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Review

Humanized cobra venom factor: Structure, activity, and therapeutic efficacy in preclinical disease models☆

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ABSTRACT

The complement system is an integral component of both innate and adaptive immunity. However, complement is also a pathogenetic factor in many diseases. The development of agents for therapeutic complement inhibition is the topic of intense investigations by many investigators. We have developed a distinctly different therapeutic approach: complement depletion rather than inhibition. This approach is based on cobra venom factor (CVF), a C3 analog known to be able to safely deplete complement. This manuscript will briefly review the structure and activity of CVF, along with its similarities and differences to C3. Exploiting the knowledge of the structure/function relationship of CVF and C3, we created derivatives of human C3 which display the CVF-like activity of depleting complement, referred to as humanized CVF (hCVF). This review describes the structure and activity of hCVF, including the important property of not cleaving C5. The efficacy of hCVF for therapeutic complement depletion in nine preclinical models diseases with complement pathology is reviewed, including reperfusion injury, age-related macular degeneration (AMD), paroxysmal nocturnal hemoglobinuria (PNH), and immunogenicity of Factor VIII in hemophilia A. Complement depletion is characterized by the absence of toxicity, even after intra-arterial injection into the pulmonary artery of primates. No immunogenicity has been observed.

1. Introduction

The complement system is part of the immune system and has important functions in both innate and adaptive immunity. However, the complement system also plays an important role in the pathogenesis of many diseases, contributing solely or significantly to the disease process and tissue injury. Table 1 lists selected diseases with confirmed complement pathogenesis. Because of its role in the pathogenesis of many diseases, the last two decades have seen multiple approaches to developing pharmacological agents to interfere with or modulate the complement cascade (Holers and Thurman, 2004; Lachmann and Smith, 2009; Morgan and Harris, 2003; Ricklin and Lambris, 2013). These anti-complement agents, small and large, can be grouped into two conceptually different categories. One group of inhibitors is aimed at a specific complement component and will inhibit its activation. Examples include a humanized antibody to C5 which will prevent its activation and therefore prevent the formation of the membrane attack complex (MAC) and ensuing tissue damage, approved for treatment of paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) (Hillmen et al., 2004; Roth et al., 2011). Another example of a complement inhibitor is compstatin, a cyclic 13-residues peptide that binds to C3 and prevents its subsequent activation (Ricklin and Lambris, 2008). A second group of complement inhibitors is represented by agents that do not prevent the activation of a complement component but inhibit the action of an activated complement component. Examples include antagonist for the C5a receptor designed to inhibit the pro-inflammatory activities of C5a (Köhl, 2006). Another example is a chimeric protein consisting of a regulatory domain of human Factor H (CCP1-5) with a C3d-binding domain from complement receptor 2 (CR2) (CCP1-4). This chimeric protein targets sites of ongoing complement activation and will cause the inhibition of

Abbreviations: CVF, cobra venom factor; rCVF, recombinant CVF; hCVF, humanized CVF; MAC, membrane attack complex; PNH, paroxysmal nocturnal hemoglobinuria; MI/RI, myocardial ischemia reperfusion injury; GI/RI, gastrointestinal ischemia reperfusion injury; AMD, age-related macular degeneration; EAMG, experimental autoimmune myasthenia gravis; AChR, acetylcholine receptor; aHUS, atypical hemolytic uremic syndrome; NCP, normal cobra plasma.

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Table 1
Diseases with complement pathogenesis.

Disease	Reference
Rheumatoid arthritis	Kerwar et al. (1981), Linton and Morgan (1999), Wang et al. (1995)
Lupus erythematosus	Buyon et al. (1992)
Myasthenia gravis	Lennon et al. (1978)
Hyperacute rejection after xenotransplantation	Dalmasso (1992), Leventhal et al. (1993)
Age-related macular degeneration (AMD)	Edwards et al. (2005), Hageman et al. (2005), Haines et al. (2005)
Ischemia/reperfusion injury	Arumugam et al. (2004), Gorsuch et al. (2012), Riedemann and Ward (2003)
Paroxysmal nocturnal hemoglobinuria (PNH)	Nishimura et al. (2001), Parker et al. (1985)
Bullous pemphigoid	Jordon et al. (1973)
Asthma	Arroyave et al. (1977)
Anti-phospholipid syndrome	Holers et al. (2002)
Atypical hemolytic uremic syndrome (aHUS)	Kavanagh et al. (2008)

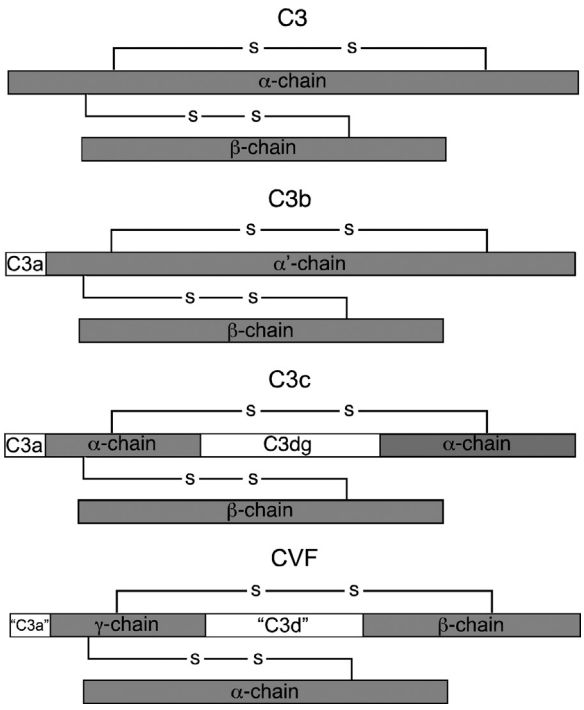

Fig. 1. Schematic representation of the chain structures of C3, C3b, C3c, and CVF. Please note the somewhat larger sizes of the CVF γ- and β-chains compared to the corresponding α′-chain fragments of C3c.

