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# Properdin: A multifaceted molecule involved in inflammation and diseases

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ABSTRACT

Properdin, the widely known positive regulator of the alternative pathway (AP), has undergone significant investigation over the last decade to define its function in inflammation and disease, including its role in arthritis, asthma, and kidney and cardiovascular diseases. Properdin is a glycoprotein found in plasma that is mainly produced by leukocytes and can positively regulate AP activity by stabilizing C3 and C5 convertases and initiating the AP. Promotion of complement activity by properdin results in changes in the cellular microenvironment that contribute to innate and adaptive immune responses, including pro-inflammatory cytokine production, immune cell infiltration, antigen presenting cell maturation, and tissue damage. The use of properdin-deficient mouse models and neutralizing antibodies has contributed to the understanding of the mechanisms by which properdin contributes to promoting or preventing disease pathology. This review mainly focusses on the multifaceted roles of properdin in inflammation and diseases, and how understanding these roles is contributing to the development of new disease therapies.

## 1. Introduction

The complement system is central to immunity and homeostasis. Over 20 candidate drugs that target complement components are being assessed in clinical trials (Ricklin et al., 2018). Since its discovery 64 years ago, properdin, the only known positive regulator of complement, has undergone significant biological characterization in its serum and microenvironment sources, physiological functions, roles in disease, and biochemical characteristics including expression, translation, post-translational modifications, oligomerization and secretion. Understanding the distinct functions of properdin and its opposing negative regulator Factor H, as well as the data surrounding the role of properdin as a potential pattern recognition molecule, will significantly contribute to our understanding of complement regulation. Herein, we review these topics, in addition to the identified roles of properdin in immunity and the status of properdin in complement therapeutics.

## 2. The complement system

### 2.1. Three pathways overview

The complement system is composed of over 40 proteins that carry out multiple functions, including participating in a cascade-like activation process, serving as cellular receptors or as important ligands for those receptors, and/or serving as essential complement regulatory proteins. The circulating complement system proteins, complement receptors expressed on human cells (CR1, CR2, CR3, CR4, C5a receptor 1and 2, C3a receptor 1, C1q receptors, and CRIg), and complement regulatory proteins (Factor H, CD35/CR1, CD55/DAF, CD46/MCP, CD59, C4BP, Factor I, C1-INH, clusterin, vitronectin, CMSD1, CRIg, Factor H-like protein 1, Factor H-related protein 1–5, and properdin), play essential roles in host defense against infection, in homeostasis through the clearance of immune complexes and cell debris, in linking innate and adaptive immunity, as well as in metabolism and in the nervous system (reviewed in (Barnum and Schein, 2018)). The complement system (Fig. 1) can be activated through three pathways:

*Abbreviations:* aHUS, atypical hemolytic uremic syndrome; AAA, abdominal aortic aneurysm; AAV, ANCA-associated vasculitis; AMD, age-related macular degeneration; AMR, antibody-mediated rejection; ANCA, anti-neutrophil cytoplasmic antibody; AP, alternative pathway; APC, antigen presenting cells; C3G, C3 glomerulopathy; C5aR1, C5a receptor 1; CP, classical pathway; DCs, dendritic cells; HUVECs, human umbilical vein endothelial cells; GAGs, glycosaminoglycans; HF, heart failure; KSHV, Kaposi's sarcoma-associated herpesvirus; LP, lectin pathway; MAC, membrane attack complex (C5b-9); MPO, myeloperoxidase; NETs, Neutrophil Extracellular Traps; NK cells, natural killer cells; $P^{-/-}$, properdin-deficient mice; $P_2$, dimeric form of properdin; $P_3$, trimeric form of properdin; $P_4$, tetrameric form of properdin; PGA, platelet-granulocyte aggregates; $P_n$, non-physiological aggregated form of properdin; PNH, paroxysmal nocturnal hemoglobinuria; sC5b-9, soluble C5b-9; TSR, thrombospondin type 1 repeat; WT, wild type

