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Characterization of the interaction between collectin 11 (CL-11, CL-K1) and nucleic acids

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ABSTRACT

Collectins are a group of innate immune proteins that contain collagen-like regions and globular C-type lectin domains. Via the lectin domains, collectins recognize and bind to various microbial carbohydrate patterns. Collectin 11 (CL-11) exists in complex with the complement activating MBL-associated proteases, MASPs. In the present work, we characterize the interaction between CL-11 and DNA, and show that CL-11 binds to DNA from a variety of origins in a calcium-independent manner. CL-11 binds also to apoptotic cells presenting extracellular DNA on their surface. The binding to DNA is sensitive to changes in ionic strength and pH. Competition studies show that CL-11 binds to nucleic acids and carbohydrates via separate binding-sites and oligomericity appears crucial for binding activity. Combined interaction with DNA and mannan strongly increases binding avidity. By surface plasmon resonance we estimate the dissociation constant for the binding between CL-11 and double stranded DNA oligonucleotides to K_D = 9–20 nM. In an *in vitro* assay we find that CL-11 binds to DNA coated surfaces, which leads to C4b deposition via MASP-2. We propose that CL-11, e.g. via complement, may play a role in response to particles and surfaces presenting extracellular DNA, such as apopototic cells, neutrophil extracellular traps and biofilms.

1. Introduction

Collectins are a group of innate immune proteins and structurally defined by including a collagen-like region and C-type lectin domain, also referred to as carbohydrate recognition domain (CRD) (Drickamer, 1988; Hansen and Holmskov, 1998). Well-studied collectins include Mannan-binding lectin (MBL), Surfactant protein A (SP-A) and Surfactant protein D (SP-D) (Benson et al., 1985; Haagsman et al., 1987; Kawasaki et al., 1983), and the group comprises a total of nine collectins, including Collectin 11 (CL-11) also known as Collectin kidney 1 (CL-K1) (Hansen et al., 2010; Keshi et al., 2006). Collectins represent an important group of pattern recognition molecules that bind to oligosaccharide structures and/or glycol-lipid moieties on the surface of microorganisms, and may facilitate their clearance through a variety of mechanisms, such as aggregation, complement activation and opsonization. In addition, collectins modulate inflammatory and allergic responses, as well as the adaptive immune system (Hansen et al., 2007; Holmskov et al., 2003; Wright, 2005). Deficiencies in MBL or other downstream complement components are known to predispose to recurrent infections (Degn et al., 2011; Gaunsbaek et al., 2012).

Many of the collectins bind not only to carbohydrate ligands but also to nucleic acids (Nakamura et al., 2009; Palaniyar et al., 2004). DNA is a pentose-based polymer with negatively charged phosphate backbone and is found extracellular in relation with bacterial biofilms, apoptotic or necrotic cells, and in neutrophil extracellular traps (NET), which consist of proteins and DNA released from activated neutrophils (Medina, 2009). Removal of DNA is essential to maintain inflammation-free tissues and further to prevent autoimmune diseases (Savill et al., 2002). In many clinical conditions, such as systemic lupus erythematosus (SLE), impaired clearance of apoptotic cells has been suggested to play an important role, as a result of increased amounts of autoantigens, such as DNA, present on apoptotic cells (Denny et al., 2006; Frisoni et al., 2005). Both C1q and nucleosomes are also known to serve as targets for complement activation leading to glomerulonephritis in SLE patients (O'Flynn et al., 2011), and alteration in expression or function of complement receptors on B and T cells may also contribute SLE pathogenesis (Erdei et al., 2009).

Abbreviations: CL-11, Collectin 11 (alias Collectin Kidney 1, CL-K1); CRD, carbohydrate recognition domain; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; RU, resonance unit; SPR, surface plasmon resonance; SP-A/-D, surfactant protein A/D.

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CL-11 is a recently characterized collectin and has a mannose-type CRD with calcium-dependent lectin activity with preference for L-fucose and D-mannose. It binds to a variety of microorganisms, including bacteria, fungi and viruses, through partially calcium-dependent interactions (Hansen et al., 2010; Keshi et al., 2006; Selman and Hansen, 2012). CL-11 is a secreted protein, with an estimated plasma concentration of approximately 0.3 μg/mL (Selman et al., 2012; Yoshizaki et al., 2012). It is found in circulation and in association with the MBL-associated serine proteases, MASPs (Hansen et al., 2010). These proteases have previously been shown to be involved in activation and/or modulation of the complement system (Banda et al., 2011; Degn et al., 2012; Skjoedt et al., 2010). Recently, it was found that human CL-11 and MASP-3 deficiency or malfunction strongly associate with the developmental midline-related defect syndrome, designated 3MC syndrome (Rooryck et al., 2011), and these findings suggest a role for complement components in developmental processes.

In our initial identification of CL-11 as a circulating plasma protein found in association with MASPs, we found that CL-11 bound in an uncharacterized way to bacterial DNA (Hansen et al., 2010). The other collectins SP-A, SP-D, and MBL bind also to DNA, and this may lead to appropriate clearance of apoptotic cells (Nakamura et al., 2009; Palaniyar et al., 2005; Vandivier et al., 2002). Furthermore, SP-D interacts with bacteria coated with NETs, which potentially restrains the bacteria in an immediate environment with a high content of NET and hence a high local concentration of antimicrobial components (Douda et al., 2011).

