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Immunogenicity of *ORFV*-based vectors expressing the rabies virus glycoprotein in livestock species

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ABSTRACT

The parapoxvirus *Orf* virus (ORFV) encodes several immunomodulatory proteins (IMPs) that modulate host-innate and pro-inflammatory responses and has been proposed as a vaccine delivery vector for use in animal species. Here we describe the construction and characterization of two recombinant ORFV vectors expressing the rabies virus (RABV) glycoprotein (G). The RABV-G gene was inserted in the *ORFV024* or *ORFV121* gene loci, which encode for IMPs that are unique to parapoxviruses and inhibit activation of the NF-κB signaling pathway. The immunogenicity of the resultant recombinant viruses (ORFV$^{\Delta 024}$RABV-G or ORFV$^{\Delta 121}$RABV-G, respectively) was evaluated in pigs and cattle. Immunization of the target species with ORFV$^{\Delta 024}$RABV-G and ORFV$^{\Delta 121}$RABV-G elicited robust neutralizing antibody responses against RABV. Notably, neutralizing antibody titers induced in ORFV$^{\Delta 121}$RABV-G-immunized pigs and cattle were significantly higher than those detected in ORFV$^{\Delta 024}$RABV-G-immunized animals, indicating a higher immunogenicity of ORFV$^{\Delta 121}$-based vectors in these animal species.

1. Introduction

Orf virus (ORFV) is the prototype of the genus *Parapoxvirus*, subfamily *Chordopoxvirinae,* family *Poxviridae* (ICTV, 2015). ORFV is ubiquitous and causes a self-limiting mucocutaneous infection in sheep and goats, known as *orf* or contagious ecthyma (Haig and Mercer, 1998). The ORFV genome consists of a double-stranded DNA molecule of approximately 138 kbp in length and contains 131 putative open reading frames (ORFs) (Delhon et al., 2004). Notably, ORFV encodes several immunomodulatory proteins (IMPs) that modulate host-innate and pro-inflammatory responses to infection (Haig et al., 2002; Weber et al., 2013). These IMPs include an interleukin 10 homologue (vIL-10; *ORFV127*) (Fleming et al., 2007), a chemokine binding protein (CBP; *ORFV112*) (Seet et al., 2003), an inhibitor of granulocyte-monocyte colony-stimulating factor (GMC-CSF) and IL-2 (GIF; *ORFV117*) (Deane et al., 2000), an interferon (IFN)-resistance gene (VIR; *ORFV020*) (McInnes et al., 1998), a homologue of vascular endothelial growth factor (VEGF; *ORFV132*) (Wise et al., 1999) an inhibitor of apoptosis (*ORFV125*) (Westphal et al., 2007), and at least three inhibitors of the nuclear factor-kappa (NF-κB) signaling pathway (*ORFV002*, *ORFV024*, and *ORFV121*) (Diel et al., 2011a, 2011b, 2010). The function(s) and/or mechanism(s) of action of these IMPs have been determined (Deane et al., 2000; Diel et al., 2011a, 2011b, 2010; Fleming et al., 1997; McInnes et al., 1998; Seet et al., 2003; Wise

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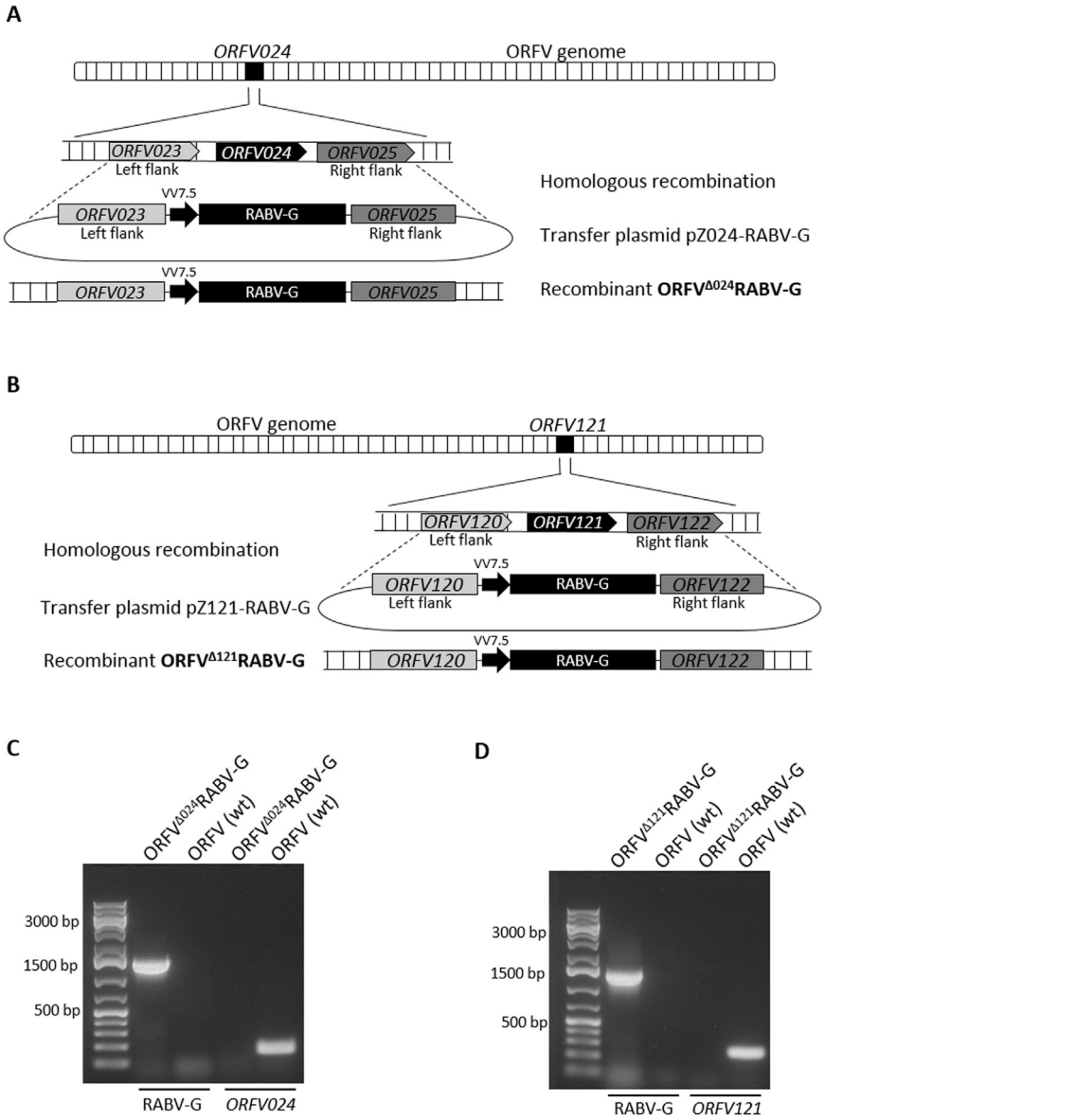

