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Properdin: New roles in pattern recognition and target clearance

Claudia Kemper[‡] and Dennis E. Hourcade^{*}

[‡]MRC Centre for Transplantation, King's College London, UK

^{*}Department of Medicine, Division of Rheumatology, Washington University School of Medicine, St. Louis, MO, USA

Abstract

Properdin was first described over 50 years ago by Louis Pillemer and his collaborators as a vital component of an antibody-independent complement activation pathway. In the 1970's properdin was shown to be a stabilizing component of the alternative pathway convertases, the central enzymes of the complement cascade. Recently we have reported that properdin can also bind to target cells and microbes, provide a platform for convertase assembly and function, and promote target phagocytosis. Evidence is emerging that suggests that properdin interacts with a network of target ligands, phagocyte receptors, and serum regulators. Here we review the new findings and their possible implications.

1. Introduction

When a pathogen breaches the host's skin/epithelial cell barriers, it faces the immune systems first-line of defense against infections: complement. The complement system, consisting of over 30 proteins that either circulate in the blood/plasma or are cell surface anchored, can recognize microbial pathogens, remove immune complexes, and participate in the selection of the antibody repertoire (Dempsey et al., 1996; Volanakis, 1998). Activation of complement on a target surface leads to target opsonization and clearance and/or lysis, and the initiation of local inflammatory reactions. Complement activation is mediated by three major pathways (Volanakis, 1998). Each pathway results in the assembly of the C3 convertases, the central enzymes of the complement cascade, which cleave the fluid phase protein C3 into the opsonin C3b, the major effector molecule of complement.

Each complement activation pathway responds to a different set of activators, ensuring that a wide range of dangerous agents are recognized: The classical pathway is triggered by antibody:antigen complexes, the lectin pathway responds to specific sugar moieties, and the alternative pathway is activated by a range of microbial surfaces. Recent investigations suggest that complement activation can occur via an additional pathway that utilizes the same components as the alternative pathway but is initiated on specific targets by the protein properdin (Hourcade, 2006; Kemper et al., 2008; Spitzer et al., 2007). The properdin-directed pathway is similar in principle to one proposed by Louis Pillemer and his collaborators over 50 years ago (Pillemer, 1954) and it may serve in the identification and clearance of pathogens, apoptotic cells, and malignant cells. This review summarizes the evidence for the properdin-directed pathway and its potential implications.

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2. Properdin and the alternative pathway

Complement was first described more than 100 years ago, when it was shown that certain microbial targets could be lysed by a mixture of antibody “complemented” by a heat-sensitive component of human serum (Whaley, 1985). This led to the discovery (elucidation) of the classical pathway. Evidence for the alternative pathway first emerged in 1954 when Louis Pillemer and his collaborators reported the partial purification of the plasma protein properdin and its apparent capacity to activate the complement system on a range of targets without antibody (Pillemer, 1954). While the “properdin system” attracted a great deal of interest as it represented the first example of “natural immunity” (Ratnoff, 1980), it also raised formidable objections, most notably by Robert Nelson, who provided strong evidence that Pillemer’s properdin preparations were likely contaminated by antibodies (Nelson, 1958). This raised the possibility that the observed complement activation that Pillemer attributed to properdin could have been due instead to antibody:antigen driven reactions. With Pillemer’s untimely death (Ecker, 1958), properdin lost its greatest proponent and was largely dismissed by the scientific community (Lepow, 1980).

Interest in properdin was renewed in the 70’s: Several reports indicated the likely existence of an antibody-independent complement activation pathway (Lepow, 1980), and new protein purification methods made it possible to reconstruct complement activation from purified components. Key contributions from several groups led to a specific model for the assembly of the alternative pathway C3 convertase (Fearon, 1979; Pangburn and Muller-Eberhard, 1984) (Fig. 1A): Nascent C3b, produced by constant turnover (“tickover”) of fluid phase C3, binds covalently to potential targets and is subsequently bound by factor B (fB), a zymogen that carries an inactive serine protease domain. The C3bB complex is cleaved in the presence of Mg^{++} at a single site in the fB subunit by factor D, releasing the factor B amino-terminal fragment (Ba), and activating the serine protease domain in the remaining C3bBb complex. The C3bBb complex ($T_{1/2} \sim 90$ sec (Medicus et al., 1976; Pangburn and Muller-Eberhard, 1986)) can be recognized by properdin and is stabilized 5–10 fold upon association with properdin (Fearon and Austen, 1975). Dissociation of convertases leads to the irreversible deactivation of the catalytic site. Regulatory proteins that inhibit convertase assembly and promote convertase dissociation serve to protect host cells from complement-mediated damage. Thus, the alternative pathway of complement activation was established and the work of Pillemer was considered vindicated (Lepow, 1980), although properdin did not play as prominent role in the new model as originally envisioned (Pillemer, 1954).

