Infection kinetics, prostacyclin release and cytokine-mediated modulation of the mechanism of cell death during bluetongue virus infection of cultured ovine and bovine pulmonary artery and lung microvascular endothelial cells

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Bluetongue virus (BTV) infection causes a haemorrhagic disease in sheep, whereas BTV infection typically is asymptomatic in cattle. Injury to the endothelium of small blood vessels is responsible for the manifestations of disease in BTV-infected sheep. The lungs are central to the pathogenesis of BTV infection of ruminants; thus endothelial cells (ECs) cultured from the pulmonary artery and lung microvasculature of sheep and cattle were used to investigate the basis for the disparate expression of bluetongue disease in the two species. Ovine and bovine microvascular ECs infected at low multiplicity with partially purified BTV were equally susceptible to BTV-induced cell death, yet ovine microvascular ECs had a lower incidence of infection and produced significantly less virus than did bovine microvascular ECs. Importantly, the relative proportions of apoptotic and necrotic cells were significantly different in BTV-infected EC cultures depending on the species of EC origin and the presence of inflammatory mediators in the virus inoculum. Furthermore, BTV-infected ovine lung microvascular ECs released markedly less prostacyclin than the other types of ECs. Results of these in vitro studies are consistent with the marked pulmonary oedema and microvascular thrombosis that characterize bluetongue disease of sheep but which rarely, if ever, occur in BTV-infected cattle.

Introduction

Bluetongue virus (BTV) is the aetiological agent of bluetongue, an insect-transmitted, haemorrhagic disease of sheep and some species of wild ruminants (Spruell, 1905; MacLachlan, 1994). The pathogenesis of BTV infection is very similar in sheep and cattle (Spruell, 1905; Moulton, 1961; Pini, 1976; Mahrt & Osburn, 1986; MacLachlan, 1994; Barratt-Boyes & MacLachlan, 1995). After initial replication in lymph nodes draining the sites of inoculation, BTV disseminates to secondary sites, principally the lungs and spleen, where it replicates in endothelium and mononuclear phagocytes (Mahrt & Osburn, 1986; MacLachlan et al., 1990; MacLachlan, 1994; Barratt-Boyes & MacLachlan, 1994, 1995; Barratt-Boyes et al., 1995). BTV infection of ruminants is characterized by a prolonged cell-associated viraemia that can persist in the presence of high titres of neutralizing antibody in the blood of infected sheep and cattle (Spruell, 1905; MacLachlan et al., 1987, 1994; Richards et al., 1988; Katz et al., 1994).

Despite the similarities in the pathogenesis of BTV infection of sheep and cattle, there are pronounced differences in the clinical manifestations of BTV infection in the two species. Cattle are considered reservoir hosts of BTV because most infections are asymptomatic, and the very sporadic occurrence of disease in BTV-infected cattle likely reflects a type-I hypersensitivity reaction (Anderson et al., 1987). In contrast, bluetongue disease of sheep and deer is characterized by virus-induced injury to vascular endothelium (Mahrt & Osburn, 1986; Howerth & Tyler, 1988) and, in fulminant cases, by disseminated intravascular coagulation that leads to ischaemic necrosis in a variety of tissues, haemorrhagic diathesis and shock (reviewed in Parsonson, 1990; MacLachlan, 1994). The lungs are the shock organ of ruminants and the organ most
susceptible to permeability disorders of the vasculature (Meyrick et al., 1989, 1991); thus severe pulmonary oedema is characteristic of bluetongue disease in sheep (Spruell, 1905; Moulton, 1961). Although high titers of BTV are present in the lungs of infected sheep (Pini, 1976), it is uncertain whether BTV-mediated injury to the pulmonary vascular endothelium is entirely the result of direct virus-induced cytopathology or also from the activity of inflammatory mediators.

Endothelial cells (ECs) from different species or tissues have diverse properties, and may respond differently to the same stimulus (Gerritsen, 1987; Kumar et al., 1987; Meyrick et al., 1989; Page et al., 1992; Craig et al., 1998). Significantly, ECs cultured from different levels of the pulmonary circulation of sheep and cattle are heterogeneous and retain their phenotype in culture (Meyrick et al., 1991). EC phenotype is central to the pathogenesis of endotheliotropic virus infections. For example, replication of American isolates of ovine lentivirus, as assessed for expression of a recently identified EC-specific adhesion molecule (GR Willebrand factor (Dako), as von Willebrand factor contained in

Methods

Isolation and propagation of ECs. Primary EC cultures were derived from the pulmonary artery and lung microvasculature of sheep and cattle. The heart and lungs of yearling animals were obtained immediately following euthanasia. The pulmonary artery and the peripheral edges of each lung lobe were washed and transported on ice in Hanks' balanced salt solution (HBSS; Sigma) that contained penicillin-streptomycin (MediaTech), gentamycin and fungizone (BioWhittaker).

