Molecular Immunology 56 (2013) 191–198

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review

Properdin in complement activation and tissue injury

Allison M. Lesher[a], Bo Nilsson[b], Wen-Chao Song[a,*]

[a] Department of Pharmacology and Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

ARTICLE INFO

Article history:
Received 15 May 2013
Accepted 22 May 2013
Available online 29 June 2013

Keywords:
Properdin
Alternative pathway
C3 glomerulopathy
Complement therapeutics

ABSTRACT

The plasma protein properdin is the only known positive regulator of complement activation. Although regarded as an initiator of the alternative pathway of complement activation at the time of its discovery more than a half century ago, the role and mechanism of action of properdin in the complement cascade has undergone significant conceptual evolution since then. Despite the long history of research on properdin, however, new insight and unexpected findings on the role of properdin in complement activation, pathogen infection and host tissue injury are still being revealed by ongoing investigations. In this article, we provide a brief review on recent studies that shed new light on properdin biology, focusing on the following three topics: (1) its role as a pattern recognition molecule to direct and trigger complement activation, (2) its context-dependent requirement in complement activation on foreign and host cell surfaces, and (3) its involvement in alternative pathway complement-mediated immune disorders and considerations of properdin as a potential therapeutic target in human diseases.

1. Introduction

Properdin (P) is a plasma glycoprotein of the complement system. It is the only known positive regulator of the complement cascade, with a history of discovery dating back to when the precise activation pathways of the complement system were still being elucidated (Lachmann, 2009; Maves and Weiler, 1993; Pillemer et al., 1954). Complement is a crucial branch of the innate immune system responsible for the recognition and removal of pathogens and cellular debris from the host organism. Over 30 proteins in circulation and on the cell surface work together in a delicate balance to drive complement activation on pathogens and cellular debris while protecting the host tissue from bystander injury. Complement activation is triggered by three main pathways – classical, lectin and alternative. While the classical and lectin pathways are initiated in response to recognition of specific markers on pathogens and susceptible surfaces (antigen–antibody complexes and pathogen-associated carbohydrates, respectively), the alternative pathway (AP) is constitutively active at a low level in circulation (Dunkelberger and Song, 2010; Lachmann, 2009). This phenomenon, referred to as the tick-over mechanism, is triggered by spontaneous hydrolysis of C3 to C3(H_2O) which, like the activated C3 fragment C3b, can bind to factor B (fB) and allow factor D to cleave fB (Lachmann, 2009; Pangburn and Muller-Eberhard, 1983). This enzymatic complex C3(H_2O)Bb acts as a C3 convertase to cleave C3 into C3a and C3b to opsonize targets and propagate further complement activation through the formal AP C3 convertase C3bBb (Lachmann, 2009). Newly formed C3bBb complex is rather labile and undergoes rapid spontaneous decay (Medicus et al., 1976a; Pangburn and Muller-Eberhard, 1986). However, it is stabilized by P. Binding of P increases the half-life of C3bBb five to ten-fold (Fearon and Austen, 1975). On the other hand, several fluid phase and membrane-bound complement regulators, including factor H (fH) and decay-accelerating factor (DAF), work to promote the decay and inactivation of C3bBb and thus help protect host cells from complement injury (Dunkelberger and Song, 2010; Liszewski et al., 1996).

While this mechanism of spontaneous complement activation and turnover – *via* the AP and involving opposing actions of P and the negative regulators on C3bBb – has been well established over the years, recently renewed interest in P has stimulated a re-examination of its mode of action and highlighted several unresolved questions regarding its biology. Among them, the concept of P acting as a pattern recognition molecule to direct and trigger AP complement activation in addition to its well established role in stabilizing the AP C3 convertase C3bBb has gained new attention. *In vitro* and *in vivo* complement assays have also shown that P is indispensible under some but not all settings of AP complement activation. It even appears to contribute, in some cases critically, to the classical and lectin pathways of complement activation. Additionally, while several studies in mice implicated P in the pathogenesis of complement-mediated tissue injury and

* Corresponding author at: Perelman School of Medicine, University of Pennsylvania, Room 1254 BRBII/III, 421 Curie Blvd., Philadelphia, PA 19104, USA. Tel.: +1 215 573 6641; fax: +1 215 746 8941.
E-mail address: songwe@upenn.edu (W.-C. Song).

