Short Communication

Wild-type *Rinderpest virus* uses SLAM (CD150) as its receptor

Michael D. Baron

Institute for Animal Health, Ash Road, Pirbright, Surrey GU24 0NF, UK

Correspondence
Michael D. Baron
michael.baron@bbsrc.ac.uk

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Rinderpest virus (RPV) is a morbillivirus, related closely to the human pathogen *Measles virus* (MV). Although cell culture-adapted strains of RPV can infect many kinds of cell from different hosts, one such strain has previously been shown to have a detectable preference for cells expressing the MV receptor CD150 (SLAM), a protein found only on certain types of activated T cells, B cells and dendritic cells. Here, it is shown that the wild-type, virulent parent of the most common vaccine strain of RPV requires CD150 as a receptor, whilst the cell culture-adapted vaccine strain has acquired the ability to use heparan sulphate as an alternative receptor.

The morbilliviruses are a compact group of viruses that includes *Measles virus* (MV), *Rinderpest virus* (RPV), *Canine distemper virus* (CDV) and *Peste-des-petits-ruminants virus* (PPRV). The use of different receptors by the human pathogen MV has been studied extensively; the current consensus is that wild-type MV uses signalling lymphocyte activation molecule (SLAM, also known as CD150) as its receptor on the lymphoid cells that are its main target during infection (Erlenhoefer *et al.*, 2001; Hsu *et al.*, 2001; Tatsuo *et al.*, 2000) and another, as-yet-unidentified protein to attach to endothelial and respiratory mucosal cells (Andres *et al.*, 2003; Takeuchi *et al.*, 2003). Cell culture-adapted strains of MV use the ubiquitously expressed protein CD46 as a receptor (Dörig *et al.*, 1993; Naniche *et al.*, 1993). Little is known about the receptors for other morbilliviruses, although we have shown previously that CD46 is not a receptor for virulent or vaccine strains of RPV (Galbraith *et al.*, 1998). Recently, Yanagi and co-workers showed that RPV (cell culture-adapted) and CDV (cell culture-adapted and wild-type) both show improved infection of Chinese hamster ovary (CHO) or African green monkey kidney (Vero) cell lines permanently expressing SLAM (Seki *et al.*, 2003; Tatsuo *et al.*, 2001). This suggested that SLAM may be a receptor for several, if not all, morbilliviruses. However, cell culture-adapted RPV efficiently infects CHO, Vero and many other common cell-culture lines; given the previous observations that the use of CD46 as a receptor by MV turned out to be a result of tissue-culture adaptation, I sought to test whether wild-type RPV uses SLAM as its receptor.

We have established rescue systems for both the Plowright vaccine strain (Plowright & Ferris, 1962) of RPV (RBOK) and the virulent parent from which it was derived, Kabete O (KO) (Baron & Barrett, 1997; Baron *et al.*, 2005). These rescue systems allow us to recover recombinant viruses with gene additions or modifications (Baron & Barrett, 2000; Baron *et al.*, 1999; Walsh *et al.*, 2000). For the virulent KO strain of RPV, the rescue uses only the lymphoblastoid line B95a (Kobune *et al.*, 1991). We have found that wild-type virus grows in these cells without prior adaptation and without apparent loss of virulence. We have used this system to create a number of chimaeric viruses in which specific genes from the RBOK strain have been replaced with the corresponding gene from the KO strain and vice versa. All of these viruses were rescued in B95a cells and stocks were grown and titrated in the same cell line.

One of the major differences between the growth of vaccine and virulent strains of RPV *in vivo* is the much greater replication of the latter (Wohlsein *et al.*, 1995). When I tried to study the growth of the KO strain in a bovine cell line, Madin–Darby bovine kidney (MDBK) cells, I was surprised to find that it grew very poorly ($<1\%$) relative to the vaccine virus, as measured by virus yield 48 h post-infection (p.i.) with virus at an m.o.i. of 0·04 (Fig. 1a). Immunofluorescence studies showed that exposure of MDBK cells to RBOK at this m.o.i. led to widespread infection, whereas, after exposure to the same amount of KO, very few cells were infected (data not shown). This suggested that the low recovery of virus was due to a defect in entry or replication. Altered cell tropism in morbilliviruses has been found to be due to the viral H gene (Moeller *et al.*, 2001; Ohgimoto *et al.*, 2001; von Messling *et al.*, 2001) or the P gene (Miyajima *et al.*, 2004). To try to identify which gene was responsible for the relative defect in infectivity of KO in MDBK cells, I tested the growth of recombinant chimaeric viruses in which the N, P or H gene had been exchanged between KO and RBOK strains. KO viruses containing the RBOK N or P gene (KO-NR, KO-PR) were still defective in growth in MDBK cells, whereas KO with the RBOK H gene (KO-HR) grew as well as RBOK (Fig. 1a). Corresponding results

