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Porcine mannan-binding lectin A binds to *Actinobacillus suis* and *Haemophilus parasuis*

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Abstract

Various collagenous lectins involved in innate immunity bind to surface oligosaccharides of bacteria and other microorganisms. We have been characterizing porcine plasma lectins that bind in a carbohydrate-dependent manner to surfaces of important bacterial pig pathogens including *Actinobacillus suis* (AS), *A. pleuropneumoniae* (APP), and *Haemophilus parasuis* (HP). A plasma protein with 32 kDa subunits (pI 5.4 and 5.75) bound most isolates of HP, AS, and some APP. Partial amino acid sequences of this protein were similar to mammalian mannan-binding lectins (MBLs). The corresponding MBL-A cDNA sequences obtained by RT-PCR on liver tissue from pigs and cattle were homologous to the *MBL1* gene of mice, rats and the *MBL1P1* pseudogene of humans and chimpanzees. While human MBL-C, the product of the *MBL2* gene, is known to bind various microorganisms, our studies in pigs provide the first direct evidence that MBL-A has bacteria-binding properties, and suggest it may have antibacterial functions in pigs.

Keywords: Mannan-binding lectin; Porcine/pigs; Collectin; *Actinobacillus pleuropneumoniae*; *Haemophilus parasuis*; *Actinobacillus suis*; Plasma proteins; Innate immunity

1. Introduction

Various evolutionarily conserved collagenous lectins implicated in innate immunity are pattern recognition molecules that can bind to microbial surface glycans [1,2]. The collagenous lectins include mannan-binding lectins (MBL), ficolins, surfactant protein A (SP-A) and D (SP-D), collectin liver 1, collectin placenta 1, conglutinin, CL-43, and CL-46 [2–4]. Collagenous lectins interact with various bacteria, viruses, fungi and protozoa, and have various antimicrobial mechanisms including neutralization, agglutination, and opsonization [2,5–8].

Abbreviations: MBL, mannan-binding lectin; APP, *Actinobacillus pleuropneumoniae*; AS, *Actinobacillus suis*; HP, *Haemophilus parasuis*; GlcNAc, *N*-acetylglucosamine; Glc, glucose; GalNAc, *N*-acetylgalactosamine; Gal, galactose; ManNAc, *N*-acetylmannosamine; Man, mannose; CRD, carbohydrate-recognition domain

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MBLs and ficolins can also activate the lectin complement pathway via interaction with MBL-associated serine proteases (MASP-1, -2, -3) and a small MBL-associated protein (sMAP) [1,9,10], and thereby contribute to microbial killing and the inflammatory response. Most collagenous lectins have been termed collectins because they bind to saccharides such as mannose (Man), fucose (Fuc), and *N*-acetylglucosamine (GlcNAc) via a calcium-dependent C-type lectin domain [11–13]. In contrast, ficolins have a fibrinogen-like domain that binds preferentially to GlcNAc on the surfaces of some microorganisms, and other synthetic matrices in a calcium-independent manner, with the *N*-acetyl groups possibly being important for binding [14,15].

Our laboratory has attempted to identify lectins in porcine plasma involved in innate defense of young pigs to common important bacterial pathogens. Members of the family *Pasteurellaceae*, including *Actinobacillus pleuropneumoniae* (APP), *A. suis* (AS), and *Haemophilus parasuis* (HP), are economically important as they cause significant production losses due to pneumonic and septic disease. Previously, we purified and characterized porcine ficolin α as the major plasma protein that binds to surfaces of APP serotype 5b [14,16]. In the current work, we identified and characterized porcine MBL-A binding to many isolates of AS, HP, and APP. As well, we identified and sequenced a cDNA encoding the porcine MBL-A precursor. This is the first report of a bacteria-binding function for an MBL-A protein in animals.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals utilized were obtained from Fisher Scientific (Ottawa, Ont., Canada) except as noted. Monosaccharides, mannan-sepharose, and oligonucleotide primers were obtained from Sigma (Oakville, Ont., Canada), and Toyopearl Epoxy AF-650 M from TosoHaas (Montgomery, PA, USA). Immobiline Drystrip Gels and IPG buffer were obtained from Amersham-Biosciences Inc. (Baie D'Urfé, PQ, Canada). RNAlater was obtained from Ambion (Austin, TX, USA) and RNA isolation was performed using the TRIzol® reagent, which was obtained from Invitrogen (Burlington, Ont., Canada), as were the Thermoscript RT-PCR System, Platinum Taq DNA polymerase and TOPO TA Cloning® Kit for Sequencing. PCR products were extracted from agarose gels with the QIAquick Gel Extraction Kit from QIAGEN (Mississauga, Ont., Canada) and all DNA sequencing was performed at the Guelph Molecular Supercentre (Guelph, Ont., Canada).

2.2. Tissue collection

Whole blood was collected from healthy adult Yorkshire cross pigs into 3.8% buffered sodium citrate (pH 7.4–9:1 blood to citrate ratio by volume). Platelet-poor plasma was isolated by centrifugation (1000*g*) for 30 min at room temperature, and stored at −70 °C until used. Prior to use in bacteria-binding assays, samples were dialyzed in 10 volumes of TBS-Ca^{2+} buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$) at 4 °C for 24 h.

Tissue samples for RT-PCR were collected from an adult Yorkshire cross male and a 5-week-old male (liver, heart, bone marrow). Liver samples from an adult Black Angus and a Hereford steer were used to amplify bovine MBL-A cDNA for comparison. Tissues were either snap-frozen in liquid nitrogen then stored at −70 °C, or collected into 1 ml RNAlater then incubated at 4 °C for 24 h, −20 °C for 24 h, then stored at −70 °C.

2.3. Bacteria-binding assays

Representative clinical isolates of *Actinobacillus suis*, *A. pleuropneumoniae*, and *Haemophilus suis* were used to identify plasma lectins that bind to the surfaces of intact bacteria in a carbohydrate-dependent manner. APP and AS isolates (Table 1) were grown overnight on blood agar plates (supplemented with 0.02% nicotinamide adenine dinucleotide (NAD) for APP); and HS isolates (Table 1) were cultured overnight on chocolate agar supplemented with 0.02% NAD. Single colonies were transferred to BHI broth, supplemented with 0.02% NAD for APP and HS isolates. Bacteria were harvested by centrifugation (10,000*g* 10 min at 4 °C) and washed three times in TBS-Ca^{2+}. At this point, some cultures were killed by incubating with TBS-Ca^{2+} containing 1% formaldehyde for 1 h at 4 °C. Subsequently live or killed bacteria were washed 3 more times in TBS-Ca^{2+}. Washed bacteria were then incubated with either dialyzed pig plasma (12 ml per ~10^{11} CFU) or TBS-Ca^{2+} as a negative control for 4 h with gentle agitation at 4 °C. Bacteria were then washed 10 times in TBS-Ca^{2+}, and then saccharide-dependent bound proteins were eluted