Veterinary Immunology and Immunopathology 174 (2016) 35–44

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Comparison of the immune competence of Turopolje, German Landrace × Turopolje, and German Landrace × Pietrain pigs after PRRSV vaccination

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 27 March 2016
Accepted 4 April 2016

Keywords:
PRRSV
Immune competence
Turopolje pig
Pig breeds
Gene expression
ELISA

ABSTRACT

The competences of the immune systems of the ancient pig breed Turopolje (T×T), German Landrace × Turopolje (L×T) and 'modern' pig breed German Landrace × Pietrain (L×P) were compared in this study. All pigs were immunized with a modified live vaccine against 'Porcine Reproductive and Respiratory Syndrome' (PRRS) virus (Ingelvac PRRS MLV®) to simulate an infection. Antibody production against PRRS MLV was evaluated in serum. Elimination of the viral infectious fragments during the experimental period was monitored in serum, leukocytes and tonsils by RT-qPCR. Furthermore relevant immune marker genes were quantified either on gene expression level using RT-qPCR [toll like receptor (TLR) 7, TLR8, TRAF6, CD163, SIGLEC1, CD4, CD8, CD14, CD19, tumor necrosis factor alpha (TNFα), interleukin (IL) 1, IL2, IL6, IL12], and on protein level using ELISA [interleukin (IL)-1, IL-2, IL-6, and IL-12]. The three breeds showed individual inactivation efficiencies as a reaction to the PRRS MLV vaccination. T×T eliminated the virus in serum within 16 days, followed by L×T (28 days) and L×P (36 days). The antibody titers against PRRS MLV of L×T and L×P were significantly higher compared to T×T ($p < 0.05$). The gene expression data and protein analysis of interleukins revealed that T×T reacted with a type 1 immune response. In contrast, the two other breeds (L×T and L×P) showed a type 2 immune response, which resulted in the higher synthesis of B-cells and an increased concentration of specific anti-PRRS MLV antibodies.

1. Introduction

The porcine reproductive and respiratory syndrome (PRRS) was first reported in the USA in the late 1980 (Jing et al., 2014). PRRS evolved to a disease with high economical influence. The PRRS virus (PRRSV) is a single stranded RNA (ssRNA) virus belonging to the Arteriviridae of the order Nidovirales (Lyoo, 2015). The typical symptoms of PRRS are reproductive disorders and respiratory inflammation; furthermore secondary symptoms are lung infections due to the massive destruction of the alveolar macrophages (Done and Paton, 1995). PRRSV infected pigs develop a specific cell-mediated immune response (Osorio et al., 2002; Lyoo, 2015). In various studies it was shown that the immune response to PRRSV, in contrast to other viruses such as Aujezky's disease, starts with a significant delay and significantly weaker (Meier et al., 2003). This delay could be a reason that PRRSV is eliminated only after months (Allende et al., 2000). The specific characteristic of the virus is that there may be a simultaneous circulation of antibodies and virus in the blood (Rossow, 1998), which means that a high titer of neutralizing antibodies is not associated with an increased protection against the virus (Nelson et al., 1994; Loving et al., 2015). Thus, it is apparent that the increase in detectable antibodies in the bloodstream is not associated with the total elimination of the virus in the organism (Bilodeau et al., 1994). This phenomenon can be explained as follows: The antibody may well eliminate the virus in the blood, but not in the lymph nodes, where PRRSV persists and therefore the infection usually lasts longer than 60 days. The interferon gamma (IFN-γ) concentration during infection is low and the virus persistence in lymph nodes shows that the early immune response cannot eliminate the virus completely (Murtaugh et al., 2002). The virus infectivity is associated with the receptor activation of CD163 and SIGLEC1, expressed by macrophages during the infection (van Gorp et al., 2008). CD163 is the best-known receptor for the virus penetration into the cells (Zhang and Yoo, 2015).

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http://dx.doi.org/10.1016/j.vetimm.2016.04.003
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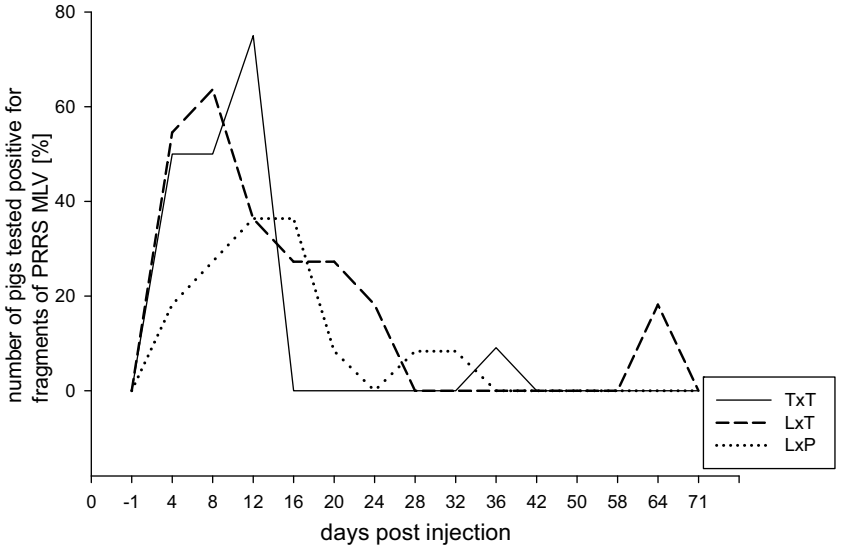

Fig. 1. Comparison of the percentage of pigs tested positive for the PRRS MLV fragments in serum between Turopolje (T×T), Turopolje × German Landrace (L×T) and German Landrace × Turopolje (L×P), PRRS MLV: modified live vaccine against porcine reproductive and respiratory syndrome virus.