0161-5890/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.molimm.2013.06.002

demonstrated a beneficial effect of its inhibition, recent experiments in mouse models of C3 glomerulopathy have revealed an unexpected protective effect of P. Thus, complete understanding of the role of P in complement activation and disease pathogenesis remains a goal to be achieved.

2. Properdin as a pattern recognition molecule to direct AP complement activation?

While the role of P as a stabilizer of the AP C3 convertase C3bBb is well established, the hypothesis that P may also work as a pattern recognition molecule to direct and trigger AP complement activation has recently gained new consideration, largely stimulated by the insightful biochemical studies of Hourcade and colleagues (Hourcade, 2006; Spitzer et al., 2007). The concept that P initiates AP complement activation harks back to the discovery of the AP itself, which was once referred to as the "properdin pathway" (Pillemer et al., 1954). However, while the existence of AP was subsequently established and the "tick-over" mechanism of activation became universally accepted, the concept of P functioning as an initiator of the pathway fell to the wayside, being replaced by the now well known activity of P as a stabilizer of preformed C3bBb (Maves and Weiler, 1993). By conjugating purified P to Biacore sensor chips, Hourcade demonstrated that P could help assemble C3bBb complexes *de novo* on a surface (Hourcade, 2006). These experiments thus unequivocally showed that surface-bound P can indeed initiate AP complement activation by providing a platform for new C3bBb formation. The investigators further demonstrated that purified P was able to bind to a variety of AP complement-susceptible particle and cell surfaces, including zymosan, rabbit erythrocytes and *Neisseria gonorrhoeae* (Spitzer et al., 2007). Significantly, P-opsonized zymosan, bacteria and erythrocytes activated AP complement in the absence of additional serum P (Spitzer et al., 2007). Subsequent studies by the same group and others extended these initial observations, demonstrating that P was also able to bind to early apoptotic T cells (Kemper et al., 2008), late apoptotic and necrotic T cells (Xu et al., 2008), plate-immobilized LPS and LOS (Kimura et al., 2008), *Escherichia coli* of the DH5α strain (Stover et al., 2008) and kidney proximal tubule epithelial cells (Gaarkeuken et al., 2008). In further studies, the P-binding ligand on several types of target cells was identified as glycosaminoglycans such as heparan sulfate proteoglycan (Kemper et al., 2008; Zaferani et al., 2011).

Despite these suggestive observations, however, one problem in envisioning P as a pattern recognition molecule to direct and trigger AP complement activation on specific surfaces was the inability to demonstrate direct binding of serum P in the absence of C3 (Kimura et al., 2008; Spitzer et al., 2007). Indeed, while purified human P bound to zymosan, P from C3-depleted human serum failed to do so (Spitzer et al., 2007). Likewise, in a functional assay, plate-bound LPS activated AP complement in P knockout ($P^{-/-}$) mouse serum after incubating with purified P but not after incubation with C3-depleted human or C3 knockout ($C3^{-/-}$) mouse serum (as a source of P) (Kimura et al., 2008). Other studies detected P binding on *E. coli* and zymosan after incubation with human serum but binding was abolished in the presence of EDTA or the complement inhibitor Compstatin (Harboe et al., 2012; Stover et al., 2008). The only published study of C3-independent P binding to susceptible cells is a report in which serum P from normal or C3-deficient serum was found to bind similarly to necrotic cells, although the binding was relatively weak (Xu et al., 2008). Thus, existing evidence overwhelmingly supports the conclusion that serum P does not bind to AP activators in the absence of surface-bound C3b.

