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The long pentraxin PTX3 is a multifunctional soluble molecule involved in inflammation and innate immunity. As an acute phase protein, PTX3 binds to the classical pathway complement protein C1q, limits tissue damage in inflammatory conditions by regulating apoptotic cell clearance, and plays a major role in the phagocytosis of selected pathogens. This study was designed to investigate the interaction of PTX3 with factor H (FH), the main soluble alternative pathway regulatory protein. We report that PTX3 binds FH with an apparent $K_d$ of $1.1 \times 10^{-7}$ M, and define two binding sites for PTX3 on FH. The primary binding site is located on FH domains 19–20, which interact with the N-terminal domain of PTX3, while a secondary binding site on domain 7 binds the glycosylated PTX3 pentraxin domain. The FH Y402H polymorphism, which affects binding to the short pentraxin CRP, did not affect binding to PTX3. Surface-bound PTX3 enhances FH recruitment and iC3b deposition and PTX3-bound FH retains its activity as a cofactor for factor I-mediated C3b cleavage. Thus, our findings identify PTX3 as a unique FH ligand in that it can bind both of the two hot-spots of FH, namely SCR7 and SCR19–20 and indicate that PTX3 participates in the localization of functionally active FH. The Journal of Immunology, 2008, 181: 8433–8440.

Pentraxins constitute a superfamily of multifunctional molecules characterized by a cyclic multimeric structure (1, 2). The classical short pentraxins, C-reactive protein (CRP) and serum amyloid P component, are produced in the liver in response to inflammatory mediators and play a major role in the innate immune response and in the scavenging of cellular debris and extracellular matrix components (3–5).

The prototypic long pentraxin PTX3 shares similarities with the classical short pentraxins. However, it has an unrelated long N-terminal domain coupled to the C-terminal pentraxin domain, and differs in gene organization, cellular source, inducing stimuli and ligands recognized (2, 6). PTX3 is rapidly produced and released by several cell types in response to primary inflammatory signals (e.g., TLR engagement, TNF-α, IL-1β) (2, 7–11) and its production is amplified by IL-10 (12). PTX3 binds to the complement component C1q, apoptotic cells, the extracellular matrix component TNF-stimulated gene 6 and selected microorganisms, including Aspergillus fumigatus and Pseudomonas aeruginosa (13–19). Recent studies in gene-modified mice have shown that PTX3 plays complex nonredundant functions in vivo, ranging from innate immunity against diverse microorganisms to the assembly of a hyaluronic acid-rich extracellular matrix and female fertility (2, 7, 14, 16, 17, 20–22).

The alternative complement pathway is activated spontaneously and continuously at a low rate in the human plasma (23). Once activated, alternative pathway may enhance its own activation through an amplification loop which can lead to massive deposition of the complement component C3b on target surfaces (24). Under normal circumstances, the activation of the amplification loop on host surfaces is tightly regulated by specific membrane-bound complement inhibitors (CD46 and CD55) and by soluble plasma proteins, factor H (FH) and Factor H-like protein 1 (25–29).

Different studies have investigated the role of pentraxins in the function of the complement system (2, 30–32). When bound to self surfaces (e.g., apoptotic cells, damaged tissue), CRP activates the classical pathway of complement through interaction with C1q, but this activation is restricted to C1, C4, C2, and C3 (33). Furthermore, surface-bound CRP has been shown to decrease the alternative pathway C3-convertase activity and inhibit the alternative pathway amplification loop (34, 35). In part, these inhibitory effects result from a direct binding of FH to surface-bound CRP (36).

Factor H, a 155 kDa plasma protein, is composed of 20 short consensus repeat (SCR) domains each consisting of ~60 amino acids (37). As a regulator of the alternative pathway, FH binds to
PTX3 interacts with complement factor H

C3b and inhibits the binding of factor B to C3b, acts as a cofactor for the factor I-mediated cleavage of C3b to iC3b (cofactor activity), and accelerates the decay of C3bBb, the alternative pathway C3 convertase (decay-accelerating activity) (25–27). Lack of or misdirected FH activity relaxes the control of the alternative pathway and may predispose to potentially hazardous consequences such as membranoproliferative glomerulonephritis type II, age-related macular degeneration (AMD), or atypical hemolytic uremic syndrome (38).

