Monomeric C-reactive protein inhibits renal cell-directed complement activation mediated by properdin

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O’Flynn J, van der Pol P, Dixon KO, Prohászka Z, Daha MR, van Kooten C: Monomeric C-reactive protein inhibits renal cell-directed complement activation mediated by properdin. Am J Physiol Renal Physiol 310: F1308–F1316, 2016. First published March 16, 2016; doi:10.1152/ajprenal.00645.2014.—Previous studies have shown that complement activation on renal tubular cells is involved in the induction of interstitial fibrosis and cellular injury. Evidence suggests that the tubular cell damage is initiated by the alternative pathway (AP) of complement with properdin having an instrumental role. Properdin is a positive regulator of the AP, which can bind necrotic cells as well as viable proximal tubular epithelial cells (PTECs), inducing complement activation. Various studies have indicated that in the circulation there is an unidentified inhibitor of properdin. We investigated the ability of C-reactive protein (CRP), both in its monomeric (mCRP) and pentameric (pCRP) form, to inhibit AP activation and injury in vitro on renal tubular cells by fluorescent microscopy, ELISA, and flow cytometry. We demonstrated that preincubation of properdin with normal human serum inhibits properdin binding to viable PTECs. We identified mCRP as a factor able to bind to properdin in solution, thereby inhibiting its binding to PTECs. In contrast, pCRP exhibited no such binding and inhibitory effect. Furthermore, mCRP was able to inhibit properdin-directed C3 and C5b-9 deposition on viable PTECs. The inhibitory ability of mCRP was not unique for viable cells but also demonstrated for binding to necrotic Jurkat cells, a target for properdin binding and complement activation. In summary, mCRP is an inhibitor of properdin in both binding to necrotic cells and viable renal cells, regulating complement activation on the cell surface. We propose that mCRP limits amplification of tissue injury by controlling properdin-directed complement activation by damaged tissue and cells.

alternative pathway; C-reactive protein; kidney; properdin; proximal tubular epithelial cell

Proteinuria is a key diagnostic marker of chronic renal injury (7, 37). Filtered complement components can become activated in the tubular lumen, resulting in tubular injury and interstitial fibrosis (1, 42). Previous studies from our research group have shown that proximal tubular epithelial cells (PTECs) are capable of activating the alternative pathway (AP) of complement on its surface in vitro (4, 8, 9). Furthermore, complement activation has been detected in the apical surface of renal tubules, as was observed for nephritic patients with C3 deposition along the brush border (9). To reenforce these findings that complement plays a role in renal injury, C6-deficient rats were protected from damage in a puromycin-induced nephritic model and in a remnant kidney model (32, 33). In addition, experiments in an in vivo model of proteinuric renal disease demonstrated that targeting complement inhibitory components to the tubules preserved renal function (15). With previous findings demonstrating that AP activation occurred on PTECs, experiments in our laboratory by Gaarkeuken et al. (14) demonstrated an integral role for properdin in mediating AP activation on PTECs, with deposition of properdin observed on the tubular luminal surface of biopsies from patients with proteinuric renal disease. Heparan sulphate on the surface of PTECs acted as a binding site for properdin, binding that can be inhibited by heparinoids (46, 47). In addition, excretion of properdin in urine was associated with complement activation products and worse renal outcome in patients with at least 1 g/day of proteinuria in diabetic nephropathy and glomerular disease cases (40).

Properdin is the only positive regulator in the complement system. It stabilizes short-lived AP C3 convertase (C3bBb) by ~5- to 10-fold (13). In recent years, it has been discovered that the role of properdin role extends beyond its stabiliser activity, as it can also act as a pattern recognition molecule. Properdin can recognize pathogenic stimuli including lipopolysaccharide and bacteria promoting C3 deposition (41). Furthermore, properdin binds to viable mammalian cells such as neutrophils or altered-self including necrotic/apoptotic cells independent of complement activation (20, 45). Previous studies demonstrating the diverse roles of properdin led to a reevaluation of its role in various AP-activated settings (19, 24).