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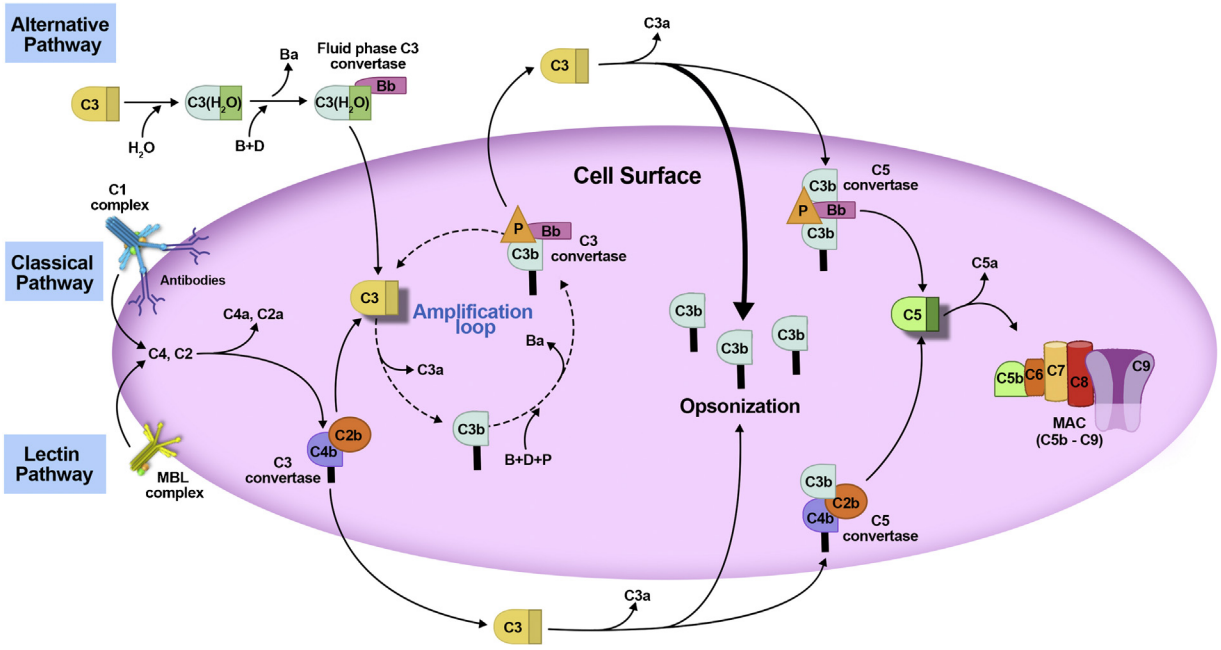


**Fig. 1.** An overview of complement system and the role of the alternative pathway (AP) in the amplification loop. Complement activation is initiated by three different pathways: classical, lectin and alternative pathway. In the classical pathway (CP), C1 complex [C1q, C1s(2) and C1r(2)] recognizes two IgGs or one pentamer IgM (not shown) to form an antigen-antibody complex. In the lectin pathway (LP), mannose-binding lectin (MBL), ficolins or collectins, in association with MBL-associated serine proteins (MASPs), recognize carbohydrates on pathogens. Recognition of these molecules leads to the generation of the C3 convertase C4b2b. Unlike the CP and LP, the alternative pathway (AP) is spontaneously activated in fluid phase through hydrolysis of C3 to C3($H_2O$). C3($H_2O$) recruits Factor B (labeled B) that is cleaved by Factor D (labeled D) to form the fluid-phase AP C3 convertase C3($H_2O$)Bb. C4b2b and C3($H_2O$)Bb cleave C3 molecules found in plasma to generate C3a and C3b, which covalently attach to the cell surface. The C3b derived from all pathways can be used to generate the AP C3b convertase (C3bBb) (amplification loop). Properdin binds to C3bBb and C3bBbC3b extending the half-life of the convertase and thus promoting efficient C3b deposition on the cell surface (represented by the thick line). If C3b binds to or near any C3 convertase, it will form the AP C5 convertase (C3bBbC3b) and the LP and CP C5 convertase (C4b2b3b). These C5 convertases cleave C5 to form C5b and C5a, leading to the formation of the membrane attack complex (MAC) that is common to all pathways. Anaphylatoxins C3a and C5a play important roles in inflammation.

