Conservation and variation of the parapoxvirus GM-CSF-inhibitory factor (GIF) proteins

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The GIF protein of orf virus (ORFV) binds and inhibits the ovine cytokines granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2). An equivalent protein has so far not been found in any of the other poxvirus genera and we therefore investigated whether it was conserved in the parapoxviruses. The corresponding genes from both the bovine-specific pseudocowpox virus (PCPV) and bovine papular stomatitis virus (BPSV) were cloned and sequenced. The predicted amino acid sequences of the PCPV and BPSV proteins shared 88 and 37% identity, respectively, with the ORFV protein. Both retained the six cysteine residues and the WSXWS-like motif that are required for biological activity of the ORFV protein. However, an analysis of the biological activity of the two recombinant proteins revealed that, whilst the PCPV GIF protein bound to both ovine and bovine GM-CSF and IL-2 with very similar binding affinities to the ORFV GIF protein, no GM-CSF- or IL-2-binding activity was found for the BPSV protein.

INTRODUCTION

The parapoxviruses (PPVs), members of the genus Parapoxvirus of the family Poxviridae, cause a contagious pustular dermatitis in ruminants. Orf virus (ORFV) is the type species of the genus and causes disease in sheep and goats, whereas bovine papular stomatitis virus (BPSV) and pseudocowpox virus (PCPV) affect cattle. All three viruses can infect humans. As is common amongst other poxviruses, ORFV has been shown to encode factors that either mimic host immunoregulatory proteins (Fleming et al., 1997; Haig & Fleming, 1999; Seet et al., 2003) or have the potential for interacting with components of the host immune system (Haig et al., 1998; McInnes et al., 1998, 2005; Deane et al., 2000). In doing so, it is thought that these virus-encoded factors are capable of subverting the host immune response to infection, creating an environment suitable for efficient virus replication. Some of the poxvirus factors, such as the double-stranded RNA-binding protein (VAC E3L), which inhibits the antiviral effects of interferon (Haig et al., 1998; McInnes et al., 1998, whilst others appear to be genus-specific, such as the orthopoxvirus protein (VAC C21L), which inhibits the activation of the complement cascade (Isaacs et al., 1992). Some, such as the vFLIP protein (MC159) of molluscum contagiosum virus, which inhibits apoptosis, may be species-specific (Shisler & Moss, 2001; Thurau et al., 2006). ORFV has been shown to encode a protein (GIF) that is capable of binding and inhibiting both of the ovine cytokines interleukin-2 (IL-2) and granulocyte–macrophage colony-stimulating factor (GM-CSF) (Deane et al., 2000; McInnes et al., 2005). Such a protein has not been found so far in any other poxvirus; we therefore investigated whether it was conserved in other PPVs.

METHODS

Viruses. ORFV strain NZ2 (Mercer et al., 2006), PCPV strains BO74 (kindly obtained from Dr Mathias Buettner, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany) and VR634 (Gassmann et al., 1985) and BPSV strains V660 (Gassmann et al., 1985) and A599 (isolated de novo in the UK) were used throughout this study and were maintained by passage in primary bovine testis or fetal lamb muscle cells.