Properdin is a highly positively charged ($pI > 9.5$) protein (Fearon and Austen, 1975) composed of identical 53 kDa subunits (Nolan et al., 1991), 26 nm in length and 2.5 nm in diameter (Smith et al., 1984). Each subunit is composed of 6 globular domains that are homologous to the thrombospondin type 1 repeat. Each subunit harbors a single glycosylation site (Nolan et al., 1991) and is C-mannosylated at ~ 14 tryptophan residues (Hartmann and Hofsteenge, 2000), making properdin one of the most highly mannosylated proteins known. Properdin subunits associate head-to-tail and form dimers, trimers and tetramers that resemble rods, triangles and squares, respectively (Smith et al., 1984). Properdin is a C3b-binding protein that protects C3b from the catalytic cleavage by the complement regulators, factor H and factor I (Medicus et al., 1976), and promotes and stabilizes C3bB (DiScipio, 1981; Hourcade, 2006) as well as C3bBb (above). Properdin appears to function optimally on surfaces, with its higher oligomers possessing the greatest activity (Smith et al., 1984). Farries *et al.* accounted for these observations in their proposal that a single properdin protein binds to multiple pre-formed surface-bound ligands (C3b, C3bB, and C3bBb) (Farries et al., 1988).

3. Evidence for a properdin-directed complement activation pathway

Most biological activities of complement require the cleavage of the C3 protein by the C3 convertases (Volanakis, 1998). Thus, for several years, our laboratory has aimed to understand the assembly and regulation of these important enzymes. Our recent studies have provided new insights into properdin function and suggest that it has biological activities beyond that of convertase stabilization. We observed that properdin, covalently attached to a biosensor surface and treated with purified complement proteins in the presence of Mg^{++} , provides a platform for the step-wise assembly of C3bBbP (Hourcade, 2006): Properdin bound to the biosensor surface associated with C3b, the C3bP complex then bound factor B, and C3bBP was cleaved by factor D, releasing Ba and generating surface-bound C3bBbP (Fig. 1B). This reaction leads to a convertase similar to that generated by the standard AP model, but one that is initiated by properdin rather than nascent C3b (compare Fig. 1A and 1B). C3b:target association is relatively non-specific since it is directed by a highly reactive but non-discriminating thioester that can form covalent bonds with target amino and hydroxyl groups (Janssen and Gros, 2007; Law and Levine, 1977). Thus, C3b binds not only to microbes but also to host cells, and further (unwanted) complement activation on host tissues is prevented by complement regulatory proteins (Fearon, 1979). In contrast, it seemed possible that properdin could direct complement activation more selectively by recognizing and binding to specific target surfaces only. We undertook further investigations to explore this possibility.

Because properdin-deficient patients are sensitive to meningococcal disease (Densen, 1989) (see below), a biological target of properdin would likely include *Neisseria* bacteria. Indeed, purified properdin readily binds the *Neisseria gonorrhoeae* surface (Spitzer et al., 2007). In addition, properdin bound to the bacterial surface (Ng-P) promotes the subsequent binding of C3b (Ng-PC3b), and treatment of Ng-PC3b with factor B and factor D leads to the generation of Ng-PC3bBbP. Thus, properdin recognizes and binds *Neisseria gonorrhoeae* and the resulting Ng:properdin complex provides a platform for the step-wise assembly of C3bBbP comparable to that shown for the properdin-coated biosensor surface (Fig. 2A–C). Moreover, Ng:properdin complexes incubated with human serum promoted the deposition of C3 fragments on the bacterial surface (Spitzer et al., 2007) (Fig. 2D). Properdin also binds to zymosan (a yeast cell wall preparation) and rabbit erythrocytes and induces C3bBbP assembly on these surfaces in a similar fashion (Spitzer et al., 2007).