classical, lectin and alternative. The classical pathway (CP) is commonly initiated by binding of the C1 complex (composed of C1q, C1r, and C1s) to immunoglobulins bound on pathogens or cell surfaces, to circulating immune complexes, or to pentraxins (e.g. C-reactive protein, pentraxin 3, serum amyloid P). The lectin pathway (LP) is initiated when mannose-binding lectin (MBL), ficolins or collectins (CL–LK), recognize certain carbohydrates and other ligands on the surface of pathogens. The activation of both the CP and LP leads to cleavage of C4 and C2 by serine proteases (C1s and MBL-associated serine protein 2 (MASP-2)) associated with the recognition molecules C1q or MBL, respectively. The C4b that is formed, binds covalently to the cell surface and the C2b fragment binds to the C4b, generating the C3 convertase of the CP and LP (C4b2b), which then converts native C3 to C3b and C3a, a chemoattractant molecule. C3b has an exposed thioester bond (Pangburn and Muller-Eberhard, 1980; Tack et al., 1980), which allows C3b to effectively tag certain molecules by covalently binding to hydroxyl (−OH) and amine (-$NH_2$) groups found on cell surfaces. Covalent binding of C3b occurs within sixty microseconds at a minimum distance of 28–30 nm (280–300 Å) (Sim et al., 1981), before the C3b thioester is quickly inactivated by hydrolysis.

Unlike CP and LP, the alternative pathway (AP) initiates spontaneously on surfaces that are not (or inefficiently) protected by complement regulatory proteins. Spontaneous hydrolysis of C3 (Muller-Eberhard, 1988) as well as contact activation on certain cells (e.g. platelets), and artificial surfaces (Hamad et al., 2015; Nilsson and Nilsson Ekdahl, 2012), forms C3($H_2O$). C3($H_2O$) is able to bind to Factor B, forming C3($H_2O$)B and Factor B can then be cleaved by the serum protease Factor D, generating the AP fluid-phase C3 convertase C3($H_2O$)Bb. C3($H_2O$)Bb can then cleave additional C3 molecules, generating C3b and C3a. C3b deposited on cell surfaces associates with Factor B to generate C3bB, in which Factor B is cleaved by Factor D to generate the membrane-bound AP C3 convertase C3bBb. C3bBb cleaves many C3 molecules to C3b allowing efficient amplification of C3b deposition on cell surfaces, which is essential for opsonization (Barnum and Schein, 2018). C3b can be further cleaved to iC3b and C3dg fragments, which can be recognized by complement receptors (CR1, CR2, CR3, CR4, and CRIg), leading to phagocytosis (Holers, 2014). When C3b binds to or near the C4b2b and C3bBb convertases, C5 convertases are formed (C4b2b3b and C3bBbC3b [$(C3b)_{2\text{-}n}Bb$], respectively). Both the C3 and C5 convertases of the AP are stabilized by properdin, which increases their half-life (Berends et al., 2015; Fearon and Austen, 1975; Schreiber et al., 1975). The C5 convertase cleaves C5 into C5a, a potent chemoattractant, and C5b. C5b can form a complex with C6, which is the base for the sequential binding of C7, C8 and C9 to form the membrane attack complex (MAC; C5b-9) (reviewed in (Holers, 2014)). MAC leads to direct microbial killing, while sub-lytic levels of MAC stimulate various cellular responses including pro-apoptotic or anti-apoptotic signaling (Nauta et al., 2002; Tegla et al., 2011), and inflammasome activation (Morgan, 2016). C3a and C5a are important factors that bind to specific G-protein-coupled receptors for C3a (C3a receptor 1) and C5a (C5a receptor 1; C5aR1; CD88), respectively. Receptor engagement promotes inflammation and other functions, including cancer progression, cerebellar development, homing of stem cells to the bone marrow, and tissue fibrosis (reviewed in (Ajona et al., 2017; Klos et al., 2009)).

### *2.2. Properdin as a soluble positive regulator via stabilizing C3 and C5 convertases*

The AP represents a true safeguard system that is always active and also accounts for approximately 80–90% of terminal pathway activation by forming a powerful amplification loop for the three complement