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Swine Immunity and Genetic Resistance to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Infection

Joan K. Lunney

APDL, BARC, USDA, Beltsville, MD, United States

Derek Petry

University of Nebraska - Lincoln

Rodger Johnson

University of Nebraska - Lincoln, rjohnson5@unl.edu

Daniel Kuhar

APDL, BARC, USDA, Beltsville, MD, United States

Ramon Molina

Iowa State University, Ames, IA, United States

See next page for additional authors

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Authors

Joan K. Lunney, Derek Petry, Rodger Johnson, Daniel Kuhar, Ramon Molina, Jane Christopher-Hennings, Jeffrey Zimmerman, and R. R. R. Rowland

4, 8 and 12 post-infection (pi) for PK-15 or 0, 12, 18 and 24 h pi for iDCs. Total RNA transcripts were labeled and hybridized onto DNA chips comprising 80 PrV amplicons covering the whole viral genome and a set of 1663 cellular genes, including 420 genes mapping to the extended major histocompatibility (MHC) locus, 73 immune genes outside the MHC and 1170 randomly chosen genes. In PK-15 cells, a high increase in viral gene expression was found 4 h pi and most viral genes were detected differentially expressed 12 h pi in both cell types. No early global cellular gene shut off occurred and the highest number of differentially expressed cellular gene was observed 8 h pi in PK-15 cells. The results showed that MHC class I genes were down-regulated in both infected cell types and that MHC class II genes were also down-regulated in iDCs. Genes that are involved in other pathways such as apoptosis, protein metabolism and modification were also identified as differentially expressed. Real time quantitative PCR experiments confirmed the down-regulation of MHC class Ia genes, as well as TAP1, TAP2, LMP2 and LMP7 all involved in class I antigen presentation pathway, the down-regulation of cyclophilin A and the up-regulation of TNFA in infected PK-15 cells. Validation of the down-regulation of class II antigen presentation pathway in infected iDCs is in progress. The present comparative study will provide new data on time-dependant differences according to the host cell during PrV infection.

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Genomic analysis revealed the duplication model of porcine CD1 genes during evolution

Tomoko Eguchi-Ogawa^{1,2,*}, Takeya Morozumi^{1,3}, Maiko Tanaka^{1,3}, Hiroki Shinkaj^{1,3}, Naohiko Okumura^{1,3}, Kohei Suzuki^{1,3}, Takashi Awata^{1,2}, Hirohide Uenishi^{1,2}

¹ Animal Genome Research Program, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

² Division of Animal Sciences, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

³ Second Research Division, STAFF-Institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan

Keyword: CD1

E-mail address: egutomo@affrc.go.jp (T. Eguchi-Ogawa).

Species: Swine

CD1 is an MHC class I-like protein that presents lipid antigens to T cell receptors. To clarify the variety and genomic structure of porcine CD1 gene, we constructed a bacterial artificial chromosome (BAC) contig, and determined 470,187 bp of the region encoding the CD1 genes. We identified 16 genes in this region and newly identified CD1A2, CD1B, CD1C, CD1D, and CD1E in addition to formerly identified porcine CD1 gene, CD1.1 (CD1A1, homolog of human CD1A). RT-PCR analysis showed CD1A1, CD1B, CD1D and CD1E were expressed, and suggested both group 1 (CD1A1, CD1B, CD1E) and group 2 (CD1D) CD1 genes

are functioned in pig. Tyrosine-containing motif involved in CD1 intracellular trafficking was conserved in the C termini of porcine CD1B and CD1D. The C terminus of porcine CD1b shared a similar motif with human CD1d, and porcine CD1d had similar motif with that of human CD1b; therefore, porcine CD1d may complement the function of human CD1b in pigs. Southern blot hybridization with several breeds of pig genome was performed using the exon 4 sequence encoding the C-like-domain [D3] of CD1.1 as a probe. Although slight difference in restriction sites was observed among the breeds, we confirmed that no more than six CD1 genes existed on the porcine genome. Genomic sequence analysis showed that porcine CD1 genes were located in clusters between KIRREL and olfactory receptor (OR) genes, as observed in humans, although they were divided into two regions by a region encoding OR genes. Comparison of the genomic structure encoding CD1 genes in pigs with other mammals showed that separation of the CD1 gene cluster by ORs was observed only in pigs. To investigate the process of evolution of the region where the porcine CD1 genes are located, we identified characteristic repetitive sequences commonly found close to the human and porcine CD1A genes. By estimation of the time of CD1A duplication by using the repetitive sequences, we conclude CD1A duplication in the porcine genome might occur after the divergence of the human and porcine. We constructed the schematic model of porcine CD1 and OR gene duplication, which indicated that the unique split structure of the CD1 cluster in the pig had been established before the shuffling of the OR genes in the artiodactyl lineage. This analysis of the genomic sequence of the porcine CD1 family will contribute to our understanding of the evolution of mammalian CD1 genes.