The AP can be activated by the spontaneous hydrolysis of C3 forming C3(H2O). This C3(H2O) associates with factor B in the presence of Mg2+. Factor B attached to C3(H2O) is cleaved by factor D with the resulting C3(H2O)Bb complex (C3 convertase) stabilized by properdin. Stabilized C3 convertase can cleave additional fluid phase C3, allowing for the generation of the amplification loop (13, 17, 19, 30, 35). Recently, another mode of AP activation was identified, termed the properdin-directed model. This mechanism is dependent on the pattern recognition ability of properdin to initially recognize and bind its target surface. Once properdin has bound to a target surface, it can bind C3b followed by factor B and Mg2+, which is cleaved by factor D to form fully stabilized and active AP C3 convertase (19, 41).

Currently, there are limited data available on modes of properdin regulation. It has been demonstrated that human serum can inhibit the ability of properdin to interact with platelets; however, the exact mechanism is still unclear (38). Intriguingly, during an acute phase response, properdin levels decrease in the circulation in contrast to various other serum proteins, such as C-reactive protein (CRP), which show an increase (36). These findings indicate the possibility that a
protein that is increased in serum during an acute phase response may regulate properdin. One such protein often used as a marker of an acute phase response is CRP. Previous studies on CRP have demonstrated its ability to interact with various complement factors including C1q and C4bp (10, 26). In humans, the normal physiological level of CRP is usually <0.5 μg/ml (11). CRP in the circulation can increase up to 500-fold after an acute phase response with its production in hepatocytes regulated by IL-6. CRP is a member of the pentraxin family, consisting of five monomeric (23 kDa) subunits forming a pentameric structure (pCRP) (36, 43). Interestingly, pCRP can dissociate into monomeric CRP (mCRP) on the surface of activated platelets or apoptotic monocytes and T cells. The mechanism of dissociation is dependent on lyso phosphatidylcholine exposure (12).

In the present study, we investigated whether CRP could affect the ability of properdin to bind to PTECs and affect properdin-directed AP activation. Our findings demonstrate that mCRP directly interacts with properdin in solution and inhibits its ability to bind to PTECs or necrotic Jurkat cells, in contrast to pCRP, which had no effect. Furthermore, mCRP inhibited properdin-directed C3 deposition and C5b-9 generation on viable PTECs, demonstrating its novel role in regulating properdin-mediated complement activation on cells and tissue.

MATERIALS AND METHODS

Reagents and cells. Properdin was obtained from Quidel (San Diego, CA), which we have previously shown to be devoid of large artificial aggregates (34). pCRP was obtained from human plasma stored in buffer containing 5 mM calcium chloride (Sigma-Aldrich), and recombinant mCRP [as previously described (5)] was a gift from Larry A. Potempa (Roosevelt University, Chicago, IL). The immortalized renal PTEC human kidney-2 (HK2) cells were a kind gift from Prof. M. Ryan (University College Dublin, Dublin, Ireland) and were cultured in medium containing 1.045 mM calcium chloride, as previously described (14). HK2 cells were cultured in serum-free DMEM-HAM-F12 supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), insulin (5 μg/ml), transferrin (5 μg/ml), and hydrocortisone (36 μg/ml). Jurkat cells were cultured in medium containing calcium nitrate (100 μg/l), as previously described (45). Properdin-deficient human serum was generated in house using zymosan depletion (6). Mouse anti-human factor P#1 was obtained from Quidel. Mouse anti-human C3 (RFK-22) antibody was an in-house reagent (14), and mouse anti-human C5b-9 (AE-11) was a kind gift from T. E. Mollnes (Bodø, Norway). Goat anti-mouse IgG Alexa 647, goat anti-mouse IgG Alexa 488, and propidium iodide (PI) were obtained from Molecular Probes (Leiden, The Netherlands). Rabbit anti-human CRP and digoxigenin (DIG)-labeled rabbit anti-human properdin were in-house reagents. Polyclonal anti-CRP was shown to interact with both mCRP and pCRP. Sheep anti-DIG peroxidase was from Roche (Mannheim, Germany).

