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Short communication

Tolerance and immune response to the porcine endogenous retrovirus in German landrace pigs immunised with viral proteins

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ABSTRACT

Immunisation of goats, mice, rats, rabbits, guinea pigs, and hamsters with the recombinant ectodomain of the porcine endogenous retrovirus (PERV) transmembrane envelope (TM) protein (p15E) induced binding and neutralising immune antibodies in all animals. In contrast, no antibodies were induced when pigs were immunised with p15E, indicating that pigs are tolerant to their endogenous retroviruses, at least to the TM protein. To answer the question of whether pigs are tolerant to other structural proteins of PERV, we immunised German landrace pigs with p15E, this time in conjunction with the surface envelope proteins gp70 and the core capsid Gag protein p27CA. To ensure that the pigs were immunocompetent and that immunisation was successful, all animals also received an injection of an unrelated protein, keyhole limpet hemocyanin (KLH). Whereas all animals produced antibodies against KLH, no animals produced antibodies against the viral envelope proteins, thus confirming previous results for p15E and extending them to the other envelope protein, gp70. However, the pigs did produce antibodies against p27CA, indicating that there is no tolerance to the core capsid protein of PERV.

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Porcine endogenous retroviruses (PERVs) pose a risk for xenotransplantation when using pig cells, tissues or organs, since they are integrated into the genome of all pigs and can infect human cells *in vitro* under certain experimental conditions (for review see Denner and Tönjes, 2012). Three subgroups of PERV, *i.e.* PERV-A, PERV-B and PERV-C are found in the porcine genome. PERV-A and PERV-B are present in the genome of all pigs (Patience et al., 1997; Le Tissier et al., 1997), and are expressed in some animals (Bittmann et al., 2012); infectious particles are released from normal porcine cells (Wilson et al., 1998; Tacke et al., 2003). PERV-A and PERV-B are able to infect transformed human cells *in vitro* (Patience et al., 1997; Wilson et al., 1998; Specke et al., 2001). In contrast, PERV-C is an ecotropic virus which is found in the genome of many but not all pigs and is unable to infect human cells. However, recombination between PERV-C and PERV-A results in replication competent human tropic PERV-A/C (for review see Denner, 2008). PERV-A and PERV-A/C serially passaged on transformed human cells were, under certain circumstances, also able to infect human primary cells (Rodrigues Costa et al., 2014; Denner, 2015).

Vaccination may be a promising approach to prevent PERV transmission to human recipients if other strategies do work. Immunisation against gammaretroviruses was successful in different *in vivo* systems: feline leukaemia virus (FeLV) and cats (Hofmann-Lehmann et al., 2007; Langhammer et al., 2011b), murine leukaemia virus (MuLV) and mice (Hunsmann et al., 1975). In addition we induced in different species antibodies neutralising FeLV (Langhammer et al., 2005, 2006, 2011a), koala retrovirus (KoRV) (Fiebig et al., 2006, 2015b; Denner, 2014) and PERV (see below). When immunisation studies with a recombinant PERV transmembrane envelope (TM, rp15E) protein were performed, binding and neutralising antibodies were induced in goats, mice, rats, rabbits, guinea pigs, and hamsters (Fiebig et al., 2003; Kaulitz et al., 2011; Denner et al., 2012; Waechter and Denner, 2014). However, when pigs were immunised with rp15E (even the same batch), no antibodies were induced, indicating that pigs are tolerant, at least to this PERV protein (Keller et al., 2014). Binding and neutralising antibodies were also induced when goats, mice, rats, rabbits, guinea pigs, and hamsters were immunised with the recombinant surface envelope (SU, rgp70/p52) (Kaulitz et al., 2011; Denner et al., 2012). Here, we investigated whether pigs are tolerant to other structural proteins of PERV such as the SU protein or the core protein p27Gag.