Fig. 1. Generation of recombinant ORFV-RABV-G viruses. (A) Schematic representation of the ORFV genome depicting *ORFV024* insertion site and flanking regions (*ORFV023* and *ORFV025*) used to generate the recombinant ORFV$^{\Delta 024}$RABV-G. (B) Schematic representation of the ORFV genome depicting *ORFV121* insertion site and flanking regions (*ORFV120* and *ORFV122*) used to generate the recombinant ORFV$^{\Delta 121}$RABV-G. The coding sequence of the RABV G was inserted into the *ORFV024* or *ORFV121* gene loci of the ORFV genome by homologous recombination between the parental ORFV IA82 and the recombination cassette pZ024-RABV-G or pZ121-RABV-G. The pZ024-RABV-G and pZ121-RABV-G transfer plasmids containing the full-length glycoprotein gene under the control of the early/late VV7.5 poxviral promoter. (C) Agarose gel demonstrating PCR amplification of an internal region of the glycoprotein gene from the genome of the recombinant ORFV$^{\Delta 024}$RABV-G virus and absence of *ORFV024* gene sequences on the recombinant virus genome. (D) Agarose gel demonstrating PCR amplification of an internal region of the glycoprotein gene from the genome of the recombinant ORFV$^{\Delta 121}$RABV-G virus and absence of *ORFV121* gene sequences on the recombinant virus genome. Wild type ORFV DNA was used as a negative and positive control on the PCR amplifications with glycoprotein specific and *ORFV024* or *ORFV121* specific primers, respectively.

et al., 1999). Most importantly, while these genes are non-essential for ORFV replication *in vitro*, the viral homologues of IL-10 (*ORFV127*) and VEGF (*ORFV132*), the CBP (*ORFV112*) and the NF-κB inhibitor *ORFV121* are virulence factors that contribute to ORFV pathogenesis in the natural host (Diel et al., 2011b; Fleming et al., 2017, 2007; Meyer et al., 1999).

Given its immunomodulatory and biological properties, ORFV has been proposed as a vaccine delivery vector for use in animal species (Rziha et al., 2000). The unique features that make ORFV an attractive vector for vaccine delivery include: 1) its restricted host range (sheep and goats); 2) its tropism for skin keratinocytes or their counterparts in the oral mucosa; 3) the absence of systemic dissemination and 4) the low or absent neutralizing activity of ORFV-induced antibodies (Amann et al., 2013; Fischer et al., 2003; Hain et al., 2016; Henkel et al., 2005; Rohde et al., 2011; Rziha et al., 2000). Additionally, the presence of well characterized IMPs in the ORFV genome provides a unique opportunity for rational engineering of a safe and highly immunogenic ORFV-based vector platform. Recently, we have shown that immunization of pigs with a recombinant ORFV with a deletion of *ORFV121* (IMP that contributes to ORFV virulence) and expressing the porcine epidemic diarrhea virus (PEDV) spike (S) glycoprotein induced neutralizing antibody responses and protected pigs from clinical signs of PED (Hain et al., 2016). Here the immunogenicity of two ORFV-based recombinant viruses with single gene deletions on NF-κB-inhibitors *ORFV024* or *ORFV121* was investigated in pigs and cattle. The rabies virus glycoprotein (RABV-G) was used as a model antigen to evaluate the immunogenicity of the recombinant vector candidates in the target animal species.