PTX3 has a tuning function under inflammatory conditions in limiting tissue damage by promoting phagocytosis of selected pathogens and in regulating apoptotic cell clearance (2, 19, 20, 39, 40). In addition, PTX3 shares with CRP and serum amyloid P component the capacity to bind C1q (13) and to participate in classical pathway activation (15). However, the role of PTX3 in the activation of the alternative pathway of complement has not been investigated. This study was designed to analyze the possible interplay between PTX3 binding to FH, and FH-dependent C3 convertase inhibition.

Materials and Methods

Complement components and PTX3

Complement factor I, C3b, C1q, and C3d were purchased from Calbiochem. FH constructs SCRs 1–4, 1–6, 1–7, 8–11, 15–20, and 8–20, as well as FHR-4 were cloned and produced in the baculovirus expression system as described previously (41). FH constructs SCRs 5–7, 5–7v, 11–15, 12–14, and 19–20 were produced in *Pichia pastoris* as described (42). Human PTX3, C-terminal, and N-terminal domains were purified under endotoxin-free conditions by immunoaffinity or ionic exchange chromatography and gel filtration from the supernatants of stably transfected CHO cells, as previously described (13, 43). Deglycosylated PTX3 was obtained by PNGase F (from *Chryseobacterium meningosepticum* (Sigma-Aldrich) digestion, as previously described (44). Purity of recombinant proteins was assessed by SDS-PAGE followed by silver staining or Coomassie blue staining. All proteins were at least 95% pure.

Microtiter plate binding assays

Microtiter plates (NUNC) were coated with FH (5 µg/ml), PTX3 (10 µg/ml), or C3b (10 µg/ml) in PBS with Ca\(^{2+}\) and Mg\(^{2+}\) (PBS \(^{++}\); contains 1.17 mM CaCl\(_2\) and 1.05 mM MgCl\(_2\); Lonza) by overnight incubation at 4°C. Nonspecific binding to the plates was blocked with 5% dry milk in PBS \(^{++}\) for 2 h at room temperature. All reaction volumes were 100 µl and plates were washed after each step with PBS \(^{++}\) containing 0.05%Tween 20 (PBS/T). For binding, the ligands were diluted in PBS/T, except for the calcium-dependency study, where the absence of calcium was assured by the use of PBS without divalent cations and the addition of 10 mM EGTA. Single binding experiments and concentrations of ligands used are specified in the respective figure legends.

The bindings were detected by incubation with rabbit anti-hPTX3 polyclonal Ab (pAb) (1/2000), goat anti-hFH pAb (1/5000; Calbiochem), or rabbit anti-C3c mAb (1/5000; DakoCytomation) followed by appropriate
secondary anti-species HRP-conjugated Abs (Jackson ImmunoResearch Laboratories) and subsequent addition of OPD substrate (Sigma-Aldrich). All Abs were diluted in PBS/T. The OD was determined at 492 nm using a Multiscan 340 MCC spectrophotometer (Labsystems). In some experiments TMB substrate (Sigma-Aldrich) was used and OD was determined at 450 nm. Microtiter plate binding assay was also used to calculate the apparent dissociation constant as previously described (13). In brief, serial dilutions of biotinylated PTX3 were incubated on FH-coated wells for 1 h at 37°C. After washing with PBS/T, wells were incubated with HRP-conjugated streptavidin (Biospa) for 1 h at room temperature. The OD results were converted to nanomolar concentration using a standard curve of biotinylated PTX3 and considering a molecular mass of 45 kDa for PTX3 monomer.

$K_d$ and $B_{\text{max}}$ were calculated by nonlinear fitting with GraphPad Prism software.

**Surface plasmon resonance**

Surface plasmon resonance measurements were performed using the Biacore 2000 instrument and analyzed with the BIAevaluation 3.0 software (Biacore AB). FH and C1q were immobilized on carboxymethylated dextran CM5 chips (Biacore AB) by using the amine-coupling procedure according to the manufacturer’s protocol. Binding analyses were performed using 1/2 veronal buffered saline (VBS; 49 mM NaCl, 0.6 mM sodium barbital, 1.1 mM barbituric acid (pH 7.4)) at a flow rate of 5 μl/min, in the presence of 2.5 mM Ca$^{2+}$. As controls, all binding tests were also performed using a blank chip (activated and deactivated without any coupled proteins). After each binding experiment, the surface was regenerated by 30 μl of 3 M NaCl in acetate buffer (pH 4.6) (regeneration buffer).