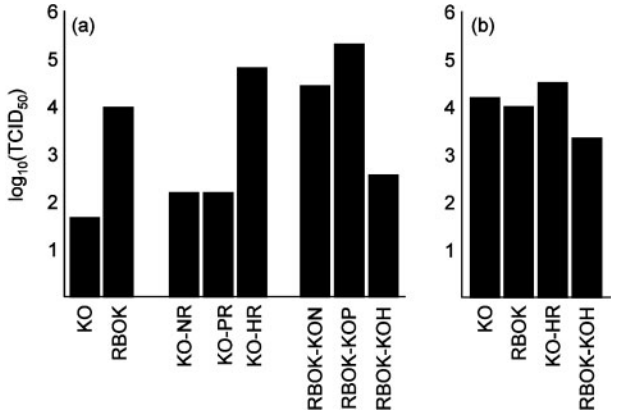

Fig. 1. (a) Growth of recombinant RPVs in MDBK cells. MDBK cells in six-well plates were infected for 1 h with recombinant RPV at an m.o.i. of 0·04. After 48 h, the cells were subjected to one freeze–thaw cycle and the titre of the released virus was determined. (b) Growth of recombinant RPVs in a bovine T-cell line. Cells were infected with recombinant virus at an m.o.i. of 0·04 and the virus yield at 48 h p.i. was determined as in (a). The results shown for (a) and (b) are the means of two experiments with independently isolated recombinant viruses.

were found with the modified RBOK viruses, in that replacing the RBOK H gene with that of KO (RBOK-KOH) resulted in greatly decreased virus yield, whilst exchanging the N or P genes had no effect (Fig. 1a). Immunofluorescence studies showed, as expected, that KO-HR infected MDBK cells readily, whereas RBOK-KOH showed very low levels of infection; similar results were also found with primary bovine skin fibroblasts (data not shown).

As the morbillivirus H protein is involved primarily in attachment of the virus to its receptor on the host cell, these observations suggested that the H protein of the wild-type KO strain of RPV was defective in binding to bovine cells of epithelial origin. As RPV is primarily a lymphotropic virus, the ability of the KO H protein to mediate the infection of Tp4/9 cells, a *Theileria parva*-transformed bovine T-cell line (kind gift of Dr Haru Takamatsu, Institute for Animal Health), was studied. Such cells have previously been found to support the replication of wild-type RPV and PPRV (Rossiter *et al.*, 1992). When the infection of these cells by RBOK, KO and the respective H protein-exchanged chimaeric viruses was compared in terms of virus yield, RBOK and KO appeared to grow equally well and the origin of the H gene in the virus had no major effect on virus yield (Fig. 1b). The KO H protein, in fact, seemed to bind to these cells better than the RBOK H protein, as KO-infected Tp4/9 cells showed cytopathic effects (primarily syncytia, which appear as giant balloon-like cells) much earlier than RBOK-infected cells (Fig. 2), and this phenotype was linked to the H gene, in that RBOK-KOH also showed rapid onset of cell fusion, whereas KO-HR did not (Fig. 2).

The proposed RPV receptor, SLAM, is found on dendritic cells and some B and T cells, particularly after activation (Cocks *et al.*, 1995), as well as on the B95a cells (Hsu *et al.*, 2001) that we use to grow wild-type RPV. RT-PCR with primers based on the published sequence of bovine SLAM (Tatsuo *et al.*, 2001) showed that Tp4/9 and other *Theileria*-transformed cells expressed SLAM (data not shown). The two cell lines that are infected efficiently by wild-type RPV therefore both express the putative receptor. In order to confirm the role of SLAM in KO infection, I used a CHO line expressing human SLAM (a gift of Dr J. Schneider-Schaulies, Institute for Virology, Wurzburg, Germany).