further complement activation by inactivating C3b (Banda et al., 2009). These approaches have shown encouraging results in multiple disease models with complement pathogenesis (Ricklin and Lambris, 2013).

We have developed a third, distinct approach that is neither based on inhibition of a complement component nor its activated fragment but on depletion of complement (Fritzinger et al., 2008b, 2009; Vogel and Fritzinger, 2007, 2010). Our concept is based on cobra venom factor (CVF), an unusual venom component of cobras. As described below, CVF is a structural and functional analog of complement component C3 that forms a stable C3/C5 convertase and exhibits resistance to the regulatory complement proteins, leading to exhausted complement activation and, therefore, complement depletion. Ever since it had been shown that CVF can be safely administered to laboratory animals for complement depletion (Cochrane et al., 1970; Maillard and Zarco, 1968; Nelson, 1966), CVF has become an important research tool to study the role of complement in both its biological functions as well as in the pathogenesis of disease. By comparing normal (complement-sufficient) animals with complement-depleted animals, the involvement of complement in physiological and pathological situations could be elucidated. Moreover, complement involvement in the pathogenesis of many diseases was first recognized by using animals depleted of their complement with CVF (Vogel, 1991). CVF has also served as the gold standard to compare the efficacy of other complement inhibitors (Hebell et al., 1991). In this manuscript, we will describe the structure and function of CVF and its homology to complement component C3 as well as our work to create human C3 derivatives with the complement-depleting function of CVF (collectively referred to as humanized CVF (hCVF)) as a novel therapeutic approach for treatment of diseases and clinical conditions with complement pathogenesis.

2. Cobra venom factor

CVF is the complement-activating protein in the venom of cobras. Whereas more recent work has established that probably all members of the *Naja* genus and other elapid snakes (*e.g.*, *Ophiophagus*, *Austrelaps*) produce CVF in their venom (Rehana and Kini, 2007; Vogel and Fritzinger, 2010; Zeng et al., 2012), the majority of previous work is based on CVF isolated from the Indian cobra (*Naja naja*) or a closely related Asian cobra species (*Naja kaouthia*) (Vogel and Fritzinger, 2010). CVF is a structural and functional analog of complement components C3 (Vogel, 1991; Vogel and Fritzinger, 2010; Vogel et al., 1984). Fig. 1 shows the schematic chain structures of C3, C3b, C3c, and CVF. CVF exhibits a high degree of sequence homology at both the DNA and protein level with C3 from mammalian and other vertebrate species; and its homology exceeds 90% with C3 from cobra (Fritzinger et al., 1992, 1994; Vogel and Fritzinger, 2010). Like C3, CVF is synthesized as a single-chain pre-pro-protein that is subsequently processed into the mature three-chain protein (de Bruijn and Fey, 1985; Fritzinger et al., 1994; Misumi et al., 1991; Vogel et al., 1996). The genes for C3 and CVF exhibit a high degree of homology with an essentially identical exon/intron structure (Bammert et al., 2002; Vik et al., 1991). The homology of the three chains of CVF with the corresponding chains of C3 has not only been established by amino acid sequence homology and immunological cross reactivity (Eggertsen et al., 1981, 1983; Lundwall et al., 1984; Vogel et al., 1984) but more recently by the crystal structures (Janssen et al., 2005, 2006, 2009; Krishnan et al., 2009). The three-dimensional structures of C3 and CVF display identical domains (Fig. 2). Whereas the three-chain CVF resembles the physiological three-chain degradation product C3c, it differs in one important aspect. In contrast to C3c, CVF contains the intact CUB domain which has been shown to be important for Factor B binding and convertase formation (Janssen et al., 2006, 2009; Vogel and Fritzinger, 2010; Wiesmann et al., 2006) (Fig. 2).

CVF is a glycoprotein. It has three glycosylation sites for N-linked oligosaccharide chains, two of which are in the α-chain and one is in the β-chain (Fritzinger et al., 1994). The overall carbohydrate content of CVF is 7.4% (w/w), which is significantly higher than that of human C3 (1.7%) and other mammalian C3 species (Tomana et al., 1985; Vogel and Müller-Eberhard, 1984). We have extensively characterized the structures of the CVF carbohydrate chains, with the major oligosaccharide chain being a symmetric fucosylated biantennary complex-type chain with an unusual α-galactosylated Le^x structure at its non-reducing end (Supplemental Fig. 1) (Gowda et al., 1992, 2001). However, the glycosylation of CVF is not required for its biological activity as partial or complete deglycosylation as