**Cofactor assay for C3b inactivation**

The effect of soluble or surface-bound PTX3 on the cofactor activity of FH in factor I-mediated cleavage of C3b was tested: 1) by incubating factor I (160 nM), FH (133 nM), and C3b (112 nM) for 30 min at 37°C in the presence or absence of PTX3 (444 nM) in final volume of 50 μl of PBS; 2) by incubating FH (133 nM) for 60 min at 37°C in PTX3-coated or BSA-coated wells, washing, and subsequent incubation for 30 min at 37°C with factor I (160 nM) and C3b (112 nM). Experiments conducted in the absence of factor I or FH were taken as negative controls. After incubation, the samples were heated to 95°C in reducing buffer (containing 2.5% 2-ME) and run in a 10% SDS-PAGE gel. C3b and its cleavage products were detected by Western blotting using a polyclonal anti-C3c Ab (DakoCytomation).

**FIGURE 2.** Mapping of the PTX3–FH interaction sites by using recombinant fragments of FH and recombinant C- and N-terminal domains of PTX3. A, Recombinant FH fragments were incubated with PTX3-coated wells. Results are expressed as the percentage of binding with respect to FH, and represent mean values ± SD from three separate experiments performed in duplicate wells. B–E, FH and fragments containing SCRs 1–7, 15–20, and 19–20 (33 nM) were incubated in PTX3, C-terminal, or N-terminal coated wells. Results are expressed as the percentage of binding with respect to the binding to full-length PTX3, and represent mean values ± SD from three separate experiments performed in duplicate wells. F, FH-coated wells were incubated with enzymatically deglycosylated with PNGase F (PNGaseF) PTX3 (111 nM). Results are expressed as the percentage of binding with respect to the binding of untreated PTX3 and represent mean values ± SD from three separate experiments performed in duplicate wells. Normalized data were compared by one-way ANOVA analysis followed by Dunnett posthoc analysis using GraphPad Prism 4.00.

**FIGURE 3.** The effect of heparin and C3d on the PTX3–FH interaction studied by a microtiter plate binding assay. PTX3 and its N-terminal fragment-coated wells were incubated with 33 nM FH (A) or 33 nM SCR 19–20 (B) in the absence or presence of 0.1–100 μg/ml heparin. C, PTX3-coated wells were incubated with 33 nM FH or 33 nM SCR19–20 alone or in the presence of 16.5–330 nM C3d. The binding of FH and SCR19–20 is shown as percentage of binding without competitors. Results are mean values ± SD from three separate experiments performed in duplicate wells.
FH deposition on apoptotic cells

Apoptosis was induced in Jurkat cells (American Type Culture Collection) by overnight treatment with 5 μM staurosporine (Sigma-Aldrich) in culture medium without FCS (Lonza). After treatment, all cells were annexin V positive, 90% were PI positive, and 70–90% bound PTX3 in flow cytometric analysis. Apoptotic cells were incubated with 2 μM PTX3 in veronal buffered saline containing 1% gelatin (GVB) for 30 min at room temperature. After incubation, unbound PTX3 was washed away and cells were incubated for 20 min at 37°C with 20% normal human serum (NHS) in GVB. In some experiments purified FH was used instead of NHS. After washing, cells were fixed with 1% formaldehyde in PBS, and stained with anti-hFH pAb (1/200 in PBS + 0.5% BSA; Calbiochem), anti-iC3b mAb (45) (1/200 in PBS + 0.5% BSA; Quidel), or anti-C3c pAb (1/200 in PBS + 0.5% BSA; DakoCytomation) followed by secondary Alexa 488-conjugated species-specific Ab (1/500 in PBS + 0.5% BSA; Invitrogen/ Molecular Probes). Negative controls were obtained by omitting the primary Abs. Samples were analyzed using FACS Canto (BD Biosciences) and FACS Diva software (BD Biosciences) or CellQuest software (BD Biosciences). NHS-PTX3 content was 40 pM.