4. Properdin binds to apoptotic T cells and promotes complement activation and phagocytosis

The new findings provided evidence that properdin can recognize a variety of non-self antigens/structures and function as an initiation point for the assembly of the alternative complement pathway on potentially dangerous targets. This working hypothesis became the basis for a new question: Can properdin recognize dangerous self antigens/structures? Several investigations have implicated complement in the recognition and removal of apoptotic T cells (Nauta et al., 2003; Roos et al., 2004). The membrane changes occurring on apoptotic T cells initiate the deposition of C1q and/or MBL leading ultimately to their opsonization with C3 activation fragments. These C3 fragments are in turn recognized by the complement receptors CR3 (CD11b), CR4 (CD11c) and CRIg, expressed by phagocytes and scavenger cells and this interaction mediates the fast uptake and clearance of dying cells. (Helmy et al., 2006; Mevorach et al., 1998; Roos et al., 2004; Wright and Silverstein, 1983). Thus, according to the current paradigm, recognition of apoptotic T cells is initiated through either the CP or LP, which then triggers AP activity.

We initiated studies to determine whether properdin might also direct the recognition and clearance of apoptotic T cells (Kemper et al., 2008). We found that properdin bound early

apoptotic T cells but neither resting T cells or activated (live) T cells. Moreover, surface-bound properdin directed deposition of C3b activation fragments on apoptotic T cells under conditions that excluded CP or LP participation. In addition, we demonstrated in this system that properdin deposition on a target cells translates into functional biological consequences: Properdin bound to apoptotic cells promoted their phagocytic uptake by macrophages and dendritic cells (Fig. 3). Although maximum uptake was dependent on complement activation, properdin bound to apoptotic T cells promoted their uptake in the absence of complement activation or other serum proteins. These results, together with the observation that macrophages and DCs can bind properdin, strongly suggest that properdin:target complexes can direct phagocytosis without further complement involvement. We proposed two different mechanisms by which properdin could promote phagocytosis of apoptotic T cells (Kemper et al., 2008): In one case, properdin binds to apoptotic T cells, and initiates *in situ* complement activation. The surface-bound C3b and iC3b that is generated then mediates contact with phagocytes via the phagocyte receptors CR3 and CR1g. It is noteworthy that in this case, complement activation proceeds even in the presence of endogenous complement regulators. This is possibly due to the down-regulation, shedding or redistribution of complement regulatory molecules observed on apoptotic cells (Elward et al., 2005; Jones and Morgan, 1995). In the second case, properdin binds to apoptotic T cells and mediates contact with phagocytes directly. In this scenario properdin functions as a bridging protein that can promote phagocytosis in the absence of further complement activation. Although studies of the interaction of properdin with apoptotic cells have been limited to T cells, it is possible that properdin plays a role in the recognition and clearance of other apoptotic cell types.

5. Properdin binds the GAG chains on surface proteoglycans

How does properdin recognize target surfaces? Glycosaminoglycans (GAGs) are linear polysaccharides composed of repeating disaccharide units that are variously modified with sulfate groups (Esko and Selleck, 2002). Proteoglycans are protein:GAG conjugates that are synthesized by and present on most mammalian cells. Proteoglycans play major roles in cell signaling and morphogenesis through their specific GAG structures. A previous study showed that properdin binds heparin, a major GAG (Yu et al., 2005). Thus, we initiated studies to examine whether properdin attaches to target cells via surface GAGs. Wild-type and GAG-defective type Chinese Hamster Ovary (CHO) cells have been used to study the effects of proteoglycan GAG structure on a wide variety of cell properties (Zhang et al., 2006). Properdin binds to the wild type CHO cells, and a comparison to mutant CHO lines demonstrated that properdin binds wild type CHO cells via heparin sulfate and chondroitin sulfate proteoglycan chains, and that binding requires sulfate moieties (Kemper et al., 2008). Interestingly, properdin recognizes apoptotic T cells via a similar mechanism: sulfated GAGs on the T cells were a prerequisite for their interaction with properdin, and soluble GAGs, especially chondroitin sulfate C and E, inhibited properdin recognition of apoptotic T cells (Kemper et al., 2008). These observations provided strong evidence that sulfated GAGs can mediate properdin:cell recognition although additional interactions may be involved.