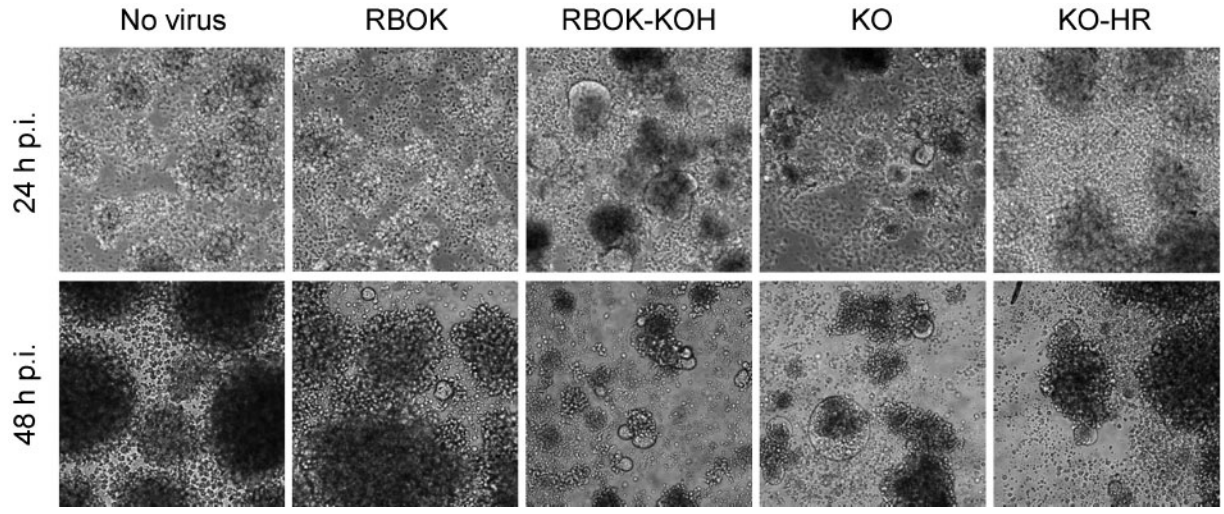

Fig. 2. Cell fusion induced by recombinant RPVs in a bovine T-cell line. Tp4/9 cells were infected with recombinant virus at an m.o.i. of 0·04 and photographed under phase-contrast microscopy at 24 and 48 h p.i.

CHO cells and CHO-SLAM cells were infected at an m.o.i. of 0·04 with RBOK, RBOK-KOH, KO or KO-HR, and the ability of the viruses to infect the different cells was determined by the appearance of viral protein, detected by Western blotting using a polyclonal rabbit antiserum against RPV P protein (Fig. 3a). Whilst viruses carrying the RBOK H protein could infect either cell line, viruses carrying the wild-type KO H protein were strictly dependent on the presence of the SLAM protein to act as a receptor. As noted previously (Tatsuo *et al.*, 2001), cell culture-adapted RPV infects CHO cells readily,but shows slightly increased infection of CHO-SLAM cells.

To extend and confirm these findings, I made two further chimaeric viruses, variants of KO-HR in which only the amino-terminal (membrane-proximal) half of the H protein (KO-RKH) or only the carboxyl-terminal (membrane-distal) half (KO-KRH) was from RBOK. As the carboxyl-terminal half contains the globular head that contains the SLAM-binding domain (Massé *et al.*, 2004; Vongpunsawad *et al.*, 2004), it was expected that KO-RKH would require SLAM, whereas KO-KRH would not; this was found to be the case (Fig. 3a). These data confirmed, therefore, that the wild-type RPV uses SLAM as its receptor.

Cell-culture adaptation has, for a number of viruses, involved the selection of mutants that can utilize heparan sulphate (HS) as a receptor (Bose & Banerjee, 2002; Goodfellow *et al.*, 2001; Jackson *et al.*, 1996; Klimstra *et al.*, 1998; Mandl *et al.*, 2001; Summerford & Samulski, 1998). To see whether this was also true of RPV, CHO cells were treated with heparinase I (Sigma; 10 U ml^{-1} for 90 min at 37 °C) and the ability of RBOK or KO-KRH (the minimal KO mutant that showed infection of ordinary CHO cells) to infect the cells was determined. As shown in Fig. 3(b), heparinase treatment of the cells greatly reduced the subsequent infection by RPV. In addition, I assessed the ability of RPV to infect the cell line *pgs*D-677 (Lidholt *et al.*, 1992) (a gift of Dr J. D. Esko, UCSD, CA, USA), a mutant CHO cell that does not make HS. Neither RBOK nor KO-KRH could infect *pgs*D-677 cells (Fig. 3c). Transient transfection of *pgs*D-677 cells with a plasmid expressing bovine SLAM (cloned by PCR from Tp4/9 cells) did allow RPV to infect and replicate in them (data not shown), confirming that there was no underlying defect in the ability of the cells to support RPV replication. These data showed that the ability of the RBOK strain of RPV to infect so many tissue-culture cell lines is due to its use of this almost ubiquitously expressed glycosaminoglycan.