Statistical analysis

Normalized data were compared by one-way ANOVA analysis followed by Dunnett post hoc analysis using GraphPad Prism (version 4.00 for Windows; GraphPad Software). Where indicated, Student’s t test has been used and results were considered significant at p < 0.05 (*), p < 0.01 (**), and p < 0.001 (***)

Results

Binding of PTX3 to FH

The binding of PTX3 to FH was examined by microtiter plate binding assays and by surface plasmon resonance. PTX3, both native and biotinylated, bound to a FH-coated microtiter plate in a dose-dependent manner. Likewise, FH bound dose-dependently to PTX3-coated plates (Fig. 1, A and B). To characterize the binding of PTX3 to FH, serial dilutions of biotinylated PTX3 were added to immobilized FH and the amount of bound PTX3 was evaluated on the basis of a standard curve of the biotinylated protein. The calculated apparent dissociation constant \( K_d \) was 1.1 × 10\(^{-7}\) ± 0.13 × 10\(^{-7}\) M with a \( B_{\text{max}} = 0.66 \) pmol PTX3/pmol FH (Fig. 1C). Surface plasmon resonance was used to confirm the interaction. PTX3 bound to FH-coupled flow-cell (Fig. 1D). As a comparison and a positive control, we analyzed binding of PTX3 to C1q coupled to the chip. PTX3 bound to C1q, in accordance with previous observations (13) (Fig. 1E).

The presence of calcium is required for PTX3 interaction with some of its ligands (18), while no calcium is necessary for an interaction with apoptotic cells (19). The lack of a general calcium requirement in PTX3 interactions prompted us to investigate the calcium dependence of the novel PTX3–FH interaction. As shown in Fig. 1F, microtiter plate binding of FH to PTX3 performed in the presence of 10 mM EGTA reduced significantly the PTX3–FH interaction, whereas C1q binding to PTX3 remained unaltered.

Mapping of PTX3–FH interaction sites

To identify the binding site(s) of PTX3 on FH, we assessed the binding of recombinant FH constructs that span through the whole molecule to PTX3-coated microtiter plate. Recombinant FH fragments consisting of SCRs 1–7, 5–7, 8–20, 15–20, and 19–20 bound to PTX3, while FH fragments encompassing SCRs 1–6, 1–4, 8–11,11–15, and 12–14 exhibited only weak nonspecific binding (Fig. 2A). These results indicate the presence of at least two PTX3 binding sites on FH, one of which is located in SCR7 and the other in SCR19–20. Even though SCR19–20 seems to account for the binding of the SCR15–20, the existence of another interaction site in SCR16–18 cannot be excluded. Interestingly, FH-related protein 4, a member of the FH-related family of proteins, did not bind to PTX3 despite its high homology with SCR19–20, suggesting a high specificity of the PTX3–FH interaction (Fig. 2A).

The common FH polymorphism Y402H (in SCR7) correlates with increased risk and incidence of AMD (46). Because PTX3 interacts with SCR7, we examined whether the Y402H polymorphism influences the PTX3–FH interaction. Recombinant SCR5–7 fragment carrying the variant amino acid 402His (5–7v), displayed no difference in binding to PTX3 when compared with the 402Tyr-containing fragment (Fig. 2A).

We examined the binding of FH and SCRs 19–20, 15–20, and 1–7 to recombinant PTX3 fragments (N terminus and C terminus) in comparison to the binding to the full length PTX3 molecule. FH did not display a clear domain preference because it bound to both N- and C-terminal fragments, albeit less than to the full length PTX3. SCR19–20 and SCR15–20 bound to the N terminus comparably to the full-length molecule but not to the C terminus, while SCR1–7 bound to C terminus and not to the N terminus (Fig. 2, B–E). PTX3 has one glycosylation site in the C-terminal domain. Enzymatic deglycosylation of PTX3 impaired the PTX3–FH interaction (Fig. 2F).

