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Binding of porcine ficolin-α to lipopolysaccharides from Gram-negative bacteria and lipoteichoic acids from Gram-positive bacteria

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Abstract

Protein(s) reactive with *N*-acetyl-D-glucosamine (GlcNAc) was isolated from porcine nonimmune serum. The molecular weight of the purified protein was found to be mainly 40 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. The N-terminal 10 amino acid sequence of the purified protein were found to be identical to that of porcine ficolin-α reported previously. In enzyme-linked immunosorbent assay, the purified protein was found to react with lipopolysaccharides (LPS) from different Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella abortus* equi, *Pseudomonas aeruginosa*, *Shigella flexeneri*, and *Serratia marcescens* and with lipoteichoic acid (LTA) from Gram-positive bacteria such as *Streptococcus sanguis*, *Bacillus subtilis*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. The purified protein also reacted with *E. coli* O26 isolated from food poisoning and bovine feces and heat-treated Gram-positive bacteria such as *S. aureus*, *B. cereus*, *B. subtilis*, *Enterococcus faecium*, and *Corynebacterum bovis*. On the other hand, porcine IgG isolated from nonimmune serum showed different reactivity with these LPS, LTA, and heat-treated bacterial cells. From the present findings, purified porcine serum protein reactive with GlcNAc is concluded to be ficolin-α playing an important role(s) in innate immunity against microbial infection with Gram-positive and -negative bacteria.

Keywords: Ficolin; Porcine; Innate immunity; Lipopolysaccharide; Lipoteichoic acid

1. Introduction

Some of serum lectins are well-known to play important roles in innate immunity of animals and human [1–7]. Mannan and/or mannose-binding lectin (MBL) is best known as an antimicrobial substance which activates the lectin pathway of complement [2,3,5,7]. *N*-acetyl-D-glucosamine (GlcNAc)-binding proteins (or ficolins) in sera of different animal species have been recently reported to play important roles in innate immunity [1,3,4,6,7]. Ficolin is primarily identified as a transforming growth factor (TGF)-β1-binding protein on porcine uterus membranes [8,9]. It also binds to cortisol, elastin, heparin, fibronectin, zymogen and GlcNAc

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in addition to TGF-β [1,3,6–8]. Ficolins of human [10–12], rodents [13,14], hedgehogs [15], and invertebrates [16] have been well characterized at the cDNA and /or protein level, indicating that they are composed of collagen-like and fibrinogen-like domains [8,9] which are structurally similar to complement 1q (C1q) and the collectins.

Three types of human ficolins have been isolated: L-ficolin/P35 from serum/plasma, Hakata antigen (H-ficolin) from serum/plasma, and M-ficolin from surface monocytes [1,6]. Human L-ficolin binds to GlcNAc of lipopolysaccharides (LPS) derived from Gram-negative bacteria [10,17] and lipoteichoic acid (LTA) derived from Gram-positive bacteria [18]. Human L- and H-ficolins have been reported to participate in complement activation upon binding to some microbial surfaces [2–4]. Furthermore, the L- and H-ficolins play an important role in innate immunity in a similar manner as found with MBL [6,7] and are complexed with MBL-associated serine proteases (MASPs and sMAP) that lead to complement activation upon binding to bacterial surfaces [3,4,7,19].

According to Ichijo et al. [8], cDNA encoding two types of porcine ficolins, named ficolin-α and ficolin-β, have been isolated. They show 81–84% identity at the amino acid level [9,13]. Porcine ficolin-α and -β show a distinct difference in their tissue distribution. Antibody against the fibrinogen-like domain of the bacterially expressed pig ficolin-α reacted with pig serum ficolin [13], suggesting that porcine ficolin-α may be mainly found in plasma and/or serum. Porcine ficolin-α mRNA is expressed preferentially in lung, liver and bone marrow but low in uterus, whereas porcine ficolin-β mRNA is abundantly expressed in skeletal muscle and bone marrow but poorly in uterus [9,14].

Less is known about the functional role(s) of porcine ficolin although porcine plasma ficolin has been reported to bind to the important pig pathogen *Actinobacillus pleuropneumoniae* serotype 5B in a GlcNAc-dependent manner [20]. From the structural similarities between human and porcine ficolins [14], the fibrinogen-like domain of porcine ficolins is suggested to contribute in innate immunity by eliminating microbial pathogens exposing GlcNAc. Therefore, in this study, attempts were made to isolate a GlcNAc-binding protein (or ficolin and/or ficolin-like substance) from porcine nonimmune serum and to study the binding of purified porcine GlcNAc-binding protein to lipopolysaccharides (LPS) derived from different Gram-negative bacteria and lipoteichoic acid (LTA) derived from different Gram-positive bacteria to finally elucidate the role of porcine serum ficolin (or GlcNAc-binding protein) in innate immunity against bacterial infection.

2. Materials and methods

2.1. Chemicals and reagents

GlcNAc-Sepharose 4B was the product of Sigma Aldrich, Inc. (St. Louis, USA). Protein A-Sepharose 4B, Sephacryl S-300, CNBr-activated Sepharose 4B, and Q Sepharose FF were obtained from Pharmacias, (Uppsala, Sweden). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG was obtained from BioSource International (Carmillo, USA). ExtrAvidin-conjugated peroxidase and alkaline phosphatase-conjugated goat anti-rabbit Igs were the products of Sigma Aldrich Co. (St. Louis, USA). Molecular weight markers for sodium dodecyl sulfate electrophoresis (SDS-PAGE) were obtained from Bio-Rad Laboratories and Pharmacia. Freund's complete adjuvant was obtained from Difco Laboratories (Detroit, USA).

The lipopolysaccharides (LPS) derived from *Salmonella typhimurium* (wild type, and Re mutant SL1181), *Salmonella enteritidis*, *Salmonella abortus* equi, *Escherichia coli* (serotype O26:B6, serotype O55:B5, serotype O111:B4, Ra mutant EH100, and Rc mutant J5), *Pseudomonas aeruginosa* serotype 10, *Shigella flexeneri* serotype 1A, *Serratia marcescens* were the products of Sigma Chemical Co. (St. Louis, USA).

The lipoteichoic acids (LTAs) derived from *Streptococcus sanguis*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus* were also the products of Sigma Chemical Co. (St. Louis, USA).

E. coli 026 strains Osaka-1, Osaka-3, Osaka-4, and Osaka-8 were isolated from outbreaks of food poisoning, whereas *E. coli* O26 strains 1–28, H-265, TN 9-15, and HB-2000 were isolated from bovine feces. All strains of *S. aureus* 209P, *B. cereus*, *B. subtilis*, *Enterococcus faecium* ATCC 19434,