Lung microvascular ECs were obtained essentially as previously described (Meyrick et al., 1989; Carley et al., 1992; Craig et al., 1998). Briefly, the visceral pleura was removed, the lung parenchyma was finely minced, washed repeatedly with HBSS, and then digested for 30 min with 2 mg/ml collagenase IA/dispase (Sigma) in HBSS. The digested tissue was filtered through a screen, washed with MEM/α-Val (Life Technologies) that contained 20% foetal bovine serum (FBS; Hyclone) and the isolated cells were stimulated with vascular endothelial cell growth factor (VEGF) and EC mitogen (Biomedical Technologies). The procedure was repeated three times on any remaining tissue too large to pass through the screen. The cells from each digest were pooled in human fibronectin-coated dishes (Becton Dickinson) and grown in isolation medium [MEM/α-Val, 10% FBS, penicillin–streptomycin, MEM vitamins, nonessential amino acids (MediaTech); 16 U/ml heparin, 1 μg/ml hydrocortisone, sodium pyruvate, t-glutamine and 5% human serum from platelet-depleted plasma (Sigma)].

Purification and characterization of ECs. Cultures of ECs were purified by fluorescence-activated cell sorting (FACS) with a MoFlo instrument (Cytomation). ECs have a scavenger pathway for low-density lipoproteins and rapidly uptake the acetylated forms (Voyta et al., 1984); thus FACS selection of ECs was based on the uptake of 1,1-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate conjugated to acetylated low-density lipoproteins (DiI-Ac-LDL; Biomedical Technologies). This method has been used previously to obtain pure cultures of ECs from a variety of species and tissues (Voyta et al., 1984; Carley et al., 1992; Visner et al., 1994; Magee et al., 1994; Craig et al., 1998).

Cultures of lung microvascular ECs were enriched by FACS at approximately 2 days after isolation and transferred to dishes coated with 1 μg/cm² natural mouse laminin (Life Technologies). Multiple colonies of microvascular ECs were then selected with cloning discs, pooled and expanded. The cultures of pulmonary artery and lung microvascular ECs were labelled with DiI-Ac-LDL and purified by FACS three additional times to remove any contaminating cells that remained after the primary isolation. Purified EC cultures were screened for contaminating cells with monoclonal antibodies (MAbs; Dako) that recognize epithelial and mesothelial cells (MAbs AE1/AE3, cytotkeratin-specific), pericytes (MAb D33, desmin-specific) and smooth muscle cells (MAb 1A4, actin-specific; Hewett & Murray, 1993; McDouall et al., 1996). The phenotype of the purified ECs was confirmed with rabbit antisera to von Willebrand factor (Dako), as von Willebrand factor contained in

Virus. BTV serotype 17 in the blood of a sheep that died of bluetongue disease after natural infection was passaged twice in
seronegative cattle and isolated from blood essentially as previously described (MacLachlan & Fuller, 1986; Richards et al., 1988). The virus was isolated in bovine lung microvascular ECs, amplified by two additional passages, and a stock of BTV-infected EC lysate was prepared. Briefly, the cells and medium were frozen at −70 °C and then thawed, homogenized, sonicated and filtered (0.2 µm). A similar lysate of uninfected lung microvascular ECs was produced for mock infection of ECs. Partially purified BTV17 was prepared from BTV-infected bovine microvascular ECs that were pelleted from the medium, washed with PBS, resuspended in ECMM, and then disrupted and filtered as above. Virus was pelleted by centrifugation at 69000 g for 3 h through a 20% sucrose cushion. The virus-containing pellets were resuspended in ECMM and the supernatant, containing cellular components of infected ECs, also was harvested. Virus titres (TCID$_{50}$) were determined in BHK-21 cells as described previously (MacLachlan et al., 1984; Barratt-Boyces et al., 1992).

**BTV infection of ECs.** All ovine and bovine pulmonary artery and lung microvascular EC cultures were infected with BTV that was derived from bovine lung microvascular ECs. Cultures of similar passage and cell density were infected at high multiplicity (m.o.i. = 3) for a one-step analysis of virus growth. Briefly, cultures were washed with DMEM and adsorbed with partially purified BTV for 1 h at 37 °C. The inoculum was then removed and the cultures were washed with DMEM. The infected cultures were maintained in ECMM and replicate cultures were harvested at 4 h intervals by scraping. Samples of each culture were frozen and thawed, sonicated, and virus titres (TCID$_{50}$) were determined.