Competition by heparin and C3d

As one of the heparin binding sites and the C3d binding site are located in SCR19–20, we wanted to assess the influence of these FH ligands on the binding of FH and SCR19–20 to PTX3 and its N-terminal domain. In a microtiter plate binding assay, PTX3 or

FIGURE 4. Influence of surface-bound or fluid-phase PTX3 on FH deposition in the presence of C3b. A, PTX3-coated wells were incubated with C3b (6.6 nM) alone or in the presence of FH (0.66, 6.6, or 66 nM), and the binding of C3b was evaluated. B, C3b-coated wells were incubated with FH (6.6 nM) alone or in the presence of PTX3 (0.66, 6.6, or 66 nM), and FH binding was evaluated. The binding of FH is shown as percentage of binding of FH alone. All results are mean values ± SD from three separate experiments performed in duplicate wells. Normalized data were compared by one-way ANOVA analysis followed by Dunnett post hoc analysis using GraphPad Prism 4.00.
N-terminal domain were coated onto the wells and incubated with FH alone or FH preincubated with 0.1, 1, 10, or 100 μg/ml heparin. Similar experiments were performed by preincubating FH with 3.3, 16.5, 33, or 330 nM C3d. All experiments were also done using SCR19–20 instead of full length FH. Heparin inhibited the binding of both FH and SCR19–20 to PTX3 and to its N-terminal fragment in a dose-dependent manner (Fig. 3, A and B). In contrast, C3d exhibited no inhibitory effect on the FH–PTX3 interaction (Fig. 3C).

Functional implications
To analyze the possible functional consequences of FH–PTX3 interaction, we explored C3b deposition on PTX3-coated surface in the presence of FH and analyzed the influence of PTX3 on FH cofactor activity for factor I in C3b inactivation.

PTX3-coated wells were incubated with C3b (6.6 nM) alone or in the presence of 0.66, 6.6, or 66 nM FH. No direct binding of C3b to PTX3 was observed, while C3b bound in the presence of FH (Fig. 4A). Also, the presence of PTX3 in the fluid phase did not influence the binding of FH to surface-bound C3b (Fig. 4B).

We next determined whether FH still exhibited its cofactor activity in the presence of PTX3, both in the fluid phase and when bound to a surface. As demonstrated by the fluid phase cofactor assay (Fig. 5A), PTX3 exhibited no intrinsic cofactor activity itself, and it had no inhibitory effect on the cofactor activity of FH in the fluid phase. Furthermore, when bound to a surface, PTX3 bound and retained FH and allowed it to perform its cofactor function (Fig. 5B).

To assess whether PTX3 can bind FH and contribute to regulation of complement activation in a cell-based setting, we used late-apoptotic Jurkat cells. Late apoptosis was induced in Jurkat cells by staurosporine, and detected by Annexin V and PI staining (Fig. 6A). As observed previously (19), PTX3 bound to apoptotic Jurkat cells (Fig. 6, B and C). The pretreatment of apoptotic Jurkat cells with 2 μM PTX3 resulted in a significant increase in the binding of purified FH (Fig. 6D). Likewise, an incubation of

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** Analysis of the fluid and solid phase cofactor activity of FH in the presence of PTX3. A, Fluid phase cofactor activity: C3b (112 nM) was incubated with or without FH (133 nM), factor I (160 nM), and PTX3 (444 nM). Lane 1 is the positive control with C3b, FH and factor I and without PTX3. Lane 4 represents a negative control. FH was excluded from lane 3 to determine a possible intrinsic cofactor activity of PTX3. B, Solid phase cofactor activity: PTX3-coated wells or control BSA-coated wells were incubated with or without FH (133 nM), washed, and then incubated with factor I (160 nM) and C3b (112 nM). The mobilities of the α- and β-chains are indicated.

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** FH binding and iC3b deposition on apoptotic cells. A, Annexin V–PI staining of apoptotic Jurkat cells indicates late apoptosis. B, Annexin V-positive cells bind PTX3. C, Late apoptotic Jurkat cells bind biotinylated PTX3 (bPTX3) in a dose-dependent manner. Filled gray histogram represents streptavidin control. D, Apoptotic Jurkat cells were preincubated with 2 μM PTX3 before incubation with 20% NHS or purified FH. Cells were stained for FH (FHserum n = 14; FHpurif n = 5). Results are expressed as mean fluorescence intensity (MFI) ± SD of single stained samples. E, Apoptotic Jurkat cells were preincubated with 2 μM PTX3 before incubation with 20% NHS or heat-inactivated (HI) NHS. Cells were stained for iC3b (n = 7; HI NHS iC3b n = 4) or total C3 (n = 4). Results are expressed as mean fluorescence intensity (MFI) ± SD of single stained samples. PTX3-pretreated vs non-PTX3-pretreated data were compared by paired t test using GraphPad Prism 4.00.
apoptotic Jurkat cells with PTX3 before the addition of 20% NHS resulted in a significant increase (28%) in FH binding (Fig. 6D), iC3b deposition (25%), and overall C3 deposition (6%) (Fig. 6E). No increase in iC3b deposition was seen when heat-inactivated NHS was used (Fig. 6E) and no cell lysis was observed in our experimental conditions (data not shown).