Properdin binding to cells. For flow cytometry, HK2 cells were trypsinized and aliquoted into respective wells. Necrotic Jurkat cells were harvested and aliquoted into respective wells. Necrotic Jurkat cells were generated by heat treatment at 56°C for a minimum of 30 min, as previously described (45). Both cell culture mediums used contained calcium as mentioned above. A fixed concentration of human properdin (5 or 10 μg/ml, both within the serum physiological normal range of 4–25 μg/ml) (38) or medium alone was preincubated in the presence or absence of normal human serum (NHS) or pCRP or mCRP for a minimum of 30 min at 4°C. Cells were washed using their respective medium and exposed to their respective preincubated mix for 60 min at 4°C. After properdin incubation, wells were washed twice using flow buffer (PBS, 1% BSA, and 0.02% sodium azide). Cells were then exposed to mouse anti-human factor P#1 (1 μg/ml) specific for human properdin diluted in flow buffer for 30 min at 4°C. After 30 min, cells were washed, and goat anti-mouse Alexa 647 in flow buffer was added for 30 min at 4°C. Cells were washed in flow buffer, and cell viability was assessed using PI added just before measurements in the flow cytometer.

For fluorescent microscopy, HK2 cells were cultured on a 48-well culture plate. Properdin (20 μg/ml) diluted in HK2 cell medium was preincubated in the presence or absence of pCRP (20 μg/ml) or mCRP (20 μg/ml) diluted in HK2 cell medium for a minimum of 30 min at 4°C. The preincubated mix was then added to HK2 cells for 60 min at 4°C. After cells had been washed, mouse anti-human factor P#1 (4 μg/ml) or mouse IgG control (4 μg/ml) was added for 60 min at 4°C. Finally, goat anti-mouse Alexa 488 was added for 45 min at 4°C.

Properdin-CRP sandwich ELISA. Rabbit polyclonal anti-human properdin was coated to a Nunc 96-well plate using carbonate buffer (pH 9.6). Wells were washed using PBS and 0.05% Tween and blocked using PBS and 1% BSA for a minimum of 30 min at 37°C. A fixed concentration of human properdin (0.5 μg/ml) or HK2 cell medium alone (control) was preincubated in the presence or absence of increasing concentrations of pCRP or mCRP for a minimum of 30 min at 4°C (as per the cell-based assay). The plate was washed in PBS and 0.05% Tween, and the preincubated mix was added to its respective well for 60 min at 37°C. After the properdin-CRP step, the plate was washed using PBS and 0.05% Tween. Properdin was detected using rabbit anti-human properdin DIG and anti-DIG peroxidase for 60 min at 37°C, respectively, using PBS Tween 1% BSA as the diluent buffer. The enzymatic reaction was developed using 3,3',5',5'-tetramethylbenzidine (TMB). The plate was washed in PBS and 0.05% Tween 20 between each step. Data are expressed as optical density values at 450 nm.

C3b-CRP sandwich ELISA. In a similar manner to the aforementioned ELISA, a rabbit polyclonal targeting human CRP was coated to a Nunc 96-well plate using carbonate buffer (pH 9.6). Wells were washed using PBS and 0.05% Tween and blocked using PBS and 1% BSA for a minimum of 30 min at 37°C. A fixed concentration of human properdin (3 μg/ml) or HK2 cell medium alone was preincubated with a fixed concentration of mCRP (3 μg/ml) or HK2 cell medium alone (control) for 30 min at 4°C. After properdin-mCRP preincubation, increasing concentrations of trypsinized C3b diluted in HK2 cell medium was added for 30 min at 4°C. The plate was washed in PBS and 0.05% Tween, and the preincubated properdin-mCRP-C3b mix was added to its respective well for 60 min at 37°C. Afterward, the plate was washed using PBS and 0.05% Tween. C3b was detected using mouse anti-human C3-DIG and anti-DIG peroxidase for 60 min at 37°C, respectively, using PTB as the diluent buffer. The enzymatic reaction was developed using TMB. The plate was washed in PBS and 0.05% Tween 20 between each step. Data are expressed as optical density values at 450 nm.