Expression of the PPV GIFs. The entire open reading frames of the three PPV GIF genes were amplified by PCR and cloned individually into the pEE14 expression vector (Celltech) (Cockett et al., 1990). The integrity of the genes was verified by sequencing prior to transfection into COS-7 fibroblasts or CHO cells. Stable cell lines expressing each of the GIF proteins were established as described previously (Deane et al., 2000). Cell lines expressing the recombinant ORFV and PCPV
GIF (rGIF) proteins were selected by assaying for GIF activity using a modification of the ovine (ov) GM-CSF-specific ELISA (Entrican et al., 1996; Deane et al., 2000). The pEE14 vector contains a glutamine synthetase (GS) gene; therefore, cell lines transfected stably with the plasmid can be selected on the basis of their survival in methionine sulphoximine (MSX; Sigma). The cell line expressing the BPSV GIF was selected based on its survival in MSX and upon reactivity with a rabbit anti-ORFV GIF serum. Verification that the anti-ORFV GIF serum recognizes the BPSV GIF was obtained by direct ELISA using an anti-ORFV GIF mAb (see Supplementary Fig. S1, available in JGV Online). The recombinant proteins used in these studies were prepared by culturing the cells for 4 days after reaching confluence in Glasgow’s modified Eagle’s medium without serum or MSX. The serum-free supernate was harvested, clarified by centrifugation at 1000 g, dispensed in aliquots and stored at −20 °C. Recombinant ORFV and PCPV GIF were purified from CHO cells by affinity chromatography using purified rovGM-CSF bound to CNBr-Sepharose (GE Healthcare Life Sciences). Cell-free supernates from the rGIF-transfected cells were applied to the GM-CSF-Sepharose affinity column. Eluted fractions were tested for GIF activity by using the ovine GM-CSF-specific ELISA. Recombinant BPSV GIF was partially purified, from CHO cell-free supernates, by Mono-Q anion-exchange chromatography followed by gel filtration on Superdex 200 (GE Healthcare Life Sciences). For ligand blotting, the purified GIF proteins were radioiodinated by the chloramine-T method and a soluble ligand-binding assay was performed for each cytokine as described previously (Deane et al., 2000). Briefly, a range of 125I–GM-CSF and 125I–IL-2 concentrations (2–20 nmol) was incubated with GIF (100 ng) containing 5% fetal bovine serum for 2 h at room temperature. Bound proteins were precipitated by the addition of 20% polyethylene glycol (PEG 6000; Sigma) in PBS and incubation on ice for 30 min. The precipitated material was collected under vacuum onto GF/C filter discs (Whatman) and washed four times with ice-cold 10% PEG 6000 in PBS. The radiolabelled complexes were detected and quantified in a gamma scintillation counter. The level of non-specific binding of 125I-labelled cytokines to the filter discs in the absence of GIF was measured and the data were adjusted accordingly. Scatchard analysis was performed on best-fit plots [bound counts min−1]/free counts min−1 (y-axis) versus bound counts min−1 (x-axis), generated by using the Origin software package (OriginLab Corporation).

### Assay of GIF activity

The presence of biologically active ORFV GIF can be measured indirectly by its ability to interfere with the detection of ovine/bovine GM-CSF by specific ELISA. Cell-free supernates and cell lysates from GIF cDNA-transfected and virus-infected cells were assayed for GM-CSF-binding activity as described previously (Deane et al., 2000). Binding of GIF to ovine/bovine IL-2 was similarly measured by ELISA. Briefly, recombinant ovine/bovine IL-2 was isolated from CHO cell-free supernates by Mono-Q anion-exchange chromatography followed by gel filtration on a Superdex 200 column (GE Healthcare Life Sciences). ELISA plates were coated overnight with 1 µg IL-2 ml−1 in 0.1 M NaHCO3 (pH 9.5) before blocking with 4% non-fat milk powder in PBS. Cell-free supernates and cell lysates from GIF cDNA-transfected and virus-infected cells were assayed for IL-2-binding activity. Bound GIF was detected with 2 µg ml−1 of an affinity-purified IgG fraction from a rabbit anti-GIF serum, followed by a 1:1000 dilution of goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP; DAKO) in wash buffer [PBS containing 0.02% Tween 20 (Sigma)]. For colour development, TMB peroxidase substrate (SureBlue; Kirkegaard & Perry Laboratories) was added; the reaction was stopped after 20–30 min by the addition of 0.1 M HCl and A450 was read. The specificity of the interaction between GIF and IL-2 was verified by pre-incubating the IL-2-coated ELISA plate with an anti-ovine IL-2 mAb, 1E10 (a gift from Dr P. Wood, CSIRO Animal Health, Parkville, Australia; Pedersen et al., 2002), before incubating with GIF.

### Western blot analysis

In order to verify protein expression from the various GIF constructs, the cell-free supernates and/or cell lysates from the COS-7/CHO cells were electrophoresed in a denaturing SDS-polyacrylamide gel (12%) and transferred to Hybond ECL nitrocellulose membranes (GE Healthcare Life Sciences) for 3 h at 2 mA (cm gel)−1. The membranes were washed in PBS containing 4% non-fat milk powder for 1 h at room temperature before being probed with a rabbit anti-GIF IgG fraction (1 µg ml−1) in blot wash buffer [PBS containing 0.35 M NaCl and 0.5% (v/v) Tween 80 (Sigma)]. Binding of antibody to immobilized proteins was visualized by a further 1 h incubation with a 1:1000 dilution of goat anti-rabbit IgG conjugated with HRP (DAKO) in wash buffer, followed by treatment with the enhanced chemiluminescence reagent ECL (GE Healthcare Life Sciences), according to the manufacturer’s instructions, and exposure to Hyperfilm ECL for 0.5–5.0 min before development.