In the present study, twelve female German landrace pigs (1 year old, 160 kg, from the experimental farm of the Institute of Farm

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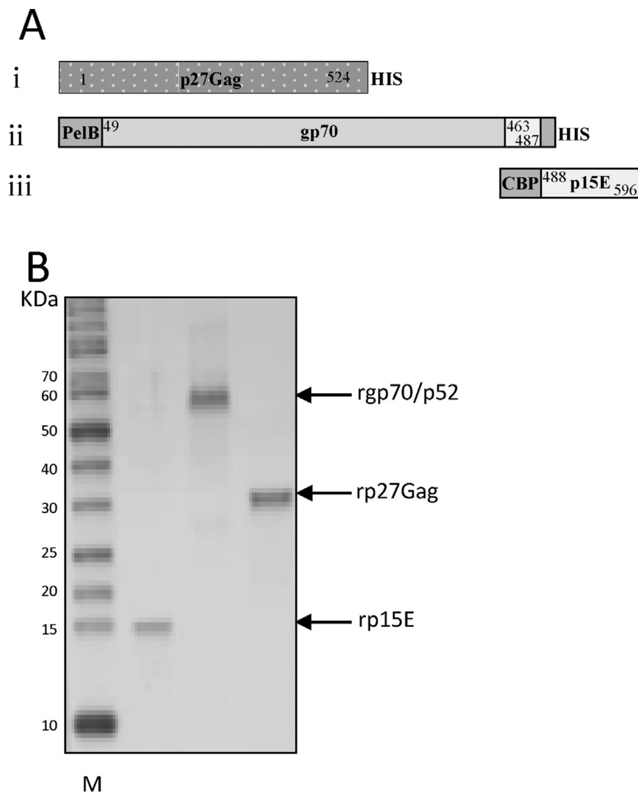

Fig. 1. Schematic presentation and levels of purity of the PERV core and envelope proteins used for immunisation. (A) (i) recombinant p27CA, His-tagged, (ii) recombinant SU envelope protein rgp70/p52, His-tagged, (iii) ectodomain of p15E fused to the calmodulin binding protein (CBP). (B) Characterisation of the antigens used for immunisation by SDS PAGE.

Animal Genetics, Mariensee, Germany) were immunised intramuscularly twice at 22 days intervals, each with 300 μg of each protein behind the ear. A group of three animals (#356, 386, 387) received the same recombinant ectodomain of p15E (amino acids 488–596, accession number HQ688786, 1–109) that had been used in the previous experiments (Keller et al., 2014) (Fig. 1). This protein was produced as a calmodulin binding protein (CBP) fusion protein in *E. coli* BL21 and purified by CBP affinity chromatography as described previously (Kaulitz et al., 2011). Another group of three animals (#339, 341, 359) was immunised with the recombinant SU protein that previously had been used for immunising other species (Kaulitz et al., 2011). The recombinant SU protein gp70 corresponds to the subgroup PERV-A (amino acids 49–487, accession number HQ688785). PERV-A are present in all pigs including the German landrace pigs used for immunisation. Since the recombinant protein is not glycosylated, it is called p52. Due to the presence of the PelB signal peptide which was attached to direct the protein into the periplasma of *E. coli* producer cells, the final size of the molecule was 58 kDa (Fig. 1B). Three animals (#390, 392, 396) were immunised with the recombinant core (capsid) protein rp27CA (AAY28927, aa1–524) (Irgang et al., 2003). In order to ensure that the pigs were immune-competent and that immunisation was successful, all animals were injected with an unrelated protein, keyhole limpet hemocyanin (KLH), which is a large, multi-subunit metalloprotein found in the hemolymph of the giant keyhole limpet, *Megathura crenulata*. A total of 100 μg of KLH were mixed with the PERV antigens and three animals (#302, 371, 376) were immunised with KLH alone. Complete Freund's adjuvant was used for the first immunisation and incomplete Freund's adjuvant for the second round of vaccinations. Pre-immune serum was taken immediately prior to immunisation. Pre-immune and immune sera after each immunisation were analysed for the presence of binding antibodies using Western blot analysis with