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Swine immunity and genetic resistance to porcine reproductive and respiratory syndrome virus (PRRSV) infection

Joan K. Lunney^{1,*}, Derek Petry^{2,3}, Rodger Johnson², Daniel Kuhar¹, Ramon Molina⁴, Jane Christopher-Hennings⁵, Jeffrey Zimmerman⁴, R.R.R. Rowland⁶

¹ APDL, BARC, USDA, Beltsville, MD, United States

² University of Nebraska, Lincoln, NE, United States

³ Triumph Foods, United States

⁴ Iowa State University, Ames, IA, United States

⁵ South Dakota State University, United States

⁶ Kansas State University, Manhattan, KS, United States

Keywords: Porcine reproductive and respiratory syndrome; Resistance/susceptibility; Immune gene expression; Cytokine regulation

E-mail address: jlunney@anri.barc.usda.gov (J.K. Lunney).

Species: Swine

Current vaccines are only partially effective against porcine reproductive and respiratory syndrome (PRRS) virus infection because they elicit a weak immune response

that is not fully protective. PRRS is the most economically significant disease facing the swine industry today, costing U.S. pork producers at least \$560 million annually. Despite substantial research efforts the exact components of a protective anti-PRRSV immune response are still not known, thus we are testing alternate approaches to evaluate immunity and genetic resistance to PRRSV. We used host genomics to compare different lines of pigs and look for factors that correlated with PRRSV resistance/susceptibility. Viremia, weight change, and rectal temperature at 0, 4, 7, and 14 days post-PRRSV infection (dpi) were recorded and genetic differences detected (Petry et al., 2005). We evaluated immune gene expression in RNA from frozen lung and bronchial lymph node (BLN) tissue of the 7 highest and lowest responders per line, and from each of their control littermates, as well as serum cytokine protein levels. Genetic analyses of this data indicated that levels of interleukin-8 (IL8) may be predictive of resistance. Additionally, low (not the expected high) levels of serum interferon-gamma (IFNG) after infection may be associated with a PRRSV resistant phenotype. These data are critical for genetic association studies to fine map candidate genes and determine causative alleles of PRRSV resistance/susceptibility. Further genetic studies are required to affirm these associations. For direct immunity studies we have assessed immune gene expression in lung, BLN, and tonsil samples, and protein expression in serum, collected from pigs infected for over 200 days after PRRSV infection. We compared pigs that apparently cleared the viral infection in the first 28 dpi to pigs that even at 150 dpi have evidence of long term persistent PRRSV infection. Results show that there is up regulation of expression of IFNG associated T helper 1 (Th1) markers from 14 to 84 dpi; regulatory IL10 and apoptosis associated markers are also increased early. To date, however, no significant differences between persistent and non-persistent PRRSV infected pigs have been discovered in immune gene expression; serum protein expression studies are underway. We hope to reveal differential protein expression associated with PRRSV clearance. Overall, by combining these diverse approaches, we expect to develop new hypotheses about protective anti-PRRSV responses and to identify novel regulatory pathways that would stimulate PRRSV immunity.

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Identification of *Boophilus microplus* phagotopes from phage displayed peptide libraries

Carlos Roberto Prudencio*, Aline Aparecida, Rezende Rodrigues, Guilherme Rocha, Lino Souza, Juliana Franco Almeida, Ana Paula, Peres Freschi, Rone Cardoso, Fausto Emílio Capparelli, Luiz Ricardo Goulart

Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia and Vallée S/A, Uberlândia, MG, Brazil

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E-mail address: crprudencio@gmail.com (C.R. Prudencio).

Species: Other

The ticks cause serious economic losses to animal production worldwide, in the order of billions of dollars. Phage display techniques have been widely employed to map the epitope structures which have served as the basis for developing molecular vaccines. In the present study, we applied this technique to map the epitopes of *Boophilus microplus* and directly evaluated the immune responses in mice to verify immunogenicity of the selected phage-displayed epitopes (phagotopes). Seven phage-displayed random peptide libraries were biopanned in different situations of stringency with the purified IgY of chicken anti-*B. microplus* hyperimmune serum and the selected phage clones were sequenced and analyzed. Some of the inserts of the selected phagotopes showed a good match with the known proteins of *B. microplus*. Others, which did not match with any known proteins, but shared extensive homology with each other, were clustered and classified as the conformational epitopes of *B. microplus*. To evaluate the potential of using these phagotopes as effective vaccines, several phage clones were chosen to immunize mice. The serum raised by the phage clones clearly recognized tick proteins indicating that the phagotope-induced immune responses were antigen-specific. The present work demonstrates that the whole epitope profile can be obtained through screening the phage displayed peptide libraries with the hyperimmune serum and reveals the potential of using epitope-displaying phages as peptide vaccines.

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