### Ligand blotting

GIF–cytokine binding was detected by ligand blotting as described previously (Deane et al., 2000). Briefly, 250–400 ng each cytokine was separated by denaturing SDS-PAGE (15% gel) and then transferred to nitrocellulose membranes. The membranes were blocked in blocking buffer, washed in PBS containing 0.05% Tween 20 (wash buffer) and then incubated with 125I–GIF (15–25 µM) for 2 h at room temperature. After washing three times with wash buffer, bound GIF was detected by autoradiography using Hyperfilm MP X-ray film (GE Healthcare Life Sciences).

### Scatchard analysis of GIF activity

Purified cytokines were radioiodinated by the chloramine-T method and a soluble ligand-binding assay was performed for each cytokine as described previously (Deane et al., 2000). Briefly, a range of 125I–GM-CSF and 125I–IL-2 concentrations (2–20 nmol) was incubated with GIF (100 ng) containing 5% fetal bovine serum for 2 h at room temperature. Bound proteins were precipitated by the addition of 20% polyethylene glycol (PEG 6000; Sigma) in PBS and incubation on ice for 30 min. The precipitated material was collected under vacuum onto GF/C filter discs (Whatman) and washed four times with ice-cold 10% PEG 6000 in PBS. The radiolabelled complexes were detected and quantified in a gamma scintillation counter. The level of non-specific binding of 125I-labelled cytokines to the filter discs in the absence of GIF was measured and the data were adjusted accordingly. Scatchard analysis was performed on best-fit plots [bound counts min−1]/free counts min−1 (y-axis) versus bound counts min−1 (x-axis), generated by using the Origin software package (OriginLab Corporation).

### Cytometric bead array (CBA) assay

Cell-free supernates were collected from virus-infected cells and concentrated approximately 10 times by using Centricon YM-10 concentrators (Millipore). These and purified rGIF proteins were assayed for chemokine-binding activity with the human chemokines IL-8, MCP-1, MIP-1α, MIP-1β and RANTES in a modified CBA assay (BD), carried out as outlined in the manufacturer’s instructions. Briefly, 50 µl aliquots of infected cell supernates, rGIFs (10–20 µg ml−1) or control samples were pre-incubated with 50 µl individual chemokine standards from Chemokine kit II (1250 pg ml−1) for 1 h at 4 °C. The mixture was then added to a 50 µl aliquot of mixed capture beads and PE detection reagent and incubated for 3 h at room temperature in the dark. After washing, the beads were analysed for bound PE in a FACScalibur flow cytometer (BD). Levels of each chemokine present in the samples were calculated from the data by using CBA analysis software (BD).

## RESULTS

The ORFV GIF gene (ORFV117) was found approximately 20 kbp from the right terminus of the genome (Deane et al., 2000; Mercer et al., 2006) and we therefore explored the corresponding regions of the PCPV, strain VR634, and BPSV, strain V660, genomes. Sequencing of the right-hand end of the PCPV EcoRI D fragment (Gassmann et al., 1985) revealed a homologue of the ORFV GIF gene. Sequencing the ends of the 20.7 kb BPSV BamHI A fragment and comparing the sequence with that of ORFV suggested that the BPSV GIF gene was likely to be located in this fragment. We found partial sequences of the BPSV GIF gene in SalI subfragments of the BPSV BamHI A fragment,
with the complete sequence being determined by the primer-walking method. Subsequent publication of the complete BPSV genome sequence (Delhon et al., 2004) confirmed this to be the equivalent of the ORFV GIF gene. The sequences of the PCPV and BPSV GIF genes have been deposited in GenBank under accession numbers EU999744 and EU999745, respectively. An alignment of the predicted GIF protein sequences from the three viruses is shown in Fig. 1.