EC cultures of similar passage and cell density also were infected at low m.o.i. (0.05) with either partially purified BTV or the BTV-infected EC lysate. Individual EC cultures were infected at 4–8 h intervals, and replicate cultures were included at 24, 48, 72 and 96 h intervals. Control flasks were inoculated (mock infected) with the uninfected EC lysate at 24 h intervals. Cytopathic effect was estimated as a percentage of the monolayer at each interval, and cultures were then harvested and simultaneously prepared for virus titration and FACS analyses. Culture medium from each flask was clarified by centrifugation at 400 g prior to virus titration. Cells remaining in each flask were removed by trypsinization, pooled with those previously pelleted from the medium, and washed once with PBS. Titres (TCID$_{50}$) of BTV in the supernatant and cell fraction of individual EC cultures were determined by microtitre assay. Infection of ECs was detected by labelling virus proteins with BTV-specific rabbit antiserum (Heidner et al., 1988, 1990), FITC- or PE-conjugated goat anti-rabbit immunoglobulin (Sigma), and FACS analyses as previously described (Barratt-Boyces et al., 1992).

**Quantification of cell death in BTV-infected EC cultures.** The incidence of cell death in BTV-infected EC cultures was quantified by determining the percentage of apoptotic and necrotic cells in each culture at 4–8 h intervals after BTV infection. Apoptosis and necrosis were quantified in EC cultures by double-label FACS analysis based on the exclusion of propidium iodide (Sigma) and the binding of annexin V (R&D Systems), as previously described (Vermes et al., 1995).

**Influence of inflammatory mediators on BTV infection of ECs.** The levels of prostacyclin released following BTV infection of ECs were determined at 24 h intervals by measuring its stable metabolite, 6-ketoprostaglandin F$_{1α}$, in clarified culture medium with a competitive ELISA (R&D Systems). The potential role of cytokine mediators in BTV-induced EC injury was investigated also. EC cultures were treated with 10 ng/ml mouse recombinant interleukin-1β (IL-1β; Sigma) and infected with partially purified BTV. Uninfected EC cultures were treated with IL-1β alone, the EC supernatant collected during the purification of BTV, or a cocktail of cytokines (10 ng/ml IL-1β, 10 µg/ml mouse recombinant tumour necrosis factor-α (TNF-α) and 1 µM platelet-activating factor (PAF); Sigma). These cytokines previously have been used to activate ECs isolated from humans and ruminants (Meyrick et al., 1991; Jutila et al., 1994; Bargatze et al., 1994; Sterner-Kock et al., 1996). The percentage of apoptotic and necrotic cells in cultures treated with cytokines then was determined at 4–8 h intervals.

**Data analysis.** Data were analysed with CELLQuest version 3.1 (Becton Dickinson), Excel 97 (Microsoft) and MINITAB (Minitab Inc.) software packages. Polynomial regression analyses were performed on FACS and ELISA data, and moving average regression analyses were done on virus titration data obtained at different times from the various EC cultures. All experiments were repeated at least once and the data from replicate experiments were compared at 0, 24, 48, 72 and 96 h post-infection (p.i.). The mean values and standard deviations were calculated for each parameter and a Student’s t-test was applied to determine differences at 0.05 considered significant. The comparison yielding the highest $P$ value ≤ 0.05 is reported in cases where multiple t-tests were used to identify statistical differences at various time-points between the four types of ECs.

**Results**

**Isolation and characterization of ovine and bovine ECs**

Primary EC cultures were established from two distinct regions of the pulmonary vasculature of sheep and cattle. Cultures were derived from several animals and the poolings of multiple independent EC colonies to minimize variation. Ovine EC cultures initially required supplementation with filtered (0.2 µm) serum obtained from the same animals from which the cultures were derived, but this was discontinued after the EC cultures were purified. The cultures of ovine and bovine lung microvascular ECs initially were extensively contaminated with pericytes and smooth muscle cells. Pure cultures were obtained after a rigorous combination of colony selection, removal of contaminating cells by aspiration, FACS, and variation of the culture medium and substrate. Pulmonary artery ECs were less fastidious and more readily purified than the microvascular ECs. Immunofluorescent staining of von Willebrand factor in perinuclear granules (Weibel–Palade bodies), uptake of Dil-Ac-LDL, and inducible expression of E-selectin (bovine ECs) and GR antigen (ovine and bovine ECs) confirmed the identity of cells in the various EC cultures. The absence of staining for cytokeratin, smooth muscle actin and desmin confirmed the purity of the cultures. All ECs were strongly contact inhibited and formed cobblestone monolayers that were maintained for up to 30 passages without contamination by other cell types.