Discussion

PTX3 is a multifunctional acute phase protein involved in inflammation, innate immunity, and fertility (2). In addition, the interaction of PTX3 with C1q and its role in the activation of the classical complement pathway have been reported (15, 39, 44, 47), while the role of PTX3 in the alternative pathway has not been investigated. In the present study, we report a novel interaction of PTX3 with factor H, the main soluble regulator of the alternative pathway of complement.

Our results show a direct interaction of FH with PTX3 using a microtiter plate-based assay and surface plasmon resonance. Recombinant FH fragments allowed us to map PTX3 binding sites on FH to SCR7 and to the most C-terminal region SCR19–20. The binding of FH is Ca2+-dependent, with an apparent 

\[ K_{d} = 1.1 \times 10^{-7} \]

M and \[ B_{\max} = 0.66 \text{ pmol PTX3/pmol FH} \], and the binding of both full length FH and SCR19–20 was dose-dependently inhibited by heparin. However, FH–PTX3 and SCR19–20–PTX3 interactions were not inhibited by C3d, suggesting that the PTX3 binding site in SCR19–20 is at least partially overlapping with the heparin binding site (48), while it is distinct from the C3d binding site (49, 50). This would allow a simultaneous interaction of FH with C3d and PTX3.

Structurally, PTX3 is a multimeric molecule that consists of protomers containing the C-terminal pentraxin domain, encoded by exon 3 and homologous to CRP, and an unrelated N-terminal domain, encoded by exon 2 (13). To determine which PTX3 domain mediates the binding with FH, we used recombinant N- and C-terminal PTX3 fragments (13, 43). Our findings indicate that the SCR19–20 interacts with the N-terminal domain of PTX3, while the SCR7 binds the C-terminal domain, consistently with a previous report of CRP interacting with SCR7 (36). Furthermore, the lower level of binding of single PTX3 fragments in comparison to the full-length protein suggests the importance of structural organization of PTX3 into a full-size molecule and/or into polymers (51).

The C-terminal domain of PTX3 contains a single N-glycosylation site on Asn 220 and the glycosidic moiety has been implied in the regulation of interaction of PTX3 with C1q and in complement activation (44). To determine whether the PTX3 glycosidic moiety plays a role in the PTX3–FH interaction, we compared the FH binding of enzymatically deglycosylated PTX3 to that of native PTX3. Surprisingly, unlike for C1q, the deglycosylated PTX3 bound weakly to the full length FH. This observation suggests an important role for glycosylation in the stabilization of the PTX3–FH interaction. Alternatively, the impaired binding of the deglycosylated molecule could be due to the impact of glycosylation on the overall PTX3 conformation and stability or on multimer formation.

As PTX3 is organized into octamers and possibly octamers can associate to form higher m.w. structures (51), it is possible that PTX3 polymers can bind multiple FH molecules. Importantly, the multimeric nature of PTX3 could allow it to interact with more than one ligand at the same time. In addition, as FH has at least two PTX3 binding sites, it is also possible that one FH molecule binds two PTX3 molecules. However, because single PTX3 domains do not fully account for the full-length PTX3 binding and given the multimeric organization of PTX3, it is more likely that a single PTX3 molecule binds to two sites on FH to increase the affinity of interaction.

In the FH molecule, the functionally important domains for cofactor activity and decay acceleration (SCR1–4) and the other two major binding sites for C3b are distinct from the observed PTX3 binding sites (52–54). In accordance, PTX3 did not affect the cofactor activity of FH in the fluid and solid phase functional assays, nor did the C3d part of C3b interfere with FH binding to PTX3. The solid phase cofactor assay suggests that PTX3 can substitute a nonactivating surface e.g., in areas of tissue damage, to suppress inflammation and control direct opsonophagocytosis.

