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Stringent regulation of complement lectin pathway C3/C5 convertase by C4b-binding protein (C4BP)

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ABSTRACT

The complement lectin pathway, an essential component of the innate immune system, is geared for rapid recognition of infections as each C4b deposited via this pathway is capable of forming a C3/C5 convertase. In the present study, role of C4b-binding protein (C4BP) in regulating the lectin pathway C3/C5 convertase assembled on zymosan and sheep erythrocytes coated with mannan (E_{Man}) was examined. While the C4BP concentration for inhibiting 50% (IC_{50}) formation of surface-bound C3 convertase on the two surfaces was similar to that obtained for the soluble C3 convertase (1.05 nM), ~3- and 41-fold more was required to inhibit assembly of the C5 convertase on zymosan (2.81 nM) and E_{Man} (42.66 nM). No difference in binding interactions between C4BP and surface-bound C4b alone or in complex with C3b was observed. Increasing the C4b density on zymosan (14,000–431,000 C4b/Zym) increased the number of C4b bound per C4BP from 2.87 to 8.23 indicating that at high C4b density all seven α-chains of C4BP are engaged in C4b-binding. In contrast, the number of C4b bound per C4BP remained constant (3.79 ± 0.60) when the C4b density on E_{Man} was increased. The data also show that C4BP regulates assembly and decay of the lectin pathway C3/C5 convertase more stringently than the classical pathway C3/C5 convertase because of a ~7- to 13-fold greater affinity for C4b deposited via the lectin pathway than the classical pathway. C4BP thus regulates efficiently the four times greater potential of the lectin pathway than the classical pathway in generating the C3/C5 convertase and hence production of pro-inflammatory products, which are required to fight infections but occasionally cause pathological inflammatory reactions.

1. Introduction

Strong similarities between the M1 complex (MBL-MASPs) of the lectin pathway and the C1 complex (C1qrs) of the classical pathway had initially implicated that the overall process of complement activation and regulation of the lectin pathway might be similar to that of the classical pathway. But studies examining different aspects of complement activation have suggested differences between the two pathways (Rossi et al., 2001; Harmat et al., 2004; Rawal et al., 2008). Recent studies by Rawal et al. (2008) have shown that complement activation via the lectin pathway has the potential of generating four times more C3/C5 convertase than the classical pathway and hence more pro-inflammatory products. This is because every C4b deposited via the lectin pathway forms a convertase in contrast to the classical pathway in which only one out of four C4b deposited forms a C3/C5 convertase (Rawal and Pangburn, 2003; Rawal et al., 2008). In addition, studies employing recombinant forms of mannan-binding lectin (rMBL) have shown that the structural variants of MBL not only form higher order oligomers similar to wild type MBL (MBL/A) but that the structural variant rMBL/D activates the complement lectin pathway to the same extent as rMBL/A while rMBL/B activates weakly and rMBL/C is ineffective (Rajagopalan et al., 2009). Together, these new findings imply that activation of the lectin pathway by MBL or its structural variant can produce significant amounts of anaphylatoxins, C3a and C5a, in a very short time. This can be particularly harmful in infants whose innate immune system relies on activation of the lectin pathway as one of its responses to fight infections (Aittoniemi et al., 1996) or under conditions when unregulated activation of the lectin pathway might be a more prominent contributor to the pathology of inflammatory reactions and tissue damage following oxidative stress (Collard et al., 2000), ischemia and reperfusion injury (Jordan et al., 2001),

Abbreviations: C4BP, C4b-binding protein; C3b, C4b and C5b, the proteolytically activated form of C3, C4, and C5, respectively; EA, antibody coated sheep erythrocytes; E_C, chicken erythrocytes; MBL, mannan-binding lectin; pMBL/A, MBL purified from human plasma; M1 complex, MBL-MASPs complex; MASP, MBL-associated serine proteases; C1 complex, consists of three components: C1q and the serine proteases C1r and C1s in the ratio 1:2:2; E_{Man}, mannan coated sheep erythrocytes; C4b,C2a, soluble C3 convertase; E_{Man}M1,C4b,C2a and ZymM1,C4b,C2a, surface-bound lectin pathway C3 convertases; E_{Man}M1,C3bC4b,C2a and ZymM1,C3bC4b,C2a, surface-bound lectin pathway C5 convertases; ZymC3b, C3b bound to zymosan particles; IC_{50}, concentration required to inhibit 50%.

