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Genetic variability in swine leukocyte antigen class II and Toll-like receptors affects immune responses to vaccination for bacterial infections in pigs

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ABSTRACT

The genes encoding swine leukocyte antigen (SLA) and Toll-like receptor (TLR) are highly polymorphic in pig populations, and likely have influences on infection and the effects of vaccination. We explored the associations of different genotypes of SLA class II and of the genes *TLR1*, *TLR4*, *TLR5*, and *TLR6* with antibody responses after vaccination against *Erysipelothrix rhusiopathiae* (ER) and *Actinobacillus pleuropneumoniae* (APP) serotypes 1, 2, and 5 in 191 Duroc pigs maintained under specific pathogen-free conditions. We demonstrated close relationships between SLA class II and ER antibody response and between *TLR* genes other than *TLR4* and APP antibody responses. Pigs with specific haplotypes in SLA class II or *TLR5* showed decreased antibody response to ER vaccination or increased responses to APP2 and APP5 vaccination, respectively. It might be possible to breed for responsiveness to vaccination and to implement new vaccine development strategies unaffected by genetic backgrounds of pigs.

1. Introduction

In the modern swine industry, intensive husbandry that invites deterioration of the feeding environment and an increase in stress increases susceptibility to respiratory and intestinal infections [1]. Antibiotics are used to suppress the infection rates and to cure affected animals. However, because of prohibition of the prophylactic use of antibiotics in many countries and consumer antipathy toward drug residues, a decrease in antibiotic use is desirable. Although the use of vaccination to prevent infection is beneficial as an alternative to antibiotic use, there are large differences in the efficacy of vaccination among individuals, even with the use of the same vaccine. This difference is caused not only by environmental factors but also by genomic diversity among vaccinated individuals [2,3], especially in terms of the genes coding for major histocompatibility complex (MHC) and Toll-like receptors (TLRs).

MHC molecules present antigens to T cells. These molecules are classified into class I and II groups, which are responsible for antigen presentation to $CD8^+$ and $CD4^+$

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T cells, respectively [4]. Antigenic peptides derived from bacteria are presented mainly on MHC class II molecules by dendritic cells (DCs) and other antigen-presenting cells and are recognized by $CD4^+$ T cells; therefore, vaccination against bacterial infection exerts its effect mainly through class II, rather than class I, molecules [5].

In contrast, TLRs play a central role in the innate immune system that recognizes the patterns of specific molecules derived from microorganisms, such as lipopolysaccharides, lipopeptides, and nucleic acids [6]. TLRs are critical proteins that link innate and acquired immunity, and TLR ligands are used as vaccine adjuvants to improve the efficiency of vaccination [7,8]. Inflammatory stimuli mediated principally by TLRs and other pattern-recognition receptors promote the accumulation of MHC class II molecules along with antigenic peptides on the surfaces of immature DCs, resulting in maturation of DCs and effective presentation of the antigens to T-cells [9,10].

The associations between polymorphisms in MHC class II genes and antibody responses after vaccination have been studied in many animals, including humans and domestic animals such as sheep, chickens, dogs, and cattle [11–17]. In contrast, similar studies in TLR genes have so far been limited to humans [18–21], and there has been limited work on the effects of genetic polymorphisms on antibody production after vaccination in pigs [22]. In pigs, MHC is designated "swine leukocyte antigen" (SLA) and is highly polymorphic, as in other vertebrates [23,24]. The latest nomenclature update report defined 125 alleles of SLA class I genes and 164 alleles of SLA class II genes [24]. Furthermore, new alleles and even new loci observed in certain haplotypes have been reported since this update [25], showing the complexity of this region. Similarly, we have reported the existence of many single nucleotide polymorphisms (SNPs) in porcine *TLR1*, *TLR2*, *TLR4*, *TLR5*, and *TLR6* genes, which are predicted to be involved in the recognition of bacteria [26]. These findings suggest that there are differences among individual pigs in the ability to recognize pathogen-derived molecules.

Here, we explored the influences of haplotypes of SLA class II and porcine TLR genes on the efficacy of antibody responses after vaccination against two pathogens that have long been problematic, namely *Erysipelothrix rhusiopathiae* (ER) and *Actinobacillus pleuropneumoniae* (APP), and we demonstrated differences in vaccination response among genotypes. ER is the main causative agent of swine erysipelas, and infections with this organism in humans have become a problem in farms and slaughterhouses [27]. APP, which is classified into 15 serotypes on the basis of capsule structure, causes porcine pleuropneumonia; serotypes 1 (APP1), 2 (APP2), and 5 (APP5) are common in Japan [28–31]. Our results show that it may be possible to produce effective vaccines against bacterial infections and to breed pigs on the basis of responsiveness to vaccination.

2. Materials and methods

2.1. Pigs

Experimental pigs were produced from 2005 to 2006 by mating of Duroc breeding stock consisting of 28 dams and 11 sires and were maintained under specific pathogen-free (SPF) conditions at the Shizuoka Swine and Poultry Experiment Center, Japan. We used 206 progeny reared under the same SPF facility for vaccination and blood collection. All experiments were approved by the Center under the bylaws of Shizuoka Prefecture.

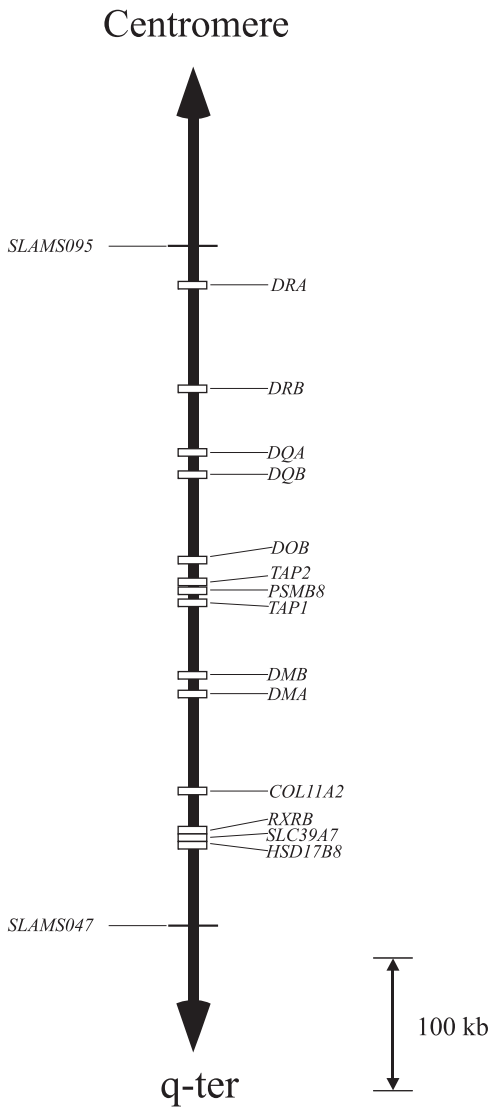

Fig. 1. Map of the SLA class II genomic region on chromosome 7. Gene loci are indicated by open rectangles and developed microsatellite markers are indicated by horizontal lines.

2.2. Vaccination and antibody titration

At 35 and 60 days after birth, progeny were inoculated with a 1 mL of combined vaccine of inactivated ER and inactivated APP1, 2, and 5 (Pigwin-EA; Kyoto Biken Laboratories, Uji, Japan) in accordance with the manufacturer's instructions. The vaccine consisted of antigens of ER (Kyoto strain) extracted with the bacterial cells by NaOH [32] and cell-free antigens of APP serotypes 1 (strain Y-1), 2 (strain G-4), and 5 (strain E-3) prepared from culture