All three GIF proteins are predicted to be 265 aa in length, with both ORFV and PCPV GIF possessing a 19 aa signal peptide, whilst that of BPSV GIF is predicted to be 20 aa in length. The ORFV and PCPV predicted proteins share 88% identity, whereas both share only 37% identity with the predicted BPSV protein. The ORFV and PCPV GIFs share four conserved potential Asn-linked glycosylation sites, only two of which are conserved in the BPSV protein, which has a further three potential sites. The mature ORFV GIF protein is predicted to contain eight Cys residues, whilst both PCPV and BPSV GIFs are predicted to contain seven. Six of these, however, are positionally conserved in all three proteins. The WDPWV motif, important for the biological activity of ORFV GIF (McInnes et al., 2005), is perfectly conserved in the PCPV GIF sequence, but although present in the BPSV GIF sequence, it shows two amino acid changes, WSPWT.

### Native and recombinant GIF activity

Native GIF activity was assessed in the supernates from fetal lamb muscle cell cultures 72 h post-infection with the three species of PPV at an m.o.i. of 10. Whilst both ORFV and PCPV native GIFs were found to bind both ovine and bovine GM-CSF and IL-2, GIF from BPSV did not bind either cytokine (Figs 2 and 3). As the BPSV strain used (V660) is a well-used laboratory strain that has been passaged in culture numerous times, a second BPSV strain (A599) that has not been repeatedly passaged in vitro was also used for comparative purposes. No GM-CSF- or IL-2-binding activity was found with this strain. To verify that the GIF gene was being expressed by the BPSV strains, a Northern blot of RNA isolated from infected cells 24 h post-infection was probed with a 32P-labelled BPSV GIF cDNA (see Supplementary Fig. S2, available in JGV Online). As had been reported previously for ORFV GIF (Deane et al., 2000), no discrete mRNA was detected, but rather a smear representative of poxvirus late mRNA transcripts was present after 24 h. This is also consistent with the T-rich late promoter-like sequences found upstream of the start codons of the GIF genes of all three viruses (data not shown).

 Supernates from CHO cells transfected with the PPV GIF cDNAs were analysed for GIF activity as above. As with the native GIF, the recombinant ORFV and PCPV GIFs bound ovine and bovine GM-CSF and IL-2, but the rGIF from BPSV bound neither (Figs 2 and 3). Again, a Northern blot of the RNA from the BPSV GIF cDNA-transfected cells indicated that the gene was being expressed (see Supplementary Fig. S3, available in JGV Online). In addition, the cell-free supernate from the BPSV GIF cDNA-transfected cells was analysed by liquid chromatography electrospray ionization tandem mass spectrometry to verify that the BPSV GIF protein was being produced (data not shown).

### Western and ligand-blot analysis

In order to verify that each of the three PPV GIF species was being expressed, partially purified proteins from the transfected CHO cells were blotted onto nitrocellulose and probed with an IgG fraction of a rabbit antiserum raised against ORFV GIF. Under reducing conditions, protein bands were detected at approximately 43 and 55 kDa (Fig. 4). The 43 kDa protein represents the native and highly glycosylated form of the predicted 28 kDa monomer, whereas the 55 kDa form is thought to be a GIF dimer (McInnes et al., 2005).

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**Fig. 1.** Alignment of the predicted mature amino acid sequences of the three PPV GIF proteins. The predicted ORFV GIF sequence is given as the consensus and identical amino acids in the other proteins are indicated by an asterisk (*). Missing amino acids are indicated by a dash (−). The predicted signal peptides are not shown. The six Cys residues and potential glycosylation sites are shaded and the WDPWV motif known to be important for biological activity of ORFV GIF is boxed.
The partially purified proteins were then radio-iodinated and tested for GM-CSF- and IL-2-binding activity by ligand blotting (Fig. 5). Under normal blotting conditions, 125I-labelled PCPV GIF bound both sets of cytokines strongly. In contrast, 125I-labelled ORFV GIF bound all four cytokines only under less stringent washing conditions and, in addition, a much longer exposure time to X-ray film was required to detect binding. No binding was observed with 125I-labelled BPSV GIF, even with mild washing conditions and extended exposure times.

**Determination of PPV GIF binding constants**

The affinity of the binding of PCPV and ORFV GIFs to both ovine and bovine GM-CSF and IL-2 was determined by Scatchard analysis (Table 1). Although no binding activity had been detected with the BPSV GIF in either the ELISA or ligand-blot assays, it was still included in this analysis. However, again no binding activity significantly above that of the non-specific control samples was detected.