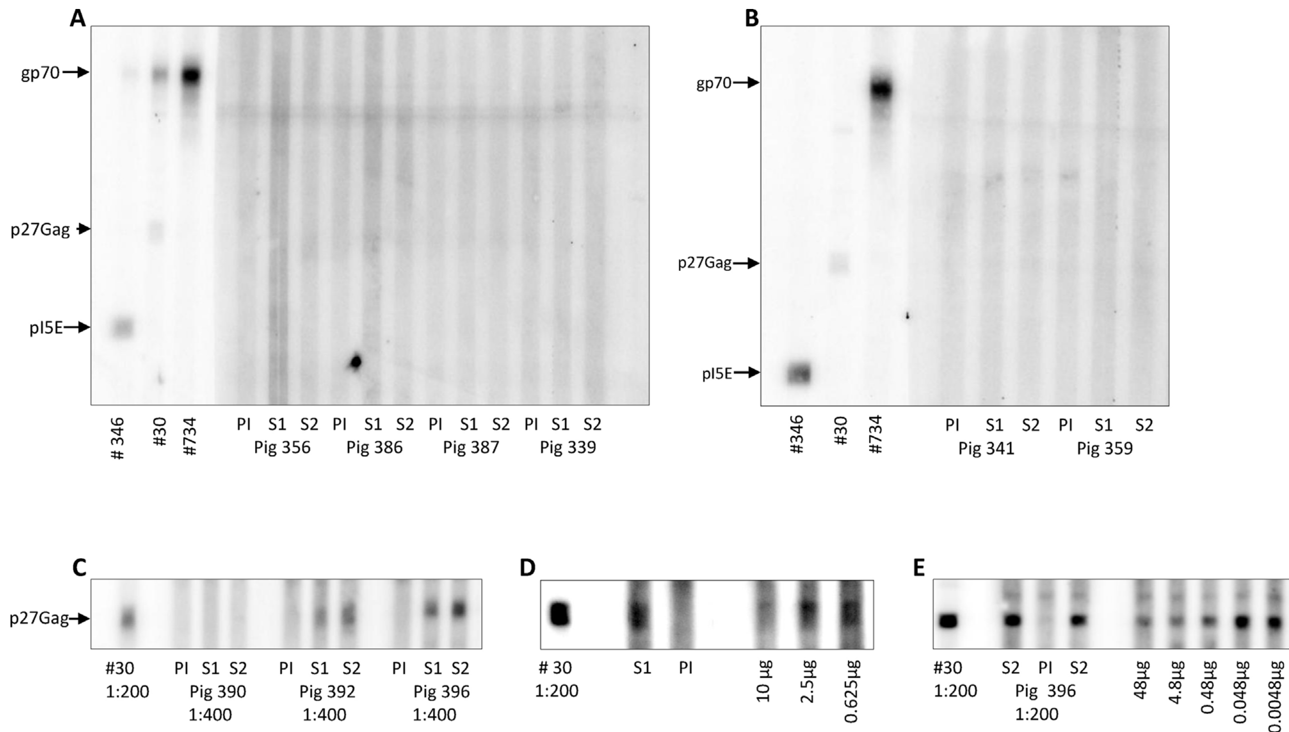

Fig. 2. Characterisation of the immune response. (A and B), Western blot analysis of the sera from pigs immunised with rp15E and rgp70/p52 and of the positive control goat sera (#346 against p15E, #30 against p27Gag, and #734 against gp70). PI, pre-immune serum; S1 and S2, sera after the first and second immunisation. Purified virus was used as antigen. Pigs 356, 386 and 387 were immunised with rp15E, pigs 339, 341 and 396 with rgp70/p52. (C) Western blot analyses of the sera from pigs immunised with rp27Gag (390, 392, 396). Recombinant rp27Gag was used as antigen and goat serum #30 was used as a positive control. (D) Absorption experiment of anti-Gag immune serum S1 from animal #396 (dilution 1:500) with different amounts of purified virus (incubation 60 min at 37 °C) and (E) the same with different amounts of recombinant rp27Gag.

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