Characterization of the interaction of CL-11 with DNA, in addition to those described for other collectins, may provide further insight into both collectin and complement biology and immunology. In this study we describe the interaction between CL-11 and DNA and find that it is a potent binding, mediated by a site distinct from the lectin binding site of the CRD.

2. Materials and methods

2.1. Buffers, reagents and materials

Unless otherwise stated, reagents were obtained from Sigma–Aldrich, Broendby, Denmark. The used buffers comprised: coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6), PBS (1.45 mM NaH_2PO_4, 6.46 mM Na_2HPO_4, 2.7 mM KCl, 137 mM NaCl, pH 8.0), HBS (10 mM HEPES, 150 mM NaCl, 0.005% surfactant P20, pH 7.4). TBS (30 mM Tris, 125 mM NaCl, pH 7.4), TBS/Ca^{2+}/Em (TBS, 2 mM $CaCl_2$, 0.05% Emulfogen (polyoxyethylene 10 tridecyl ether), TBS/Ca^{2+}/Em/BSA (TBS/Ca^{2+}/Em with 0.1% BSA), TBS/EDTA/Em (TBS, 5 mM EDTA, 0.05% Emulfogen), TBS/EDTA/Em/BSA (TBS/EDTA/Em with 0.1% BSA), substrate buffer (50 mM citric acid, titrated to pH 5.0 with Na_2HPO_4).

2.2. Cloning and expression of recombinant human CL-11

Full length human CL-11 (hCL-11) was cloned and expressed recombinantly in DG44 CHO cells using the pOptiVEC™TOPO® TA Cloning system (Invitrogen, Hellerup, Denmark) as described previously (Hansen et al., 2010). The neck/CRD region of hCL-11, including the Cys^{113}–Glu^{114}–Cys^{115} residues, was cloned and expressed using the same cloning system. In brief, the sequence encoding the neck/CRD region from Cys^{113} to the C-terminal residue Met^{271} was amplified by PCR using the forward primer 5′-**ACCATGG**AGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACTGTGAGTGCGACCAGCTGC-3′ and the reverse primer 5′-TCACATGTTCTCCTTGTCAAACTCACAC-3′. The forward primer included an optimized Kozak consensus sequence (shown in bold) and a mammalian Ig-secretion signal (underlined) upstream of the CL-11 complementary sequence. Cloning and transfection were performed according to the manufacturer's recommendations (Invitrogen).

2.3. Purification of recombinant CL-11 from mammalian cell lines

Full-length CL-11 and neck/CRD were purified by affinity chromatography using a monoclonal anti-CL-11 antibody coupled to CNBr-activated Sepharose. The mAb binds exclusively to CL-11 in the absence of calcium. Culture supernatant from CHO cells expressing full-length CL-11 or neck/CRD was applied onto the mAb-conjugated column in the presence of 5 mM EDTA. Bound CL-11 was eluted with TBS containing 5 mM $CaCl_2$. The purity was analyzed by silver staining and the concentration of full-length CL-11 was determined by ELISA (Selman et al., 2012).

2.4. Generation, purification, biotinylation and coupling of antibodies against hCL-11

Monoclonal mouse- and polyclonal rabbit-anti-human CL-11 Abs were raised against purified full length recombinant CL-11 expressed in DG44 CHO cells (Hansen et al., 2010; Selman et al., 2012). Antibodies were purified from serum-free hybridoma media by Protein G affinity chromatography and labeled with biotin using Biotin N-hydroxysuccinimide ester according to the manufacturer's recommendations. Monoclonal antibodies were coupled to CNBr-activated Sepharose beads according to the manufacturer's recommendations (GE Healthcare, Broendby, Denmark; 5 mg antibody/mL beads).

2.5. SDS-PAGE and Coomassie/silver staining

SDS-PAGE was performed using precast NuPAGE 4–12% Bis-Tris gradient gels (Invitrogen) and NuPAGE MOPS SDS Running Buffer according to the manufacturer's recommendations (Invitrogen). Protein bands were visualized with Coomassie brilliant blue R-250 or by silver staining according to the method of Nesterenko and coworkers with modifications described previously (Hansen et al., 2002; Nesterenko et al., 1994). A molecular-mass marker, Unstained Precision Plus Protein Standards, was used to estimate the molecular masses (Bio-Rad, Hercules, USA).

2.6. ELISA

Unless otherwise stated, the following general ELISA procedures and materials were used in all assays. MaxiSorp™ 96-well plates (Nunc A/S, Roskilde, Denmark) were used for all ELISAs. One M NaCl was used for coating of the various types of DNA and mannan. Coating buffer was used for coating of antibodies and streptavidin (100 μL/well, overnight, 4 °C). Plates were washed with TBS/Ca^{2+}/Em or TBS/EDTA/Em three times between each step in the assay. After coating, wells were blocked in washing buffer for 10 min. Incubation steps were made in TBS/Ca^{2+}/Em or TBS/EDTA/Em with 0.1% BSA. Unless otherwise stated, incubations were made at room temperature and biotinylated antibodies were used in combination with HRP-conjugated streptavidin, 0.25 μg/mL, with incubation times of 1 h or 30 min, respectively. Plates were developed using 0.5 mg/mL *o*-phenylenediamine in substrate buffer with 0.03% H_2O_2, according to the manufacturer's recommendations (Kementech, Taastrup, Denmark). Development was stopped with 1 M H_2SO_4 and plates were read at OD 490 nm with OD 650 nm as subtractive reference.