Properdin is composed of 6 thrombospondin type 1 repeats (TSRs) (Nolan et al., 1991). Thrombospondin repeats in thrombospondin, thrombospondin related adhesive protein (TRAP) of *Plasmodium falciparum*, and other proteins bind GAGs (Panetti et al., 1999; Tossavainen et al., 2006). While the detailed 3D structure of properdin has not yet been resolved, structures of other TSRs have been attained. In the case of TSR 2 and 3 of thrombospondin, a protein surface containing disulfide-stabilized alternating arginine and tryptophan side chains has been proposed as a GAG-binding face (Tan et al., 2002; Tossavainen et al., 2006). In particular, the distance between the positively charged arginines (~9 Angstroms) matches closely the length of a GAG disaccharide unit, and so the interaction between TSR and sulfated GAG could be determined in part by electrostatic interactions

between the TSR arginine side chains and adjacent GAG sulfates (Fig. 4). It is tempting to speculate that similar structures could mediate properdin:GAG interactions since the critical arginines and tryptophans are conserved in 5 of 6 properdin TSRs (Nolan et al., 1991).

While properdin:GAG interactions may account for properdin recognition of mammalian cells, there are no GAGs on *Neisseria* or the enteric bacteria used in our studies. Thus, properdin must recognize other biochemical targets on those microbial surfaces. The enteric Gram negative bacteria are surrounded by lipopolysaccharide (LPS), consisting of an inner lipid A anchor followed by inner and outer core oligosaccharides and terminating in the O-antigen, a highly repeated pentasaccharide (Heinrichs et al., 1998). The corresponding *Neisseria* surface molecule lacks the O-antigen and is referred to as lipooligosaccharide (LOS) (Griffiss et al., 1988). Kimura *et al.* (Kimura et al., 2007) have shown that properdin can bind microtiter wells pretreated with bacterial LPS or LOS. While properdin does not bind wild type *E. coli* K12 or *S. typhimurium* (Spitzer et al., 2007), the observations of Kimura *et al.* could simply indicate that properdin binds the LOS on the *Neisseria* surface. However, properdin binds *E. coli* and *S. typhimurium* LPS mutants missing the O-antigen, the outer core, and most of the inner core oligosaccharides (Spitzer et al., 2007). Those results do not rule out the possibility that properdin binds to portions of the LOS structure that is proximal to the bacterial membrane, but they do raise the possibility that properdin might bind *Neisseria* via surface ligands that have yet to be identified.

6. Target-binding activity of serum properdin versus properdin released from neutrophils

Unlike most complement components, which are made in the liver, the biosynthesis of properdin occurs in monocytes/macrophages, T cells and neutrophils (Schwaebler et al., 1993; Schwaebler et al., 1994; Wirthmueller et al., 1997). In the case of neutrophils, properdin is stored in granules and rapidly released upon cell stimulation (Wirthmueller et al., 1997). A recent study also suggests mast cells as a properdin source (Stover et al., 2008). Given that properdin is found at relatively low concentration in plasma (4–6 µg/ml, (Nolan and Reid, 1993)) compared with the other AP C3 convertase components (C3, 1000–1500 µg/ml, (Kohler and Muller-Eberhard, 1967)) and factor B, 74–286 µg/ml, (Oglesby et al., 1988)), changes in its local concentration could significantly impact local levels of AP activation, and for that reason it has been proposed that the release of properdin from neutrophils is potentially a major determinant of local AP activity (Schwaebler and Reid, 1999).

To address whether neutrophils could indeed play a role in the recognition of apoptotic T cells as a source of properdin, we examined the capacity of properdin released from activated neutrophils to bind apoptotic T cells (Kemper et al., 2008). We found that incubation of apoptotic T cells with freshly activated neutrophils led to deposition of properdin on the T cell surface. Importantly, properdin-binding was independent of complement activation since concomitant C3b deposition was not observed. Only minimal amounts of properdin were deposited on non-apoptotic T cells incubated with activated neutrophils or when apoptotic T cells were incubated with non-activated neutrophils. These results indicate that properdin freshly expelled by degranulating neutrophils strongly binds apoptotic T cells (Fig. 5). The recent discovery of mast cells as a potential local source of properdin (Stover et al., 2008) warrants further investigation of a possible role for these cells in the recognition or ‘tagging’ of potentially dangerous agents.