**Investigation of chemokine binding by PPV GIFs**

It had previously been suggested that the ORFV GIF protein showed significant similarity to a family of poxvirus chemokine-binding proteins; more specifically, those found in members of the genera *Orthopoxvirus* and *Leporipoxvirus* that bound a variety of CC chemokines (Seet et al., 2003). In addition, significant similarity was also found between it and another ORFV protein, encoded by a gene found upstream of the GIF gene in the ORFV genome. This protein also displayed chemokine-binding activity, but in addition to binding CC chemokines, was also shown to bind the C chemokine lymphotactin. As a consequence of the similarity between the sequences of these proteins, it had been suggested that the GIF protein may also exhibit chemokine-binding activity. As no GM-
CSF- or IL-2-binding activity had been detected for the BPSV GIF, we explored the possibility that it could bind CC chemokines. This was done by using a modified CBA assay. Chemokine-binding activity was not detected with any of the recombinant GIF proteins or supernates from the BPSV-infected cells. However, chemokine binding was detected in both ORFV- and PCPV-infected cells (Fig. 6). This, however, differed between the two viruses, with MCP-1, MIP-1α, MIP-1β and RANTES binding being found with supernates from ORFV-infected cells, whilst only RANTES binding was detected with the supernates from PCPV-infected cells. This suggests that a factor(s), other than GIF, produced by ORFV and PCPV is responsible for binding these chemokines.

DISCUSSION
Orthologues of the ORFV GIF protein were found in the two bovine-specific PPVs BPSV and PCPV. In addition to sharing 88% overall amino acid identity with the ORFV GIF protein, the PCPV protein was found to contain the six Cys residues, the Asn-linked glycosylation sites and the WDPWV (WSXWS-like) motif, all of which were shown to be necessary for the biological activity of ORFV GIF (McInnes et al., 2005). As might have been predicted from the presence of these motifs, we were able to show that the PCPV protein was able to bind GM-CSF and IL-2.

Although it is only 37% identical to the ORFV and PCPV proteins, the BPSV GIF protein shares approximately 60% amino acid similarity with them. In addition, some of the features that are key to the biological activity of GIF, such as the six Cys residues, two of the putative Asn-linked glycosylation sites and the WSXWS-like motif, are conserved in the BPSV protein. However, we did not detect any interaction between it and any of the cytokines tested. This may be due to a variety of factors. BPSV V660 is a laboratory strain of the virus that has undergone serial passage in culture and could therefore potentially have lost the ability either to express the GIF gene or, as a result of cumulative mutations to the gene sequence, to produce...
biologically active GIF. We demonstrated, however, that the GIF mRNA from V660 and A599 was expressed similarly to that of PCPV GIF. We also tested cell-free supernates from cells infected with A599, a different strain of BPSV that has been passaged fewer than 10 times since its isolation. However, again no GM-CSF- or IL-2-binding activity was detected with this virus. The V660 sequence reported here is 95.5% identical to that of the published BPSV sequence (Delhon et al., 2004) and 98% identical to another isolate (B177; kindly provided by H.-J. Rziha, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Tübingen, Germany), which we have sequenced (results not shown). Therefore, it is likely that the sequence reported here is representative of the true BPSV GIF sequence and it is considered unlikely that the few changes in amino acids noted would have resulted in an inactive protein. It is worth noting, however, that the WSXWS-like motif that is found in the BPSV GIF protein is different from those found in the ORFV and PCPV proteins. This motif is found in a number of cytokine receptors and is thought to be linked to the tertiary structure of the receptor molecules (Bazan, 1990; Quelle et al., 1992; Baumgartner et al., 1994; Ronco et al., 1995).

### Table 1. Binding affinities (in pM) of PPV GIF proteins with ovine and bovine GM-CSF and IL-2

<table>
<thead>
<tr>
<th>GIF</th>
<th>Ovine GM-CSF</th>
<th>Ovine IL-2</th>
<th>Bovine GM-CSF</th>
<th>Bovine IL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>rGIF-ORFV</td>
<td>405–500</td>
<td>1110–1390</td>
<td>400–468</td>
<td>496–556</td>
</tr>
<tr>
<td>rGIF-PCPV</td>
<td>248–278</td>
<td>536–714</td>
<td>237–263</td>
<td>250–338</td>
</tr>
<tr>
<td>rGIF-BPSV</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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NA, Not applicable.