Complement activation assays. For the C3 activation assay, HK2 cells were harvested and aliquoted in HK2 cell medium containing 1.045 mM calcium chloride. A fixed concentration of human properdin (10 μg/ml) or medium alone was preincubated in the presence or absence of pCRP or mCRP for a minimum of 30 min at 4°C. Cells were washed using medium and exposed to their preincubated mix for 60 min at 4°C. After properdin incubation, wells were washed twice using cell medium. A fixed concentration of properdin-deficient serum (5%) diluted in HK2 medium with MgEGTA (10 mM) was added to HK2 cells for a minimum of 30 min at 37°C. After serum incubation, wells were washed twice using flow buffer. C3 deposition was detected using monoclonal antibody specific for C3 (RFK-22) diluted in flow buffer and incubated with cells for 30 min at 4°C. After 30 min, cells were washed twice, and anti-mouse Alexa 647 in flow buffer was added for 30 min at 4°C. Cells were washed in flow buffer, and cell viability was assessed using PI added just before measurements in the flow cytometer.
For the C5b-9 deposition assay, the protocol was similar to the aforementioned C3 activation assay. HK2 cells were washed using their culture medium. A fixed concentration of human properdin (10 μg/ml) or medium alone was preincubated in the presence or absence of pCRP or mCRP for a minimum of 30 min at 4°C. The preincubated properdin-CRP mix was added to its respective cells for a minimum of 60 min at 4°C. After a wash, a fixed concentration of NHS (10%) diluted in HK2 medium with MgEGTA (10mM) was added to cells for 120 min at 37°C. After serum incubation, wells were washed twice using flow buffer. C5b-9 detected using a monoclonal specifically targeting the neoepitope [AE-11 (1 μg/ml)] was diluted in flow buffer and incubated for 30 min at 4°C. After incubation of the primary antibody, cells were washed twice with flow buffer. Goat anti-mouse IgG Alexa 647 diluted in flow buffer was added for 30 min at 4°C. Cells were washed in flow buffer, and cell viability was assessed by PI.

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA) using a two-tailed t-test. P values of ≤0.05 were considered statistically significant.

**RESULTS**

Properdin binding to HK2 cells is inhibited by normal human serum. Recently, we have demonstrated the ability of properdin to bind viable HK2 cells, thereby acting as a focal point for complement activation (14). HK2 cells were incubated with physiological concentrations of properdin (5 μg/ml) and, after the viable cell population was gated (Fig. 1A), showed a strong and homogeneous staining for properdin (Fig. 1B). In line with a previous report (13), binding of properdin could be inhibited in a dose-dependent manner when properdin was preincubated with NHS (Fig. 1, B and C). Properdin binding was significantly inhibited after preincubation with 10% or 20% human serum (Fig. 1D).

**CRP as an inhibitor of properdin binding to viable HK2 cells.** Based on the demonstration that the acute phase protein CRP can interact with various complement proteins (28), we investigated whether CRP could interfere with properdin binding. Initially, properdin was preincubated in the presence or absence of pCRP or mCRP followed by addition to HK2 cells. Fluorescent microscopy showed that properdin deposition on HK2 cells cultured on a plate was inhibited by preincubation with mCRP (Fig. 2A). In contrast, properdin binding appeared unaffected by the addition of similar concentrations of pCRP (Fig. 2A). To further quantify this, we performed flow cytometry on PI-negative viable HK2 cells. The properdin binding could be specifically and significantly inhibited by mCRP but not by pCRP (Fig. 2, B–D). Increasing mCRP concentrations showed a dose-dependent inhibition of properdin binding, whereas pCRP even showed an increased properdin binding at the highest concentrations (Fig. 2E). These data indicate both qualitatively and quantitatively that preincubation with mCRP but not pCRP can inhibit properdin binding to HK2 cells.