While apoptotic T cells readily bind purified properdin derived from serum as well as properdin freshly released from neutrophils, they unexpectedly bind poorly to properdin present in unfractionated serum (Kemper et al., 2008). Similarly, certain Gram negative bacteria bind purified properdin but not the properdin present in serum (Spitzer et al., 2007). These

observations suggest serum-level inhibition of properdin:target recognition. Thus, it is possible that properdin-dependent recognition of apoptotic cells/targets is mediated largely by neutrophil-derived properdin, while the properdin in the plasma functions primarily to stabilize C3 convertases. Properdin discharged from neutrophils at sites of infection would either direct complement activation appropriately to target surfaces or would diffuse in the plasma and be inactivated by regulatory proteins. Alternatively, properdin may compete for surface sites with other serum proteins and properdin-binding may prevail when local properdin concentration is relatively high.

Apoptosis plays a critical role in shaping and controlling the immune response: T cell populations that have expanded in response to specific antigens and have performed their effector functions finally undergo apoptosis, thereby limiting their physiological impact (Kerr et al., 1972; Savill et al., 2002). Thus, effector T cells, expanded through antigen presentation, simultaneously create a local and transient milieu that supports their rapid tagging for disposal once apoptosis commences: Activated T cells secrete the chemokine IL-8 (Wechsler et al., 1994), and the anaphylatoxin C5a is generated in high amounts during complement activation (Volanakis, 1998; Walport, 2001a; Walport, 2001b). Both factors are known to induce and attract neutrophil migration (Wagner and Roth, 2000). Activated T cells also produce TNF- α and GM-CSF (Daser et al., 1995), factors that can induce the release of properdin from neutrophils (Wirthmueller et al., 1997). Thus, we propose that activated T cells attract and activate neutrophils at sites of inflammation. Activated neutrophils then release properdin, which tags apoptotic cells for the safe removal by phagocytes.

A similar scenario would also hold for infections with *Neisseria* or other pathogens that might interact directly with properdin: Invading pathogens generally trigger a number of danger signal cascades including the toll-like receptor and defensin systems (Medzhitov and Janeway, 2002). Activation of these systems initiate the release of inflammatory mediators by surrounding tissues and cells as well as the migration of immune competent cells including macrophages, neutrophils etc. to the site of infection. Here, as in the setting described for apoptotic T cells, properdin released by neutrophils could tag *Neisseria* directly for clearance by phagocytes.

7. Properdin and microbial pathogens

Properdin binds *Neisseria gonorrhoeae* and the resulting bacteria:properdin complexes direct C3 deposition to the microbial surface (Spitzer et al., 2007). These observations may account for why properdin-deficient individuals are particularly sensitive to meningococcal disease. If the properdin-dependent activation pathway plays a significant role in the protection of host from pathogen, then one might expect that pathogens have evolved measures to interfere with properdin recognition. Indeed, the O-antigen of Gram-negative bacteria clearly inhibits properdin:bacterial interactions. Other pathogens may have developed different strategies to block properdin activity: The *Streptococcus pyogenes* exotoxin B (SPE B) is a cysteine protease that degrades properdin and inhibits complement activation and complement-mediated phagocytosis (Tsao et al., 2006). While SPE B appears to be a critical virulence factor, it digests a number of other host proteins and thus, the biological significance of its anti-properdin activity is not clear. In addition, the properdin-regulative activity of SPE B was analyzed using the purified protein, it is not known whether properdin can bind this pathogens' surface. Interestingly, the *Ixodes* ticks, vectors of various pathogens including *Borrelia burgdorferi*, the causative agent of Lyme disease, carry several salivary proteins that inhibit complement activation (Couvreur et al., 2008; Tyson et al., 2008). Some of them are properdin-binding and block AP activity by interfering with properdin:C3b interactions and by dissociating the AP C3 convertase, C3bBbP.

8. Properdin and disease

The prompt clearance of cells early in apoptosis is essential to avoid the harmful inflammatory and autoimmune reactions that occur when cell integrity is breached during late apoptosis and secondary necrosis (Savill et al., 2002). Thus, if properdin promotes the uptake of apoptotic T cells by phagocytes *in vitro*, one might expect that in the absence of properdin, an increased proportion of apoptotic cells would advance to secondary necrosis, which could lead to autoimmunity, most notably the development of systemic lupus erythematosus (SLE), *in vivo* (Korb and Ahearn, 1997). While properdin-deficient individuals lack serum AP activity and are exquisitely sensitive to *Neisseria meningitidis* (Braconier et al., 1983; Densen, 1989; Densen et al., 1987; Kolble and Reid, 1993), so far only isolated reports have suggested a possible relationship between properdin deficiency and autoimmune disease (Holme et al., 1989; Sjoeholm et al., 1988). There are possible explanations for the lack of an obvious autoimmune phenotype in properdin-deficient individuals:

