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ScienceDirect
Developmental and Comparative Immunology 31 (2007) 830–846
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Gene polymorphisms associated with reduced hepatic expression of porcine mannan-binding lectin C

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Received 16 October 2006; received in revised form 10 November 2006; accepted 14 November 2006
Available online 11 December 2006

Abstract

Previous studies showed that low expression of mannan-binding lectin C (MBL-C) in pigs was not due to single-nucleotide polymorphisms (SNPs) in the coding region of pig *MBL2*. In these studies, we compared the 5′ flanking regions of porcine *MBL1* (1907 bp) and *MBL2* (1880 bp) in normal and diseased pigs with low or high hepatic expression of *MBL2*. Hepatic expression of MBL-C was very low in all pigs submitted for postmortem diagnosis. In various European pig breeds, a G(–1081)A substitution was linked to very low hepatic MBL-C expression, and was more frequent in diseased pigs. A C(−251)T substitution with less influence on MBL-C expression was more common in various breeds but was not associated with disease. *MBL2* polymorphisms were associated with some disease groups and with the presence of some etiologic agents. These findings indicate that some promoter polymorphisms impair MBL-C expression in pigs and may increase their susceptibility to disease.

Keywords: Mannan- (mannose-) binding lectin; Collectin; Innate immunity; Polymorphism; Porcine/pig; Promoter

1. Introduction

Mannan-binding lectins (MBLs) are collagenous proteins that play various normal roles in innate immunity. They can bind carbohydrate patterns found on various microbial surfaces and host macromolecules, activate the lectin complement pathway, enhance phagocytosis, and contribute to specific immune responses [1–3]. Most mammalian species, including pigs, have 2 forms of MBL (A and C) that are encoded by the *MBL1* and *MBL2* genes, respectively [4–8]. Plasma of humans and chimpanzees contains only MBL-C because their *MBL1* gene is mutated to an expressed pseudogene (*MBL1P1*) [9,10]. In humans, single-nucleotide polymorphisms (SNPs) in structural and promoter sequences of the *MBL2* gene can affect the assembly or production of MBL-C and contribute to low plasma MBL and innate immune dysfunctions [11–13]. Combinations of *MBL2* SNPs are haplotype markers for increased susceptibility to various infectious, autoimmune and other noninfectious conditions in humans [14–19].

Abbreviations: MBL, mannan-binding lectin; SNP, single-nucleotide polymorphism; MASP, MBL-associated serine protease; RT-PCR, reverse-transcriptase polymerase chain reaction; B2M, β2-microglobulin

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0145-305X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2006.11.002

MBL mutations are also predicted to contribute to the variation in susceptibility of animals to various infections [20,21] but it has not been thoroughly investigated how mutations in one MBL gene affect the immune response of mammals that express two MBL genes. However, in mice, MBL-A and MBL-C have different oligomeric structures that might suggest different functions [22]. Mice with deletions of both *MBL1* and *MBL2* genes had increased susceptibility to various pathogens [23–25], whereas deletion of only *MBL1* either had no change in susceptibility [26], or had enhanced survival in an acute septic peritonitis model [27]. Interestingly, while neither MBL-A knockout mice nor MBL-C knockout mice were similar to wild-type mice in a *Staphylococcus aureus* infection model, complement activating activity of MBL-A was four times that of MBL-C, but their total complement activating activity in serum was approximately equal as serum levels of MBL-C were three times higher than MBL-A [28]. Binding ligands such as mannan and intact bacteria extract different proportions of MBL-A and MBL-C from pig serum [4,5,7,29] but it is not clear how this relates to genetic or acquired differences in amounts or functions of these proteins in pig plasma. Polymorphisms in the collagen-like domain of *MBL2* affect plasma MBL levels in humans [11,30,31], and have recently been found in the *MBL1* gene of various breeds of pigs in Canada [7]. Such miscoding or possibly promoter polymorphisms might account for low serum MBL-A concentrations that are genetically linked in Danish pigs [5]. Low serum MBL correlates with increased disease in horses but the genetic basis of this is not known [32].

Variable expression of MBL-C associated with gene promoter polymorphisms like those described in humans [11–13], has not yet been identified in animals. However, hepatic expression of MBL-C is variable among pigs with similar expression of MBL-A [33]. Expression of MBL-C is induced by inflammatory stimuli of the acute phase response in humans [34] but not in mice, in which only MBL-A is a mild acute phase responder [22]. However, expression of porcine MBL-C and MBL-A did not correlate with expression of haptoglobin or pig major acute phase protein (Pig MAP or inter-α-trypsin inhibitor heavy chain 4) which are acute phase-inducible in this species [33,35]. In this study, we compared the 5′ flanking sequences of the pig *MBL1* and *MBL2* genes to identify those SNPs that correlated with low hepatic expression or increased illness. We also quantified mRNA expression of MBL-A and MBL-C in relation to expression of haptoglobin. Expression and SNP frequencies were then compared between pig breeds and between healthy and diseased pigs.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were obtained from Fisher Scientific (Ottawa, ON, Canada) except as noted. The GenomeWalker Universal kit and Advantage 2 PCR kit were obtained from BD Biosciences (Mississauga, ON, Canada). Oligonucleotide primers and the GenElute Mammalian Genomic DNA Miniprep Kit were obtained from Sigma (Oakville, ON, Canada). RNAlater was obtained from Ambion (Austin, TX, USA) and RNA isolation was performed using the TRIzol® Reagent (Life Technologies, Burlington, ON, Canada). The Thermoscript RT-PCR System and Platinum Taq DNA polymerase were obtained from Invitrogen (Burlington, ON, Canada). The Fast Start SYBR Green Plus reagents from Roche Diagnostics (Laval, PQ, Canada) were utilized in the Real Time PCR assays. All restriction endonucleases and associated buffers were obtained from Fermentas Canada Inc. (Burlington, ON, Canada). The DNeasy Tissue Kit was obtained from QIAGEN (Mississauga, ON, Canada). DNA sequencing was performed at the Guelph Molecular Supercentre (Guelph, ON, Canada).

2.2. Tissue collection

Since the liver is the main site of expression for both porcine MBL-A [7] and porcine MBL-C [4], liver samples for RNA isolation were collected from a range of clinically healthy pigs from multiple commercial breeders in Ontario [36] and diseased pigs submitted for euthanasia and necropsy to the Animal Health Laboratory (AHL), University of Guelph, Guelph, ON, Canada [33]. Tissues were either snap-frozen in liquid nitrogen then stored at −70 °C, or collected into 1 ml of RNAlater then incubated at 4 °C for 24 h, then −20 °C for 24 h, before being stored at −70 °C. Total RNA (~1.8 μg/μl) was isolated from liver samples with the TRIzol® Reagent (Life Technologies), based on the phenol-guanidine isothiocyanate method [37] and stored at −70 °C. Tissues for DNA extraction