But it appears that the viral glycoprotein Gp5 binds to SIGLEC1 and therefore the complex can penetrate into the cytoplasm of macrophages or monocytes (Zhang and Yoo, 2015; Loving et al., 2015). SIGLEC1 modulates the immune response and is found in tissue macrophages (Zhang and Yoo, 2015). These cells activate the Toll-like-Receptor (TLR) pathway. Consequently the concentration of interleukin (IL)-4, IL-10 and IL-12 are increased and cytotoxic T-cells are reduced (Dwivedi et al., 2012). Gomez-Laguna et al. (2010) found that genotype 1 PRRSV promoted the secretion of IFN-α by macrophages, but despite the higher concentration of proinflammatory cytokines, replication of the virus was not minimized. It is also discussed that the IL-10 production as an anti-inflammatory cytokine is up-regulated by proinflammatory cytokines, such as IL-6, IL-12, TNF-α and IFN-γ to inhibit immune defense (Liu et al., 2010; Gomez-Laguna et al., 2010; Renukaradhya et al., 2010). The immune response against genotype 2 PRRSV of different pig breeds showed that the vaccination with a live attenuated vaccine led to an efficient higher concentration of antibodies against the PRRSV in the pig breed 'Wiesnauer miniature pigs' than in the Pietrain pigs (Reiner et al., 2010). Halbur et al., (2011) were able to discover a similar phenomenon with a crossbreed of Large White × Duroc, though the study was conducted with the American virus strain. These reported studies show that the genetic background has a major impact on the immune defense against PRRSV. The aim of our study was to evaluate, whether there are differences in the immune defense against PRRS MLV between the 'ancient' pure Turopolje breed (T×T) and the 'modern' German Landrace × Pietrain (L×P) or German Landrace × Turopolje (L×T). Turopolje is an endangered pig breed from Croatia listed on the FAO watch list (FAO, 2000). Breeding programs in Germany and Austria try to conserve the genetic background of this robust and frugal breed to a potential use for meat production in the future. Since there is no knowledge about the immune defense in Turopolje pigs, our study was designed to investigate the ability of Turopolje and their crossbreed to cope with PRRS MLV. If these results can be transferred to a genotype 2 field PRRSV infection needs to be evaluated in further studies. But maybe it is possible to use the genetic background of the ancient breed to improve the immune defense against PRRSV by farming crossbreeds with modern breeds.

2. Material and methods

2.1. Animal experiment

The animal trial was approved by the government of Upper Bavaria (AZ 55.21-54-2532.3-68-11). Ten piglets per race were produced at the 'Experimental Station Thalhausen', Technische Universität München (Kranzberg, Germany). Two Turopolje boars and 3 Turopolje sows were borrowed from the Tierpark Arche Warder e.V., Zentrum für alte Haus- und Nutztierrassen e.V. (Warder, Germany) to breed the piglets. Six German Landrace sows were provided by the 'Experimental Station Thalhausen'. The sperm of one Pietrain boar was purchased from an artificial insemination station (Schweineprüf-und Besamungsstation Oberbayern-Schwaben e.V., Bergheim, Germany). All animals of the F0 generation were tested prior to the commencement for PRRSV antibodies to ensure that all animals were PRRSV negative (Society for Innovative Veterinary Diagnostics GmbH, Hannover, Germany).

The sows were oestrus synchronized (2 ml of PGF Veyx forte, Veyx, Schwarzenberg, Germany). Two different Turopolje boars were used for the insemination of 3 Turopolje sows (T×T) and 3 German Landrace sows (L×T), respectively. Both breeds were occupied by a natural mating. Another 3 German Landrace sows were artificially inseminated with semen from one Pietrain boar (L×P). The insemination was carried out in crates from the herd manager of the experimental station. After a positive pregnancy check by ultrasound scan, the sows were placed in a herd system with outdoor area and an ad libidum feeding. One week before the expected birth date, the sows were placed in the farrowing pens. The compartments were divided into eight farrowing pens. Each farrowing pen was equipped with a nipple drinker and automatic feeding system for the sow and a piglet pen with red light (30 °C). The birth of all piglets was monitored and documented. The piglets were weighed and immediately post nartum (p.n.) marked with earmarks. On the third day p.p. all piglets were given 2 mg/kg Iron Dextran (Eisen-Dextran, Serumwerk Bernburg AG, Bernburg, Germany). The males were castrated on the same day and treated with 0.4 mg/kg Metacam (Boehringer Ingelheim, Ingelheim, Germany). The piglets were weaned in the fourth week p.p. and stabled in the flat deck sorted by race. Randomly eight piglets were arranged per experimental group. The pens were equipped with feed dispenser (ad libidum