The above findings led to the hypothesis that some serum factors may compete or inhibit P binding to cell surfaces, perhaps as a protective mechanism to prevent constitutive AP complement activation (Kemper et al., 2008, 2010). Given that properdin is largely made and released from leukocytes, the idea was tested that freshly secreted properdin in the local microenvironment may escape sequestration of serum inhibitory factors and be able to bind and opsonize target cells. In one study, it was shown that while P from C3-depleted human serum failed to bind to early apoptotic T cells, both purified P and freshly released P from activated neutrophils were able to bind to these cells (Kemper et al., 2008). This mechanism, however, may not operate in other settings of AP complement activation, as other investigators found freshly released P from activated neutrophils to be incapable of binding to zymosan or *E. coli* (Harboe et al., 2012). It is interesting that P opsonization of apoptotic T cells facilitated their phagocytosis through a complement-independent mechanism, raising the possibility that recognition and binding of certain target cells by neutrophil-derived P represents a physiological function of P unrelated to AP complement activation (Kemper et al., 2008).

The biological relevance of binding of purified P to the tested AP complement activators was also called into question by the demonstration of Ferreira and colleagues who showed that polymeric forms of properdin- artifacts of purified properdin from prolonged storage- may have accounted for much of the observed binding (Ferreira et al., 2010). Under physiological conditions, P exists in the plasma as cyclic dimers, trimers and tetramers (P_2, P_3 and P_4) in a fixed ratio (Pangburn, 1989). Repeated freezing and thawing of purified P during storage may cause the oligomeric P to aggregate and form polymers of undefined sizes (P_n) (Farries et al., 1987; Pangburn, 1989). Historically, P_n was referred to as "activated P" because unlike the native P_2–P_4 it caused AP complement activation and C3 consumption when added to normal serum (Farries et al., 1987). Using freshly separated P, it was demonstrated that P_2–P_4 could bind specifically to zymosan, necrotic Raji and Jurkat cells and *Chlamydia pneumoniae*, but not to rabbit erythrocytes or live cells (Cortes et al., 2011; Ferreira et al., 2010). In contrast, P_n or unfractionated P bound to almost all AP activating cells or surfaces tested (Ferreira et al., 2010). Notably, even though purified native P_2–P_4 was able to bind *C. pneumonia*, no P binding could be detected using human serum in the presence of EDTA (Cortes et al., 2011). Similarly, Agarwal et al. found that while serum P played a critical role in AP complement activation and killing of diverse strains of *Neisseria meningitidis* and *N. gonorrhoeae*, purified native P_2–P_4 did not bind to any of the strains. However, unfractionated P and purified P_n did bind to these bacteria (Agarwal et al., 2010).

Taken together, it seems clear that in most settings P does not work independently of C3 as a pattern recognition molecule to direct and initiate AP complement activation. Thus, for a given target cell or pathogen, susceptibility to AP complement attack is unlikely to be determined by its affinity for C3-independent P binding. It should be pointed out that even if serum P initially binds to a target surface in a C3-independent manner, subsequent *de novo* assembly of C3bBb would still require the tick-over mechanism to provide C3b molecules as P itself, whether in solution or surface-bound, does not possess protease activity. Therefore, the question of whether P can bind target cells independently of C3 or not is perhaps of little relevance to complement activation under typical conditions *in vivo*, because the presence of P and C3 tick-over in plasma and other biological fluids means that iterative interaction between P and C3b on target cells is a default outcome. How should these findings and considerations shape our understanding of P function in AP complement activation? We envision that for most target cells the C3 tick-over mechanism provides the initial surface ligand (C3b) for P binding. In some settings, cell surface glycosaminoglycans may also serve as P liagnds (Kemper et al., 2008; Zaferani et al., 2011). Once surface-bound, the oligomeric P can then serve as a platform for *de novo* C3bBb convertase assembly