**mCRP complexes with human properdin in solution.** Previous studies have demonstrated that CRP, both pCRP and mCRP, can interact with various complement proteins (5, 27, 28). To investigate whether pCRP or mCRP can interact with properdin in solution, we used an ELISA system. Properdin (0.5 μg/ml) was preincubated in the presence or absence of increasing concentrations mCRP or pCRP. This preincubated...
Fig. 2. C-reactive protein (CRP) as an inhibitor of properdin binding to HK2 cells. In A, properdin (20 μg/ml) was preincubated in the presence or absence of monomeric (m)CRP (20 μg/ml) or pentameric (p)CRP (20 μg/ml) for 30 min, which was then added to HK2 cells followed by properdin or control staining. B and C: flow cytometry staining for properdin on viable HK2 cells exposed to preincubation with properdin (5 μg/ml) in the presence or absence of pCRP (15 μg/ml; B) or mCRP (15 μg/ml; C). In B, cells were exposed to medium alone (open region) or properdin preincubated in the presence of pCRP (dashed line) or medium (shaded region) was added. In C, cells were exposed to medium alone (open region) or properdin preincubated in the presence of mCRP (dashed line) or medium (shaded region) was added. The ability of mCRP (15 μg/ml) to inhibit properdin (5 μg/ml) binding is clearly demonstrated in D compared with medium or pCRP (15 μg/ml) added to properdin. Data are means ± SD. mCRP significantly inhibited properdin binding to HK2 cells compared with pCRP. ***P = 0.0003 using a two-tailed t-test. Data are means ± SD of 3 independent experiments. In E, increasing concentrations of mCRP or pCRP were preincubated with properdin (5 μg/ml) followed by the detection of properdin binding to HK2 cells.
mix was added to an ELISA plate coated with polyclonal anti-human CRP antibody followed by detection using a specific polyclonal antibody targeting properdin. The results showed that properdin could complex with mCRP in a dose-dependent manner, whereas this could not be demonstrated with pCRP or the individual components alone (Fig. 3A). Similar to what was described above, a fixed concentration of human properdin (3 μg/ml) was preincubated in the presence or absence of a fixed concentration of mCRP (3 μg/ml) followed by the addition of increasing concentrations of human trypsinized C3b (Fig. 3B). The preincubated mix was added to an ELISA plate that was coated with rabbit anti-human CRP followed by the detection of bound human C3. These data demonstrated that the mCRP-properdin complex is still capable of binding purified C3b (Fig. 3B). In summary, this strongly indicates that mCRP can regulate properdin binding by complexing with properdin in solution. In addition, it could also be hypothesized that mCRP can interfere with properdin-binding sites on HK2. Indeed, we observed that mCRP is able to bind to HK2 cells more efficiently than pCRP (data not shown).

Properdin-directed C3 deposition on viable HK2 cells is inhibited by mCRP. We have previously shown that properdin can bind HK2 cells, inducing C3 deposition on the cell surface (14). To elucidate the effect of CRP on this properdin-directed C3 deposition, we preincubated properdin with or without increasing concentrations of mCRP or pCRP and exposed it to HK2 cells. Cells were washed, and a fixed concentration of properdin-deficient serum was added followed by the detection of C3 deposited using a monoclonal antibody. Properdin clearly directed C3 activation on the surface of viable HK2 cells compared with the condition exposed to serum alone (Fig. 4A). pCRP was incapable of inhibiting properdin-directed C3 deposition (Fig. 4A), whereas mCRP clearly inhibited complement activation (Fig. 4B). Increasing concentrations of mCRP strongly inhibited C3 deposition, almost returning to levels of serum alone, whereas pCRP did not affect C3 deposition (Fig. 4C). This indicates that mCRP can modulate properdin-directed C3 deposition on viable HK2 cells.

mCRP inhibits properdin-directed C5b-9 generation on viable HK2 cells. A previous study (40) in patients with kidney disease demonstrated the presence of properdin in their urine, which was associated with higher levels of urinary soluble C5b-9 and worse renal function. Properdin bound to HK2 cells can induce the generation of C5b-9 on its surface, as previously shown (14). We investigated whether the ability of mCRP to inhibit properdin-directed C3 deposition would effect C5b-9 generation. The experiment was performed as similar to that described above and gated on PI-negative HK2 cells for analysis. We observed that the addition of properdin alone before the serum step (shaded region) induced C5b-9 generation on viable HK2 cells compared with the serum alone condition (open region), with no significant effect observed with pCRP (dashed line; Fig. 5A). mCRP under similar conditions inhibited properdin-directed C5b-9 deposition, as observed in the overlay (Fig. 5B). This inhibition was observed with increasing concentrations of mCRP with the presence of pCRP unaffected C5b-9 generation on viable HK2 cells (Fig. 5C). In short, mCRP can inhibit properdin-directed AP C3 deposition and C5b-9 generation.

