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Research paper

The initiating proteases of the complement system: Controlling the cleavage

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Abstract

The complement system is a vital component of the host immune system, but when dysregulated, can also cause disease. The system is activated by three pathways: classical, lectin and alternative. The initiating proteases of the classical and lectin pathways have similar domain structure and employ similar mechanisms of activation. The C1r, C1s and MASP-2 proteases have the most defined roles in the activation of the system. This review focuses on the mechanisms whereby their interaction with substrates and inhibitors is regulated.

Keywords: Complement system; C1s; C1r; MASP-2; Protease; Serpin; C1-inhibitor

1. Complement pathways

Complement, an essential system for both innate and adaptive immunity, responds to the presence of a foreign pathogen within the vertebrate host. As well as directly affecting the pathogen, the complement cascade functions in inflammation and phagocytosis by acting as an opsonin, enhancing the migration of phagocytic cells to the infected area [1], as well as initiating adaptive immune responses and regulating of T- and B-cells [2,3]. The complement system consists of over 30 proteins and glycoproteins which generate a proteolytic cascade that initiates via one of three pathways of activation [4]: classical, lectin or alternative (Fig. 1). Although the initiation of complement can occur via these three distinct pathways, all converge at the formation of a C3 convertase complex (C4bC2a or C3bBb) that cleaves the C3 component into C3a and C3b. The C3b molecule has the ability to bind to multiple targets via a trans-esterification reaction between hydroxy and amino groups and an internal thioester [5]. Using this mechanism, C3b binds to the C4b component of the C3 convertase covalently, forming the C5 convertase complex that cleaves C5 into C5a and C5b. A complex array of proteins, C5b-9, form the final structure, the membrane-attack complex (MAC) [6], which penetrates the pathogen's surface, usually causing lysis. In relation to disease, complement pathways have been associated with both unnecessary activation, causing inflammation in host tissues [4], and deficiencies, which contribute to autoimmunity and chronic infections [7].

1.1. Classical pathway

The classical pathway is initiated by the recognition molecule C1q, which is part of the 790 kDa C1 complex that not only assists in clearing infection, but also performs a role in immune tolerance and xenograft transplantation rejection [8,9]. Activated C1q engages an associated Ca^{2+}-dependent tetramer comprised of the serine proteases, C1s-C1r-C1r-C1s, the overall structure being designated the C1 complex [10]. Autoactivation of C1r is the first step following binding of C1 and it most likely occurs due to mechanical stress induced in C1q upon binding to multiple antigens on the pathogen surface. C1r then cleaves proenzyme C1s at the Arg-Ile cleavage point in the serine protease domain [11]. The highly specific C1s binds and cleaves its substrates,

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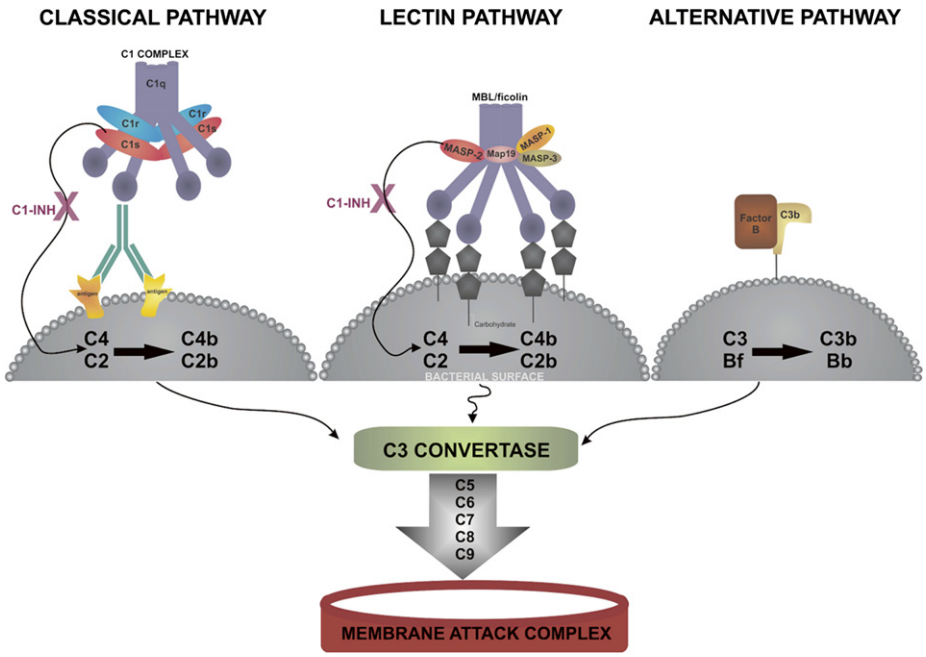