1. As the rapid clearance of dying cells is vital, it is not surprising that there are multiple (partially redundant) pathways in place to ensure the constant functionality of this process. Within the complement system alone, at least three proteins are involved in the recognition of apoptotic T cells: properdin, which recognizes relatively early apoptotic T cells (Kemper et al., 2008), while C1 and MBL recognize late apoptotic/necrotic cells (Trouw et al., 2008) (Fig. 6). Late apoptotic and necrotic cells are generally considered more dangerous as compared to early apoptotic cells, because they lose membrane integrity and release intracellular antigens which are thought to trigger an autoimmune response. Thus, even in the absence of properdin, a progressing apoptotic cell should be detected by the 'second guard', C1q and/or MBL, and tagged for removal. In addition, several other proteins such as the class-B scavenger receptor CD36, the classic phosphatidyl serine receptor, β_2 -glycoprotein and milk-fat globule epidermal growth factor 8 (MFG8) recognize apoptotic cells and are instrumental in the swift removal of early apoptotic cells (Fadok et al., 2000; Ren et al., 1995; Verhoven et al., 1995). Therefore, the effects of properdin-deficiency on autoimmunity might become apparent only when one or more of the additional 'back up' pathways are also affected.
2. Properdin-deficiency might cause several and possibly contradictory effects: Renal disease in the mouse MRL/lpr lupus nephritis model is dependent on the AP convertase components factor B (Watanabe et al., 2000) and factor D (Elliott et al., 2004) for pathologic complement deposition and cell death. Given that properdin stabilizes the AP convertases, it is possible that while properdin deficiency could permit the generation of more necrotic cells, properdin deficiency could at the same time diminish the capacity of necrotic cells to activate complement. One might speculate that properdin mutations permitting convertase stabilization but specifically interfering with the recognition of apoptotic cells would be more likely risk factors for autoimmunity than those that cause complete properdin deficiency. Such mutations would not likely have been identified in previous studies because they would not necessarily cause sensitivity to *Neisseria* nor would they inhibit properdin function in the standard properdin assays.

It is important to note that our properdin work has so far only been performed with T cells (Kemper et al., 2008), and it has not been established whether properdin binds other apoptotic cell types. In addition, autoimmune diseases occur most frequently in women (Hochberg, 1992), but the impact of properdin-deficiency in females is virtually unknown because properdin is encoded by the X chromosome (and so nearly all known properdin-deficient individuals are male) (Figuerola and Densen, 1991; Holme et al., 1989; Sjoeholm et al., 1988). Very recently, Xu et al. also examined the role of properdin in apoptosis (Xu et al.,

2008). Utilizing Jurkat cells, those investigators also observed a marked increase in properdin-binding accompanying apoptosis. In contrast to our observations with primary T cells (Kemper et al., 2008) and Fig. 6, Xu et al. reported that, in the Jurkat model system, properdin recognition was directed to late apoptotic cells and necrotic cells rather than early apoptotic cells. Jurkat cells undergoing apoptosis or necrosis may express a pattern of GAG structures distinct from those of primary T cells, and for that reason a different chronology of properdin-binding could occur. On the other hand, the numerous methodological differences that exist between the two studies, specifically in the induction of apoptosis and necrosis, the preparation of properdin, and the reagents used for properdin detection, could also contribute to the apparent disparity. We anticipate that future biochemical investigations with these and other cell types in conjunction with *in vivo* model studies will elucidate further the role and mechanism(s) of properdin activity during apoptosis.

If properdin indeed plays a role in the recognition of general danger signals its range of specific targets could also include pathogen-infected as well as malignant cells. Sjoblom *et al.* (Sjoblom et al., 2006) compared the sequences of over 13,000 genes from primary breast tumors and lines to equivalent gene sequences from matching normal tissue. Genes were identified that had mutated to a significant degree during tumorigenesis and therefore may normally play a protective role. The properdin gene was in this group. Thus, it is possible that in the case of breast cancer, the properdin gene is activated during tumorigenesis, with the resulting properdin protein exported to the cell surface, tagging it for clearance. Breast cancer cells are known to possess modified GAG structures that play critical roles in their proliferation (Fuster and Esko, 2005) and possibly form properdin-binding sites. Mutations that accrue in the properdin gene during tumor development could disable this protective mechanism.

