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Evaluation of viral peptide targeting to porcine sialoadhesin using a porcine reproductive and respiratory syndrome virus vaccination-challenge model

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ABSTRACT

Targeting antigens to professional antigen presenting cells resident at the sites where effective immune responses are generated is a promising vaccination strategy. As such, targeting sialoadhesin (Sn)-expressing macrophages, abundantly present in spleen and lymph nodes where they appear to be strategically placed for antigen capture and processing, is recently gaining increased attention. Previously, we have shown that humoral immune responses to the model antigen human serum albumin can be enhanced by using a porcine Sn-specific monoclonal antibody to target the model antigen to Sn-expressing macrophages. To date however, no studies have been performed to evaluate whether targeted delivery of a pathogen-derived antigen can enhance the pathogen-specific immune response. Therefore, we selected a linear epitope on glycoprotein 4 of porcine reproductive and respiratory syndrome virus (PRRSV), which is known to be a target of virus-neutralizing antibodies. This paper reports on the targeted delivery of this viral peptide to porcine Sn-expressing macrophages and the evaluation of the subsequent immune response in a vaccination-challenge set-up.

Four copies of the selected PRRSV epitope were genetically fused to a previously developed porcine Sn-targeting recombinant antibody or an irrelevant isotype control. Fusion proteins were shown to be efficiently purified from HEK293T cell supernatants and subsequently, only Sn-specific fusion proteins were shown to bind to and to be internalized into Sn-expressing cells. Subsequent immunizations with a single dose of the fusion proteins showed that peptide-specific immune responses and neutralizing antibody responses after PRRSV challenge were enhanced in animals receiving a single 500 μg intramuscular dose of the Sn-targeting fusion protein, although correlations between the two read-outs were hard to effectuate. Furthermore, a minor beneficial effect on viral clearance was observed. Together, these data show that viral peptide targeting to porcine Sn-expressing macrophages can improve the anti-viral immune response, although more research will be needed to further explore vaccination potential.

Abbreviations: Sn, sialoadhesin; pSn, porcine sialoadhesin; HSA, human serum albumin; DC, dendritic cell; CHO-pSn, CHO cells expressing pSn; mAb, monoclonal antibody; PRRSV, porcine reproductive and respiratory syndrome virus; VN, virus-neutralizing; GP4, glycoprotein 4; LV, Lelystad virus; i.m., intramuscular; GS, glycine-serine; AUC, area under the curve; IPMA, immunoperoxidase monolayer assay.

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1. Introduction

Traditionally, vaccines are based on inactivated pathogens, live attenuated pathogens or pathogen-derived toxins (Black et al., 2010; Purcell et al., 2007). Although successful in many instances, there may be significant drawbacks of these approaches, including the risk of reversion to virulence, unwanted host reactions such as inflammation or the induction of an autoimmune response. In addition, manufacturing constraints may occur with microorganisms that are hard to cultivate *in vitro*. For veterinary vaccines, also the need to discriminate between infected and vaccinated animals contributes to the growing interest in a more sophisticated vaccine design (Uttenthal et al., 2010). Starting from the exclusion of specific protein antigens in vaccine formulations, research has evolved towards the development of true subunit vaccines, containing only fragments of a pathogen. Going even further, the selection of a minimal, protective and immunogenic region of a protein antigen has led to the development of epitope-based peptide vaccines, allowing a precise direction of immune responses. This reductionist approach, although challenging, can result in vaccines that are more immunologically defined and with a better safety profile compared to vaccines generated by traditional empirical approaches.

Although promising, peptides are traditionally poorly immunogenic on their own (Skwarczynski and Toth, 2011). Therefore, there is a clear need for potent immunostimulatory adjuvants. In addition, these adjuvants also need to be safe. An alternative to this approach is making the peptide-based vaccines self-adjuvanting by the use of nanoparticular carriers (Skwarczynski and Toth, 2011), or by targeting the peptides directly to antigen-presenting cells. While most targeting strategies aim at delivery of the antigens to dendritic cells (DCs) (Brown and Jackson, 2005; Caminschi and Shortman, 2012; Noessner et al., 2002), sialoadhesin (Sn)-expressing macrophages, among other situated in spleen and lymph nodes, appear to be strategically placed for antigen capture and processing and may be an attractive target for vaccination strategies as well (Martinez-Pomares and Gordon, 2012). Previously, we have made use of a porcine Sn (pSn)-specific monoclonal antibody (mAb) to target the model antigen human serum albumin (HSA), which was chemically cross-linked to the mAb, to pSn-expressing macrophages. In a porcine model, this resulted in an enhanced immune response to HSA (Delputte et al., 2011). To circumvent the drawbacks associated with the chemical linkage of antigens, we recently developed a pSn-specific recombinant antibody (Ooms et al., 2013). This antibody allows targeted delivery of peptides or proteins towards pSn-expressing macrophages and thus represents an elegant tool to evaluate Sn targeting as a vaccination strategy.

Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded, positive sense RNA virus that belongs to the family of the *Arteriviridae*, order *Nidovirales* (Gorbalenya et al., 2006). PRRSV is the causative agent of porcine reproductive and respiratory syndrome, a disease present in the majority of swine-producing countries around the world which causes major economic losses (Holtkamp et al., 2011; Neumann et al., 2005). Although protective PRRSV immunity is a complex matter, it has been shown that sufficiently high titres of virus-neutralizing (VN) antibodies in serum can offer *in vivo* protection against PRRSV (Delputte et al., 2004; Lopez et al., 2007). Currently used inactivated vaccines however, do not induce VN antibodies and are of limited efficacy at best (Hu and Zhang, 2013; Zuckermann et al., 2007). To test if targeting to Sn increases the induction of VN antibodies, we selected an epitope on glycoprotein 4 (GP4) of the European Lelystad virus (LV) strain which is known to be a target for VN mAbs in continuous cell lines as well as in porcine alveolar macrophages (Costers et al., 2010a; Meulenberg et al., 1997). In addition, during the course of an infection pigs produce antibodies against this linear epitope which are able to neutralize the virus *in vitro* (Vanhee et al., 2010). The GP4 epitope was genetically linked to the pSn-specific recombinant antibody rec41D3, and the resulting product was used to immunize pigs, evaluate the induction of peptide-specific antibodies and assess the efficacy upon a viral challenge.

2. Materials and methods

2.1. Ethics statement

Animal experiments were approved and supervised by the Ethical and Animal Welfare Committee of the Faculty of Veterinary Medicine of Ghent University. The named institution approved the experiments and provided a permit for this study (Permit Numbers: EC 2011/105 and 2012/038). The experimental procedure for the collection of porcine alveolar macrophages was authorized and supervised by the named institution as well.

2.2. Cells and virus

Primary macrophages were isolated by bronchoalveolar lavage from four- to six-week-old conventional Belgian Landrace pigs as described before (Wensvoort et al., 1991), and cultivated in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 2 mmol l^{-1} L-glutamine, 1% non-essential amino acids and 1 mmol l^{-1} sodium pyruvate. HEK293T were grown in DMEM medium supplemented with 10% heat-inactivated FBS, 2 mmol l^{-1} L-glutamine and 1 mmol l^{-1} sodium pyruvate. CHO-K1 cells and CHO-K1 cells stably expressing recombinant pSn (CHO-pSn) (Delputte et al., 2007) were cultivated in F-12 medium supplemented with 10% FBS and 1 mmol l^{-1} sodium pyruvate. All culture media were supplemented with a mixture of antibiotics and cell cultures were kept in a humidified 5% CO_2 atmosphere at 37 °C.

The PRRSV LV strain was propagated in primary macrophages that were derived from gnotobiotic piglets. The fifth passage of infected cell culture supernatant was purified by ultracentrifugation as previously described by Vanhee et al. (2009).

2.3. Construction, production and purification of rec41D3- and rec13D12-GP4

Construction of the plasmids encoding pSn-specific rec41D3 and isotype matched (IgG1) control rec13D12, directed against pseudorabies virus glycoprotein gD (Nauwynck and Pensaert, 1995), was described previously (Ooms et al., 2013). To generate both rec41D3- and rec13D12-GP4 plasmids, a $(G_4S)_2$-GP4 DNA sequence was synthesized (Genscript Inc.) and introduced at the 3′ end of the heavy chain sequence by restriction enzyme digestion and ligation. Sequencing confirmed the fusion constructs were correctly assembled. HEK293T cells were transiently transfected with the plasmids using calcium phosphate and cultured in DMEM supplemented with 10% IgG depleted, heat-inactivated FBS, 2 mmol l^{-1} L-glutamine, 1 mmol l^{-1} sodium pyruvate, and a mixture of antibiotics in a humidified 5% CO_2 atmosphere at 37 °C. Culture supernatant was collected and recombinant IgG were purified from the supernatant using standard protein G sepharose chromatography following the manufacturer's instructions (GE Healthcare). Fractions of the eluate containing the purified protein were pooled and the buffer was exchanged to DPBS (Life Technologies) by dialysis. Purified protein was stored at −70 °C until use.