Fig. 1. Overview of complement activation. The complement cascade is activated via (A) classical, (B) lectin or (C) alternative pathways, which converge at the C3 convertase complex, leading ultimately to the formation of membrane attack complexes. C1-INH (C1-inhibitor) is essential in regulating both classical and lectin pathways.

C4 and C2, which consequently form the C3 convertase (C4bC2a) [5]. The pathway continues via the sequential activation of complement molecules until the MAC is formed, as mentioned previously.

1.2. Lectin pathway

The lectin pathway of complement activation acts following the recognition of carbohydrate molecules (*N*-acetyl-glucosamine, mannose, fucose or glucose) on the surface of viruses, bacteria and other pathogenic organisms [7]. This occurs through the interaction of lectin domains found on the C-terminal end of the mannose-binding lectin recognition subunit [MBL] [4] or ficolins [12]. MBL comprises an N-terminal cysteine-rich domain, a collagenous domain, a neck region with an α-helical coiled-coil organisation, followed by globular lectin domains [13]. In the lectin pathway, there are homologues of C1r and C1s, named MBL-associated serine proteases (MASPs), of which three are found (MASP-1, -2, and -3) [14]. The MASPs, although homologous to their classical counterparts, have been shown to interact with the recognition molecules, MBL and ficolins, differently. Unlike the requirement of sequential cleavage and activation of two serine proteases, C1r and C1s, for the commencement of the classical pathway, the lectin pathway can be initiated with the minimal requirement of autoactivating MASP-2 [15]. The proteolytic cascade continues with the cleavage of C2 and C4 substrates by MASP-2 [15,16]. Like the classical pathway, the formation of the C3 convertase is via C4b and C2a binding, with the final stages of complement again concluding with the establishment of the membrane attack complex (MAC). The role/s of the other two MASP enzymes are presently unclear. MASP-1 is able to cleave C2 with moderate efficiency and thus may play an indirect role in complement activation by serving to amplify the reactions initiated by MASP-2. There is still some conjecture over the efficiency of C3 cleavage by MASP-1 under physiological conditions [14]. MASP-1 has also been shown to cleave fibrinogen and factor XIII, pointing to a possible involvement of the enzyme in coagulation processes [17], although the significance of this is not presently well understood. MASP-3 is the product of alternative splicing from the MASP1/3 gene and thus shares identity with the MASP-1 N-terminus, but has an altered serine protease domain [18]. To date this enzyme has been little characterised, with only insulin growth factor binding protein-5 identified as a substrate thus far [19]. In a recent study it was shown that MASP-3 did not cleave C2, C3 or C4 and was not inhibited by C1-inhibitor (C1-inh), hence its role in complement, like MASP-1, remains unclear [20]. Finally, the smallest of the proteins of the lectin complexes, sMAP or MAp19, is a truncated form of MASP-2, which is thought to modulate binding of the MASPs to the lectin recognition moieties [21].

1.3. Alternative pathway

C3 molecules are abundant in the plasma of a host; the covalent attachment of the C3b molecule, on a wide variety of pathogen surfaces, initiates the spontaneous activation of the alternative pathway [22]. The attachment of C3b is achieved through a transacylation reaction, hydrolysing the thioester bond in the C3b molecule, through interactions with hydroxyl and amino groups on the pathogen surface [23]. The activation of the thioester group induces a conformational change and, in the presence of Mg^{2+}, plasma protein factor B binds to the C3b molecule [23]. Once bound to C3b, factor B is cleaved and activated by the serine