As discussed above, the lack of properdin or its ability to recognize potentially dangerous targets may contribute to a number of diseases or chronic conditions. However, properdin-initiated AP activation might not always be beneficial, and in some cases it might be detrimental: The target-binding capacity of properdin derived from freshly released neutrophil granules (in contrast to endogenous serum properdin) is particularly potent and could account for the critical roles of neutrophils observed in several AP-dependent disease models such as rheumatoid arthritis (Ji et al., 2002; Wipke and Allen, 2001) and anti-phospholipid syndrome (Girardi et al., 2003). Neutrophil-derived properdin could also promote AP activation in cases of glomerulonephritis and vasculitis associated with anti-neutrophil cytoplasmic autoantibodies (ANCA disease) (Xiao et al., 2007) and may play a role in the rejection of kidney transplants which is characterized by massive infiltration with neutrophils in the setting of dying tissue (Sacks and Zhou, 2003). Mast cells have been recently described as new properdin source (Stover et al., 2008). Thus, properdin-directed complement activation could be involved in cases in which mast cells play an important role.

9. Properdin: a therapeutic target?

Recently, it has become evident that the AP C3 convertase plays critical roles in the exacerbation of injury and disease (above). For these reasons, the APC3 convertase has become a promising therapeutic target. Antibodies that block factor B (Thurman et al., 2005), factor D (Fung et al., 2001), and properdin (Gupta-Bansal et al., 2000) have been proposed, in “humanized” form, as potential therapeutics. Given the important roles of the AP in maintaining health, there is concern over possible side effects of this approach.

We are just beginning to explore the biological consequences of properdin:target recognition. It is possible that this newly-appreciated properdin activity might play an important role in one or more of the many known AP-dependent neutrophil-dependent diseases (above). Properdin is composed of 6 TSRs, and most of its TSRs appear to be potential ligand-binding sites. Thus,

each target may be recognized by a different array of TSRs. This scenario would suggest the possible production of reagents which block specific properdin:target interactions without interfering with remaining properdin activities - thus reducing undesirable side-effects and limiting impact to the appropriate therapeutic intervention. To this end, structural studies which examine properdin:target recognition, and investigations designed to elucidate the inhibition of properdin:target recognition in the serum, could be critical. In addition, the availability of a small animal model will complement such investigations.

10. Conclusion and Future Outlook

In light of the potential multi-layered and complex involvement of properdin in several essential immune reactions, new inquiries are warranted. Those would include a re-evaluation of disease prevalence in properdin-deficient patients/families and an examination of the impact of properdin-deficiency in animal models. An analysis of properdin DNA polymorphisms in patients with autoimmune diseases commonly associated with defects in the clearance of apoptotic cells or in patients with certain cancers would be of great interest, especially focusing on those polymorphisms that might affect target recognition.

Innate Immunity and specifically complement is the body's way to rapidly recognize and destroy pathogens and other harmful entities. It was long thought to be a primitive "stop-gap" measure to slow down infection until the more sophisticated adaptive immune system can undertake its highly potent attacks using antibodies and cells tailored to the specific target. It has finally become clear how naïve this view had been: the successful immune defense is now seen as a complex process in which the innate system plays vital roles in instructing and guiding the adaptive system. The recent discovery of novel complement receptors, i.e. CRIg (Helmy et al., 2006), and the unveiling of unexpected new roles for long known complement proteins such as for C1q in the development of the neuronal synapse (Stevens et al., 2007), the importance of DAF, CD46 and C5a in the modulation of T cell responses (Hawlish and Kohl, 2006; Heeger et al., 2005; Kemper and Atkinson, 2007; Liu et al., 2005; Strainic et al., 2008) and the ability of properdin to recognize dangerous antigens (Kemper et al., 2008; Spitzer et al., 2007) demonstrates how deeply complement and adaptive immunity are intertwined – with the promise of many more exciting discoveries at the complement/adaptive immunity interface to come.