Properdin binding to necrotic Jurkat cells is impaired by mCRP. Properdin has been shown to act as a pattern recognition molecule and can bind altered-self such as necrotic cells (45). To investigate whether our findings with mCRP acting as an inhibitor of properdin binding was unique for viable HK2 cells or more generally applicable, we investigated whether it would control properdin binding to necrotic Jurkat cells. Jurkat cells were made necrotic by treating them at 56°C, resulting in a homogenous population of PI-positive cells (Fig. 6A). The strong binding of properdin to necrotic cells could not be inhibited by pCRP (Fig. 6B) but was strongly inhibited after preincubation of properdin with mCRP (Fig. 6C). This significant inhibition was specific and dose dependent with maximal inhibition obtained with 10 μg/ml mCRP (Fig. 6, D and E). In summary, mCRP can inhibit properdin binding to viable HK2 cells and necrotic Jurkat cells. Similar results were obtained with necrotic HK2 cells (data not shown).
DISCUSSION

Our data demonstrate that mCRP can bind properdin in solution and inhibit properdin binding on PTECs and hence properdin-directed AP activation. The ability of mCRP to inhibit properdin was not unique to PTECs, as properdin binding was also inhibited with necrotic Jurkat cells. Since properdin bound to mCRP is still capable of binding C3b, this indicates that mCRP notably targets the pattern recognition ability of properdin, independent of complement activation. The amounts of properdin used for experiments were within the normal physiological levels of properdin in the circulation, which are \( \sim 4 - 25 \) \( \mu \)g/ml (22, 38). The level of 3.3 \( \mu \)g/ml mCRP induced significant inhibition of properdin binding. One could hypothesize that this may be a mechanism by which properdin decreases in the circulation during an acute phase response (36). Recent findings have shown that properdin-induced complement deposition on PTECs can suppress cell viability (31). Additionally, mCRP counteracts properdin-mediated complement activation and complement-dependent injury of host cells and tissue. This would suggest that mCRP would limit the inflammatory potential of properdin-mediated tissue injury. We have focused on the control of initiation of properdin-directed complement activation on the cell surface. However, the downstream effects of activation, including sub-

Fig. 4. Properdin-directed C3 deposition on HK2 cells is inhibited by mCRP. A and B: flow cytometry staining for C3 deposition on viable HK2 cells exposed to preincubation with properdin (10 \( \mu \)g/ml) in the presence or absence of pCRP (5 \( \mu \)g/ml; A) or mCRP (5 \( \mu \)g/ml; B) followed by the addition of 5% properdin-deficient serum as a source of complement. In A, cells were exposed to medium alone (open region) or properdin preincubated in the presence of pCRP (dashed line) or medium (shaded region) was added. In B, cells were exposed to medium alone (open region) or properdin preincubated in the presence of mCRP (dashed line) or medium (shaded region) was added. In C, C3 deposition was quantified after increasing amounts of mCRP or pCRP were preincubated with properdin (10 \( \mu \)g/ml). The dashed line shows medium alone. Data shown are a representative experiment of 3 independent experiments.

Fig. 5. mCRP inhibits properdin-directed C5b-9 generation on viable HK2 cells. A and B: flow cytometry staining for C5b-9 deposition on viable HK2 cells exposed to preincubation with properdin (10 \( \mu \)g/ml) in the presence or absence of pCRP (10 \( \mu \)g/ml; A) or mCRP (10 \( \mu \)g/ml; B) followed by the addition of 10% NHS as a source of complement. In A, cells were exposed to medium alone (open region) or properdin preincubated in the presence of pCRP (dashed line) or medium (shaded region) was added. In B, cells were exposed to medium alone (open region) or properdin preincubated in the presence of mCRP (dashed line) or medium (shaded region) was added. C: representative results of three C5b-9 experiments, whereby C5b-9 deposition was quantified when increasing amounts of mCRP or pCRP were preincubated with properdin (10 \( \mu \)g/ml). The dashed line shows medium alone. Afterward, all were treated with 10% NHS.
lytic concentrations of C5b-9 and generation of anaphylotoxins such as C3a and C5a on the local tissue or on surrounding cells expressing receptors for C3a and C5a, would be an important area of future investigations.