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and in some types of renal diseases (Matsuda et al., 1998; Lhotta et al., 1999; Endo et al., 2000). These reports when considered with the potential therapeutic application of recombinant MBL in MBL-deficient patients emphasize the need to understand the processes of complement activation and regulation via the lectin pathway.

The C3/C5 convertase of the lectin pathway and the classical pathway assembled with C4b as the noncatalytic subunit (C4b,C2a) cleave both C3 and C5 (Rawal and Pangburn, 2003; Rawal et al., 2008). But C4b,C2a mainly cleaves C3 because it has a weak affinity for C5 and is therefore called a C3 convertase (Rawal and Pangburn, 2003; Rawal et al., 2008). Deposition of additional C3b molecules by C4b,C2a converts the low affinity C3 convertase to a high affinity C5-binding convertase (C3bC4b,C2a) (Rawal and Pangburn, 2003; Rawal et al., 2008), which in the present study has been called a C5 convertase. Cleavage products of C3 (C3b and C3a) and C5 (C5b and C5a) have important biological activities that help fight infections. The products recognize targets, mediate inflammatory responses by stimulating neutrophils and phagocytes to the site of injury or infection, and help kill and/or clear microorganisms and altered host cells (Ember et al., 1998). However, these products may also contribute to the pathophysiology of several diseases and conditions such as inflammatory diseases, reperfusion injury and xenotransplantation rejection (Rother, 1998).

Studies analyzing regulation of the lectin pathway of complement have mainly focused on the regulatory role of C1-INH (Wong et al., 1999; Petersen et al., 2000; Nielsen et al., 2007; Kerr et al., 2008). C1-INH has been shown to be a major regulator of MASP-2 than of C1s (Nielsen et al., 2007; Kerr et al., 2008) and based on different sensitivities to various synthetic inhibitors, differences in control of complement activation via the lectin pathway and the classical pathway have been suggested (Petersen et al., 2000). Although the efficiency of formation of the C3/C5 convertase via the two pathways has been reported to be different (Rawal et al., 2008) and regulation of the classical pathway C3/C5 convertase examined by several groups (Parker, 1992; Liszewski et al., 1996; Morgan and Harris, 1999), studies on the regulation of the lectin pathway C3/C5 convertase are limited (Suankratay et al., 1999).

The soluble complement regulator, C4BP is a large plasma protein that exists in many forms with varying subunit composition. The major isoform is made up of seven α-chains (70 kDa) and one β-chain (45 kDa) (α7β1) (Hillarp and Dahlbäck, 1988). The α-chain of C4BP has binding sites for many ligands (Blom et al., 2004a), which include C4b, heparin, serum amyloid protein (SAP), CD40, and CD154 while the β-chain has been reported to bind the vitamin K-dependent anticoagulant protein S. Many pathogens have been reported to bind to the α-chain of C4BP and evade complement-mediated killing (Blom, 2002; Blom et al., 2004b). C4BP controls complement activation by interacting with the noncatalytic subunit C4b of the C3/C5 convertase and thereby inhibits assembly of the convertase, enhances its decay, and exhibits cofactor activity for factor I in inactivating both fluid phase and cell-bound C4b (Fujita et al., 1978; Fujita and Tamura, 1983; Blom et al., 1999; Rawal and Pangburn, 2007).