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A Standard model: convertase assembly initiated by covalent attachment of nascent C3b to target surface

B Properdin-Directed model: convertase assembly initiated by non-covalent attachment of properdin to target surface

Fig. 1. Two models are presented for the initiation and assembly of the AP C3 convertase on a target surface

(A) Assembly is initiated via nascent C3b (C3b*) which is generated by non-specific fluid phase activation of C3 and then covalently binds to nearby surfaces. (B) Assembly is initiated via properdin, which binds targets directly and then recruits fluid phase C3b (nascent C3b is not required). In each case, the Ba fragment is released when factor B is cleaved by factor D. (Modified from (Spitzer et al., 2007); Copyright 2007 The American Association of Immunologists, Inc.).

Fig. 2. Properdin directs AP C3 convertase assembly and function on *Neisseria* surfaces
(A) *N. gonorrhoeae* was incubated with properdin (black line) or buffer alone (gray shaded area) and analyzed by FACS with anti-P mAb. **(B)** *N. gonorrhoeae* was incubated with properdin and C3b (black line), or buffer alone (gray shaded area) and analyzed by FACS with an anti-C3d mAb. Bacteria incubated with C3b alone (gray line) served as a negative control. **(C)** *N. gonorrhoeae*-C3bP complexes generated as in (B) (black line) or bacteria alone (dark gray line) were incubated with factor B plus factor D and analyzed by FACS with anti-Bb mAb. Bacteria-C3bP incubated with buffer served as a negative control (gray shaded area). **(D)** *N. gonorrhoeae*-P complexes generated as in (A) (black shaded area) or *N. gonorrhoeae* alone (dark gray line) were incubated in 5% properdin-deficient human serum under conditions

that permit AP activation only, and were analyzed by FACS with an anti-C3d mAb. *N. gonorrhoeae*-P complexes incubated in buffer served as a negative control (gray shaded area). (Figure from reference (Spitzer et al., 2007); **Copyright 2007 The American Association of Immunologists, Inc.**).

Fig. 3. Properdin bound to apoptotic T cells promotes their phagocytosis by macrophages

Apoptotic T cells were either pre-incubated with purified properdin (*d*) and (*e*) or left untreated (*a*), (*b*) and (*c*). T cells were washed and then added to human macrophages grown on slides in media containing normal human serum (NHS) or heat-inactivated human serum (Δ HS). Slides with cell mixtures were incubated for 1 hr, washed, stained with Hematoxylin and analyzed microscopically. As seen, macrophages ingested apoptotic T cells more readily than non-apoptotic control cells [Compare (*a*) and (*b*)] and the uptake of apoptotic T cells by macrophages was increased by about 100% in the presence of normal human serum [Compare (*b*) and (*c*)]. Uptake was greatest when apoptotic T cells were first pretreated with properdin [Compare (*b*) and (*d*)]. Pretreatment of apoptotic T cells with properdin alone increased their

ingestion even in the absence of complement activation substantially [Compare (c) and (e)]. Results shown are representative of four independent experiments and were confirmed by a quantitative FACS assay (Kemper et al., 2008). Red *, indicates ingested T cells. Original magnification, $\times 100$. Bar in panel (e) = 50 μm . (Figure derived from (Kemper et al., 2008)).

Putative
GAG-binding
Face

Arginine
sidechains

Fig. 4. Putative thrombospondin repeat 2 GAG-binding face

The conserved tryptophan residues (orange) and disulfide bridges (yellow) position the two arginine residues (blue) ~9 Angstroms apart. The positively-charged arginine side chains have been proposed to interact electrostatically with a similarly positioned pair of negatively-charged GAG sulfate moieties (Tossavainen et al., 2006). The structure is of thrombospondin repeat 2 (Tossavainen et al., 2006) derived with the Protein Data Bank accession code 1LSL coordinates using the program PyMole (DeLano, 2002).

Fig. 5. Properdin released from activated neutrophils binds to "dangerous self" (apoptotic cells) and "dangerous non-self" (microbial pathogens), marking them for complement activation and phagocytosis.

Fig. 6. Properdin, C1q and MBL recognize distinct target cell populations

(A) Analysis of properdin, C1q and MBL binding to either apoptotic or necrotic T cells (shown as mean $\pm$ s.d.) of three separate experiments. Statistical difference between the binding of properdin and C1q and properdin and MBL to either apoptotic or necrotic T cells was $p < 0.005$ in all cases as determined by the paired Student's t test. (B) Summary/model of the contributions of the three complement activation pathways in the recognition of human apoptotic and necrotic primary CD4⁺ T cells. (Figure derived from (Kemper et al., 2008)).