Upon its formation, C3b binds to diverse nearby surfaces, including autologous cells. Alternative pathway nonactivating surfaces are typically coated by polyanions, like sialic acid, heparan sulfate, or other glycosaminoglycans (28). FH may recognize nonactivator surfaces via its heparin binding sites (48, 55, 56). Results presented in this study show that PTX3 shares with heparin its binding site on SCR19–20 and that FH deposited to PTX3-coated surfaces is functionally active.

We observed an increased binding of FH to PTX3-coated apoptotic cells as well as an increase in iC3b deposition on these cells, suggesting a possible physiological role for the PTX3-FH interaction. The plasma level and tissue production of PTX3 are low under normal conditions. Thus, PTX3 probably does not take part in the regulation of steady state apoptotic cell clearance. However, PTX3 is likely to play a significant role in damage control upon tissue injury or in apoptotic cell clearance during inflammation-induced apoptosis. In fact, leukocyte-derived PTX3 (57) together with locally produced PTX3 could create an inhibitory environment at sites of injury and inflammation. Such an environment would contribute to the FH-mediated control of the alternative pathway and formation of iC3b, thus preventing excessive inflammatory responses and regulating the clearance of apoptotic cells and debris. In line with this hypothesis is the recent observation that PTX3-deficient mice display increased tissue damage in a model of myocardial infarction (58), as well as earlier reports of improved survival of PTX3-transgenic mice following exposure to endotoxemia and a protective role of PTX3 in seizure-induced neurodegeneration (20, 40).

Mutations and polymorphisms of FH have been suggested to predispose to different pathological conditions. We compared the binding to PTX3 of recombinant SCR5–7 wild-type (402Tyr) and recombinant SCR5–7 carrying the 402His variant allele that has been associated with the risk of AMD (59). Interestingly, unlike for CRP (42), the polymorphism did not influence FH binding to PTX3. An altered interaction between variant FH and CRP has been proposed as a pathogenetic mechanism in AMD causing decreased removal of drusen deposits and increased inflammation (42). In this case, PTX3 could substitute CRP that is not capable of exerting its full function in individuals carrying the 402His variant.

As another very interesting example of FH abnormalities, mutations in the SCR19–20 region are involved in the pathogenesis of atypical hemolytic uremic syndrome (60). It has been reported that renal mesangial and epithelial cells produce PTX3 (61, 62). It is possible to hypothesize that a mechanism involving a disturbed interaction of locally produced PTX3 with FH could be involved in the pathogenesis of this condition. Studies are underway to determine the effects of C-terminal FH mutations on the FH-PTX3 interaction.

Endothelial cells interact with complement components and the terminal complex activates the proinflammatory and procoagulant properties of vascular endothelium (63, 64). Endothelium is a major source of PTX3 in vitro (8) and in pathological conditions (65, 66). Thus, an interaction with PTX3 may be a
part of the tuning by FH of the interplay between complement and vascular endothelium.

The short pentraxin CRP and the long pentraxin PTX3 belong to the same family, but have considerable differences in the regulation of their expression, cellular sources, ligands recognized, and pathophysiology (2, 6, 13). In this study, we report that, similarly to CRP (36), PTX3 interacts with FH, though with different structural requirements. Given the different regulation and production (liver and systemic for CRP; DC, macrophages, and endothelial cells in tissues for PTX3), CRP and PTX3 are likely to exert their regulatory function on complement activation predominantly in different body compartments and in distinct time frames. Furthermore, our findings identify PTX3 as a unique FH ligand that it can bind both of the two hot-spots of FH, namely SCR7 and SCR19–20.

The results reported in this study show that PTX3 binds FH without interfering with its complement inhibitory function. Therefore PTX3 may contribute to focusing FH regulatory action, prevent excessive complement activation, and thus exert an important function in the control of inflammation in response to tissue injury.

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Disclosures
The authors have no financial conflict of interest.

References

PTX3 INTERACTS WITH COMPLEMENT FACTOR H