In the present study C4BP regulation of the lectin pathway C3/C5 convertase assembled with purified complement components on zymosan particles, a natural surface rich in mannose residues was examined. For comparison with published studies on the classical pathway C3/C5 convertase that was assembled on sheep erythrocytes (E_S) coated with antibody (EA) (Rawal and Pangburn, 2007), regulation of the lectin pathway C3/C5 convertase assembled on sheep erythrocytes coated with mannan (E_{Man}) was also examined. The regulatory role of C4BP in inhibiting enzyme formation and enhancing enzyme decay was determined by hemolytic assays measuring the C5 cleavage activity of the lectin pathway C3/C5 convertase. Our findings show that C4BP controls the assembly and decay of the lectin pathway C3/C5 convertase more stringently than the classical pathway C3/C5 convertase. The greater efficiency of C4BP in regulating the C3/C5 convertase of the lectin pathway than the classical pathway is attributed to its higher affinity for C4b deposited via the lectin pathway than the classical pathway. In addition, the data show that on zymosan the polymeric regulator mediates efficient control by utilizing all seven α-chains, each capable of binding one C4b, to regulate the four times greater potential of the lectin pathway than the classical pathway in generating C3/C5 convertase and hence production of pro-inflammatory products.

2. Materials and methods

2.1. Reagents

Sheep erythrocytes (E_S) and chicken erythrocytes (E_C) were isolated from whole blood purchased from Colorado Serum Co. (Denver, CO). Mannan, chromium chloride, NP-40 (nonidet P-40), Tween 20, and EDTA were purchased from Sigma Chemical Co. (St. Louis, MO). Veronal buffered saline (VBS) contained 5 mM barbital, 145 mM NaCl and pH 7.4. Gelatin veronal-buffered saline (GVB) was VBS containing 0.1% gelatin, while GVB^{++} was GVB containing 0.5 mM $MgCl_2$ and 0.15 mM $CaCl_2$, GVBE was GVB containing 10 mM EDTA.

2.2. Purified proteins

Complement proteins, M1, C2, C3, C4, C5, C4b monomer, and C6 were purified from normal human plasma as described (Rawal et al., 2008). Complement components, C4, purified C5b,6, and C4BP were obtained from CompTech (Tyler, TX). All proteins were homogenous by polyacrylamide gel electrophoresis. Functional activity of C2, C4, C5, C6, and C4BP was determined as explained previously (Rawal et al., 2008). Protein concentrations of C2, C3, C4, C5, C6, C5b,6, and C4BP were determined spectrophotometrically as described (Rawal et al., 2008). All purified proteins were stored at −76 °C.

2.3. Preparation of ZymM1,C4b, ZymM1,C3bC4b, and ZymC3b cells

ZymM1,C4b was made as described (Rawal et al., 2008). Briefly, zymosan was incubated with M1 in the presence of $CaCl_2$ and $MgCl_2$ to make ZymM1. ZymM1,C4b was made by incubating ZymM1 with radiolabeled (^{125}I) C4. Total number of C4b deposited on washed ZymM1 was determined by the uptake of radiolabeled C4 as C4b. Zymosan having no M1 was employed as control for background C4b binding. ZymM1,C4b was washed and the process repeated until the desired numbers of C4b/Zym were obtained. ZymM1,C3bC4b was made by incubating ZymM1,C4b with C3 and C2 as described (Rawal et al., 2008). Number of C3b molecules bound to ZymM1,C3bC4b was determined by using ^{125}I-Factor B as described (Rawal and Pangburn, 1998). The procedure was repeated until the desired numbers of C3b/Zym were obtained. Zymosan having no M1 was employed as controls for background C3b binding. Zymosan bearing only C3b (ZymC3b) was prepared as described (Rawal and Pangburn, 2007) and zymosan bearing no C3b was employed as control.

2.4. Preparation of E_{Man}M1,C4b and E_{Man}M1,C3bC4b cells

Sheep erythrocytes (E_S) were coated with mannan (E_{Man}) by the method of Ikeda et al. (1987) as described (Rawal et al., 2008). E_{Man}M1 was made by incubating E_{Man} with M1 in the presence of $CaCl_2$ and $MgCl_2$. E_{Man}M1 cells were incubated with ^{125}I-C4 to