Previous in vitro studies have demonstrated that complement activation occurs on PTECs, which is mediated by the AP (4, 8, 9). This mechanism is believed to be involved in proteinuria-induced renal injury (23). In particular, the exposure of the apical membrane of PTECs to atypically filtered complement components can result in the activation of complement and may be due to reduced expression of regulators on the surface; however, this requires further investigation due to conflicting observations (18, 25). Properdin can bind to PTECs by interactions with cellular heparan sulphates, acting as a platform for complement activation inducing C3 and C5b-9 deposition (14, 46). Interestingly, in patients with diabetic chronic kidney disease, biopsy analyses revealed tubular mCRP staining in the kidney, which is the same location observed for properdin staining in biopsies of proteinuric patients (14, 39). These data are suggestive that mCRP may be allowed to interact with properdin; however, it could also possibly bind factor H (5, 28), a negative regulator of complement activation, which has also been shown to bind PTECs (47). This would be of interest to study in kidney disease patients with proteinuria, staining for the colocalization of factor H or properdin with mCRP to see how it relates to tubular injury.

Traditionally, native CRP (pCRP) is associated with its ability to induce classical pathway activation by binding of a single C1q to two CRP molecules (10). Interestingly, pCRP activates early classical pathway components (C4 and C2) but has limited terminal pathway activity (3). The ability of pCRP to dissociate into mCRP may explain the pleiotropic range of capabilities of CRP. pCRP can undergo dissociation to mCRP by means of chemical treatment or by a new biological mechanism involving lysophosphatidylcholine, as observed on activated platelets or apoptotic monocytes and T cells (12). The conversion of pCRP to mCRP results in the generation of neoepitopes with a range of new biological functions (5).

However, the two forms of CRP differ functionally as much as structurally. In contrast to pCRP, mCRP promotes neutrophil

Fig. 6. Properdin binding to necrotic Jurkat cells is impaired by mCRP. A–C: flow cytometry staining for properdin binding on PI-positive Jurkat cells (A) exposed to preincubation with properdin (10 μg/ml) in the presence or absence of pCRP (10 μg/ml; B) or mCRP (10 μg/ml; C). In B, cells were exposed to medium alone (open region) or properdin preincubated in the presence of pCRP (dashed line) or medium (shaded region) was added. In C, cells were exposed to medium alone (open region) or properdin preincubated in the presence of mCRP (dashed line) or medium (shaded region) was added. In D, properdin binding was quantified when mCRP or pCRP (10 μg/ml) was added to properdin (10 μg/ml). The dashed line shows medium alone. Data are means ± SD. mCRP significantly inhibited properdin binding to necrotic Jurkat cells compared with pCRP. **P = 0.0022 using a two-tailed t-test. Data are means ± SD of 3 independent experiments. In E, increasing concentrations of mCRP or pCRP were preincubated with properdin (10 μg/ml) diluted in Jurkat cell medium followed by addition to necrotic cells. The dashed line shows medium alone. Data are means ± SD of duplicate measurements.
survival due to its antiapoptotic activity and binds CD16 on human neutrophils (16, 21). In thrombus development, pCRP appears to have no role, whereas mCRP enhances platelet deposition (29). For endothelial progenitor cells, mCRP induced a proinflammatory response in particular in interferon-responsive genes, with pCRP exhibiting no such response (2).

With regard to complement, complement factor H-related protein 4 binds pCRP but not mCRP (27). Furthermore, mCRP has been shown to interact with other complement proteins, including C4bp, FH, and HFH-1 protein, indicating its diverse role in the complement system (5, 26, 28). The ability of mCRP to interact with FH and promote C3 inactivation is intriguing as both properdin and factor H bind heparin sulphonates on PTECs (28, 47). This may suggest a means by which mCRP can aid in regulating AP activation on PTECs, by interacting with FH and promoting C3 inactivation and inhibiting properdin-directed complement activation. The unique capability of a fragment of CRP in solution to inhibit properdin and downstream AP activation compared with its multicomplex pentameric structure demonstrates the pleiotropic capabilities of this molecule. In particular, one could speculate that a possible mechanism by which CRP produces limited terminal pathway activation is due to regulation of the AP at both the FH-C3 level and controlling of properdin-directed complement activation.

These findings would be of particular interest in the clearance of apoptotic/necrotic cells whereby properdin has been shown to bind and pCRP has been shown to dissociate to mCRP by means of an apoptotic cell surface. Furthermore, the properdin-mCRP complex may have a role in the neutrophil arm, such as antineutrophil cytoplasm antibody vasculitides. The ability of mCRP to control properdin-directed complement activation is of particular interest for future studies in proteinuric renal disease and in the clearance of apoptotic material.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