**Fig. 6.** An adaptation of the CBA assay (BD) was used to assess chemokine-binding activity in cell-free supernates of PPV-infected cells or PPV GIF-transfected cells. Supernates were pre-mixed with a known amount of the standard chemokines MCP-1 (diagonally hatched bars), MIP-1α (filled bars), MIP-1β (horizontally hatched bars), RANTES (shaded bars) or IL-8 (empty bars) before mixing with the capture beads and detection reagent. This was incubated for 3 h at room temperature before binding was measured using a FACScalibur flow cytometer (BD). A high reading indicates no binding activity in the supernates, whereas a low reading indicates interference by the cell-free supernates of binding between the chemokines and the capture beads. Data represent mean ± SEM binding of quadruplicate samples. *P<0.001 compared with medium taken from untransfected CHO cells.
binding specificity in comparison to the ORFV and PCPV proteins.

The GIF proteins of the PPVs remain unique, with no corresponding proteins yet found in any of the other genera of the subfamily Chordopoxvirinae. This is despite many computer-based structural-analysis programs suggesting a close relationship with the family of type II chemokine-binding proteins (vCCI) encoded by members of the genera Orthopoxvirus and Leporipoxvirus (Carfi et al., 1999; Seet & McFadden, 2002). This relationship is primarily due to sequence similarity found between the GIF protein and a chemokine-binding protein also encoded by the PPVs (ORF 112). This protein has been shown to be related to the vaccinia virus (VACV) chemokine-binding protein C23L and to possess both C- and CC-chemokine-binding activity (Seet et al., 2003). As we did not find any GM-CSF- or IL-2-binding activity with the BPSV GIF protein, we investigated whether the GIF proteins exhibited CC chemokine-binding activity. No such activity was detected with any of the rGIF proteins, despite the fact that the supernates from both ORFV- and PCPV-infected cells appeared to interact with human CC chemokines. This activity was presumably due to the PPV chemokine-binding protein characterized previously. Interestingly, no activity was detected from BPSV-infected cells. The putative BPSV chemokine-binding protein (Delhon et al., 2004) is predicted to be only 41% identical to the ORFV protein (the PCPV protein is yet to be characterized), with even lower similarity to the vCCI orthopox proteins and, as a result, could have a different binding specificity. It has already been suggested that the ORFV protein has shifted its binding specificity in comparison to the orthopox and leporipox family of proteins, as it was shown to bind C chemokines as well as the CC chemokines.

Our findings suggest that the ORFV and PCPV GIF proteins behave similarly in terms of their ability to bind the cytokines GM-CSF and IL-2. However, the same activity was not found with the BPSV GIF protein. It is interesting that the pathological appearance of orf and pseudocowpox are also similar and that they both differ from bovine papular stomatitis, most notably in the proliferative nature of their lesions. Whilst we are not suggesting that the GIF protein has a role in the proliferation of ORFV and PCPV lesions, we do think that it is possible that the BPSV GIF protein has evolved a quite separate spectrum of activity more suited to its biological niche.

The full genomic sequences of ORFV and BPSV have been published and show approximately 67–75% nucleotide identity across the whole genome, with amino acid identities between proteins ranging from 34 to 93%, with an average of 71% (Delhon et al., 2004; Mercer et al., 2006). Unfortunately, the full genomic sequence of PCPV is not known, but the small amount of sequence that has been published appears to be more similar to ORFV than it is to BPSV. For example, the major envelope protein (corresponding to VACV F13L) shares approximately 95% identity with the equivalent ORFV protein, but only 84% identity with that of BPSV (Inoshima et al., 2001). Similarly, there is approximately 68% identity between ORFV and PCPV VEGF proteins, but only 55% between the PCPV and BPSV VEGFs (Ueda et al., 2003). This difference is also reflected in the biological activities of the virus-encoded VEGF proteins. In addition to binding VEGFR-2, the BPSV VEGF also has the distinct capability, amongst the poxviral VEGFs, of binding VEGFR-1 and inducing monocyte migration (Inder et al., 2007). It had originally been thought that the two cattle viruses would be more similar to each other than either would be to ORFV. However, the data published here for the three GIF proteins would appear to reinforce the suggestion that PCPV is related more closely to ORFV than to BPSV. As it would appear that PCPV does not cause disease in sheep or goats and ORFV does not cause disease in cattle, a more detailed comparison of the ORFV and PCPV genomes may reveal critical elements that help to dictate host range within the PPVs.

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