the removal of cell debris [19]. A recent report suggested that CRP activates the classical pathway on nucleated cells without activating the membrane attack complex (MAC) or causing cytolysis [20]. Another report also showed that CRP binds apoptotic cells and protects the cells from assembly of the terminal complement components by recruiting complement factor H (CFH) [21]. A similar study showed that PTX3 binds CFH without interfering with its complement inhibitory function and may contribute to focusing CFH regulatory action, preventing excessive complement activation, and thus has an important function in the control of inflammation in response to tissue injury [22]. Unlike CRP and PTX3, SAP binds C4 binding protein (C4BP) and plays a potential role for the regulation of the classical complement pathway [23].

We recently identified collectin placenta 1 (CL-P1) [24], also known as a scavenger receptor (SCAR4 and SRCL), which is a hybrid protein with the structural traits of both collectins and scavenger receptors (SR). CL-P1 is a type II membrane glycoprotein and is structurally characterized by an N-terminal intracellular domain, a transmembrane region, an α-helical coiled-coil region, a collagen-like region and a C-terminal carbohydrate recognition domain (CRD) [24]. The combination of a C-type CRD and a collagen-like region defines it as a collectin, whereas the extra-cellular projection of an α-helical coiled-coil and a collagen-like region with exposed polycationic residues is a classical trait of a class A SR [25,26]. We found that CL-P1 is an endothelial receptor that can endocytose and phagocytose Gram-negative and -positive bacteria and yeast as well as oxidized low density lipoprotein (OxLDL) in vascular endothelial cells [27]. Initially, lectin-like oxidized LDL receptor (LOX-1) was shown to interact with CRP to mediate some vascular effects. Recently, another study from the same group showed the involvement of LOX-1 in CRP-mediated complement activation through the classical pathway [28,29]. Very recently, we investigated the ability of CL-P1 to interact with CRP and drive the classical complement pathway [30]. Furthermore, CRP recruited CFH to protect the cells from the formation of terminal complement complex (TCC).

Based on existing information, it is logical to speculate that SAP and PTX3, structurally similar to CRP, might also interact with CL-P1. In this study, we examined the interaction of CL-P1 with CRP, SAP and PTX3 as well as their ability in CL-P1 mediated complement activation. We have also examined the role of CFH, C4BP and CFI in CL-P1 and PTX mediated complement regulation in protecting the cells from the formation of TCC.

2. Materials and methods

2.1. Cells and reagents

CHO/ldlA7 cells, which lack functional LDL receptors, were kindly provided by Dr. M. Krieger (MIT). Human embryonic kidney (HEK293) cells were from ATCC. The following reagents were used: native CRP, C1q depleted serum, and anti-rabbit IgG HRP (Merck Millipore); recombinant human pentraxin 2 (SAP), recombinant human pentraxin 3 (PTX3), recombinant human CD35, biotinylated anti-mouse pentraxin 2 (cross reacted with human pentraxin 2), and biotinylated anti-human pentraxin 3 (R&D Systems); purified native C1q, purified human factor H, purified human properdin, purified human factor B, rabbit anti-human C5b-9, goat anti-human factor H, goat anti-human factor B, goat anti-human properdin, properdin depleted serum, factor B depleted serum and factor H depleted serum (Complement Technology); murine anti-human C4BP, purified human C4BP, HAM's F-12, Dulbecco's minimal essential medium (DMEM)-high glucose, fetal bovine serum (FBS), and human complement serum (Sigma-Aldrich); anti-myc monoclonal antibody, Alexa Fluor 555 antibody-labeling kit, EZ-Link sulfo-NHS-LC-LC-biotin, and Alexa Fluor conjugated antibodies (Invitrogen); and rabbit anti-human C3d complement (Dako).

2.2. Cell culture and transfection

CHO/ldlA7 cells were cultured in HAM's F-12 medium supplemented with 5% heat inactivated FBS. HEK293 cells were maintained in DMEM-high glucose supplemented with 10% FBS. Cells were maintained in a humidified incubator at 37 °C and 5% CO_2. 24 h before the transfection, cells were seeded onto poly-L-lysine-coated 35 mm glass based dishes (CHO-ldlA7) or collagen-coated dishes (HEK293) (Iwaki, Japan). Constructs containing *myc*-tag were transiently transfected into cells using Lipofectamine LTX transfection reagent (Invitrogen) in accordance with the manufacturer's instructions. At 24 h after transfection, cells were used for the binding and complement related assay.

2.3. Construction of expression vectors and recombinant CL-P1 expression and purification

A cDNA encoded full length human CL-P1 was introduced into pcDNA3.1/myc-HisA expression vectors as previously described [30]. The extracellular domain of human CL-P1 (59–742) containing an insulin leader peptide and FLAG tag was produced as described earlier. Expi293F™ cells (Invitrogen) were used for transient expression of recombinant CL-P1. The cell line had been adapted to grow in a serum free medium (Expi293™ Expression Medium) (Invitrogen) and transfected using an ExpiFectamine™ 293 transfection reagent. After growth of the cells for 7 days, the culture supernatant was harvested after centrifugation at 3500 rpm for 30 min and stored at 4 °C. Anti-FLAG M2 affinity gel (Sigma) was used to purify the recombinant CL-P1 from the culture supernatant.

2.4. Binding of CRP, SAP and PTX3 to cell surface CL-P1

The binding of Alexa 555-CRP and non-labelled SAP and PTX3 with CL-P1 was performed as previously described with little modification [31]. Briefly, CHO/ldlA7 cells transfected with the indicated cDNAs were incubated with 10 μg/ml Alexa 546-CRP or SAP or PTX3 in ice-cold HAM's F12 medium/10 mM HEPES at 4 °C for 1 h. The cells were fixed with 4% phosphate-buffered formalin (Wako Pure Chemical Industries) for 30 min at room temperature, washed and incubated with anti-*myc* antibody or anti-*myc* antibody combined with biotinylated anti-mouse pentraxin 2 (cross reacted with human pentraxin 2) or biotinylated anti-human pentraxin 3 for 30 min at room temperature. After being washed, the cells were incubated with Alexa 488-anti-mouse IgG or Alexa 488-anti-mouse IgG with streptavidin Alexa 555 conjugate for 30 min at room temperature. The cells were counterstained with Hoechst 33342 (Invitrogen). The cells were photographed under a fluorescent microscope (BZ-9000, Keyence) at 40× magnification.

2.5. Binding of pentraxins to CL-P1 in solid phase ELISA

Assays were conducted in TBSTC (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, 5 mM $CaCl_2$, pH 7.4), using MaxiSorp microtiter plates (Nunc). Proteins (0.1 μg) were immobilized overnight at 4 °C. Remaining binding sites were blocked with BlockAce/PBS (DS Pharma Biomedical) at 37 °C for 1 h. If not stated otherwise, ligands were added for 1 h at 37 °C. Biotin conjugated antibodies were added for 1 h at 37 °C. For competition assay we used 1 μg/ml SAP or PTX3 for binding and 10 μg/ml for inhibition. Elite ABC kits (Vector Laboratories) were used to detect the biotin conjugated antibodies. SureBlue TMB microwell peroxidase substrate (Kirkegaad & Perry Laboratories) was used for the