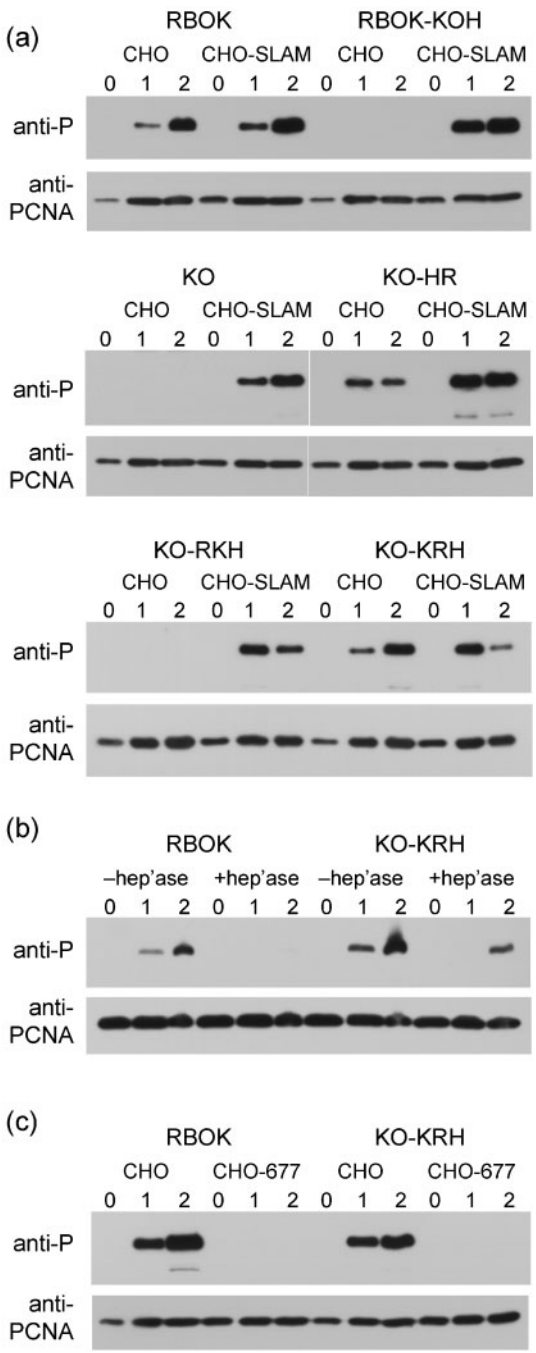

Fig. 3. Growth of recombinant RPVs in normal and mutant CHO cells. (a) CHO and CHO-SLAM cells in 12-well dishes were infected for 1 h with the indicated recombinant virus at an m.o.i. of 0·04. At 0, 1 and 2 days p.i., cells were recovered by trypsinization and dissolved directly in SDS-PAGE sample buffer. Samples (approximately one-eighth of the total) were analysed by SDS-PAGE and Western blotting. The top half of each blot was developed with rabbit anti-RPV P serum MB18 (Baron & Barrett, 1997) and the bottom half with mouse anti-proliferating cell nuclear antigen (PCNA; Santa Cruz Biotechnology) as a loading control. (b) CHO cells were incubated at 37 °C for 90 min in complete PBS containing 0·5 % BSA with or without 10 U heparinase ml^{-1}; the cells were washed twice in PBS before infection with the indicated virus and harvesting and analysis were performed as in (a). (c) CHO cells and CHO *pgs*D-677 cells (CHO-677) were infected with the indicated virus, harvested and analysed as in (a).

Comparison of the sequences of KO and the minimal HS-using mutant KO-KRH showed just four changes to the protein sequence (T496I, R556K, I586V and E603K) (the sequences of the H genes of all of the viruses used in these studies were checked to ensure that no mutations were acquired during rescue and stock amplification). Previous studies with *Foot-and-mouth disease virus* (Baranowski *et al.*, 1998; Fry *et al.*, 1999), *Sindbis virus* (Klimstra *et al.*, 1998) and *Tick-borne encephalitis virus* (Mandl *et al.*, 2001) have indicated that adaptations to use HS as a receptor are mutations that increase the positive charge in positions at the surface of the virus. Assuming that the RPV H protein structure is similar to that modelled for the MV H protein (Massé *et al.*, 2004), the E603K mutation, which changes the charge at that point on the surface from negative to positive, is situated in a very flexible region on the surface of the globular head, close to the putative CD46-binding site on the MV H protein, and therefore presumably in a suitable position to mediate virus binding to a host-cell surface protein. Interestingly, this residue is also lysine in the H protein of RPV KO grown in bovine kidney cells (Yamanaka *et al.*, 1988), which is therefore presumably able to enter cells in a SLAM-independent manner. The arginine at 556 is also in a surface-exposed position, close to the CD46-binding site. The sequence around and including R556 is conserved in morbillivirus H proteins, suggesting that it plays an important part in the structure, although it does not appear to be a part of the SLAM-binding domain (Massé *et al.*, 2004). The R556K mutation will not change the surface positive charge, but may alter internal hydrophobic interactions or hydrogen bonds, allowing greater flexibility of the side chain. Interestingly, this position is also lysine in the bovine kidney cell-adapted KO virus (Yamanaka *et al.*, 1988). Further mutations would be needed to identify the exact mutation(s) that allows RPV H to bind to HS; it would be interesting to see whether a similar mutation would allow MV to use the same receptor.

Acknowledgements

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