**Infection of ECs with partially purified BTV**

The various EC cultures were inoculated at both high and low m.o.i. to identify any differences in the kinetics of BTV infection of ovine and bovine microvascular and pulmonary artery ECs. The kinetics of BTV infection were similar in the four types of EC cultures inoculated at high m.o.i. with partially purified BTV, and there were no significant differences in the
maximum titres of BTV produced in each type of EC (Fig. 1). In contrast, the species and site of origin of the ECs clearly influenced the kinetics of BTV infection in cultures inoculated at low m.o.i. (Fig. 2). Specifically, bovine microvascular EC cultures produced significantly higher titres of virus than did the ovine microvascular EC cultures \( (10^{8.9} \text{ versus } 10^{6.9} \text{ TCID}_{50} \text{ respectively}; \ P = 0.0014) \), whereas the maximum titres of BTV in ovine and bovine pulmonary artery EC cultures were not significantly different. Furthermore, the percentage of BTV-infected cells, as determined by FACS, was significantly lower in ovine lung microvascular ECs at 24 h p.i. than in any of the other EC types \( (P \leq 0.011) \), and was significantly higher in both types of bovine ECs at 72 and 96 h p.i. than in either of the ovine EC types \( (P \leq 0.033) \). Similar results were consistently obtained in replicate experiments, confirming that both the m.o.i. of the cultures, and the species and site of origin of the ECs can significantly influence the kinetics of BTV infection of cultured ovine and bovine pulmonary ECs.

The incidence of cell death (sum of apoptotic and necrotic cells) in the four types of EC cultures was not significantly different at 24, 48, 72 and 96 h p.i. with partially purified BTV at low m.o.i. The incidence of cell death determined by FACS also was very similar to the estimated incidence of cytopathic effect observed microscopically (data not shown). With the notable exception of ovine pulmonary artery ECs, death of ECs was due almost entirely to necrosis and not apoptosis, and the percentage of apoptotic cells in EC cultures other than ovine pulmonary artery ECs was similar to that in mock-infected cultures (Fig. 2; Table 1).

**Infection of ECs with BTV-infected EC lysate**

Cultures of the four types of ECs were inoculated with a lysate of BTV-infected ECs to determine the effect of EC-derived inflammatory mediators on the kinetics of BTV infection. Components of the virus-infected EC lysate significantly changed the kinetics of BTV infection in each EC culture as compared to infection with partially purified BTV (Figs 2 and 3). Specifically, the percentage of virus-infected cells was significantly reduced in each type of EC culture at 24, 48, 72 and 96 h p.i. \( (P \leq 0.050) \) and EC cultures inoculated with the BTV-infected EC lysate also produced less virus than did similar cultures infected with partially purified BTV. For example, there were significant differences in the titres of BTV in the bovine pulmonary artery EC cultures at 48 h p.i. \( (P = 0.029) \) and in the maximum titres of virus produced by ovine pulmonary artery EC cultures \( (P = 0.012) \).

Components of the BTV-infected EC lysate also modulated the incidence and mechanism of cell death in the various EC cultures. Apoptosis was a major mechanism of cell death in lung microvascular EC cultures inoculated with the BTV-infected EC lysate, which is in distinct contrast to infection of microvascular ECs with partially purified BTV where necrosis was the major mechanism of cell death. Furthermore, the incidence of cell death in ovine lung microvascular and bovine pulmonary artery EC cultures was significantly reduced at 24, 48, 72 and 96 h p.i. as compared to similar EC cultures infected with partially purified BTV \( (P \leq 0.034; \text{ Figs } 2 \text{ and } 3) \). The data indicate that mediators contained in the BTV-infected EC lysate significantly altered the course of infection in the various EC cultures by modulating the incidence and mechanism of cell death as well as the kinetics of BTV infection. The sensitivity/susceptibility of ECs to BTV infection in the presence of inflammatory mediators also varied with the species and site of origin of the ECs.

**Prostacyclin production in BTV-infected ECs**

Levels of prostacyclin were determined at regular intervals after inoculation of the various EC cultures to further investigate the role of inflammatory mediators in the in vitro pathogenesis of BTV infection of ECs (Fig. 4). Ovine ECs released significantly less prostacyclin than did bovine ECs at 48, 72 and 96 h p.i. with partially purified BTV \( (P \leq 0.036) \). With the notable exception of ovine microvascular ECs, ECs inoculated with the BTV-infected EC lysate also released considerable amounts of prostacyclin (data not shown). Ovine lung microvascular ECs consistently released relatively little prostacyclin after infection with either BTV inoculum, indicating for the first time that their antithrombotic response to BTV infection is distinctly different from that of other types of ECs.

**Influence of inflammatory mediators on the mechanism of EC death**

The incidence of apoptosis and necrosis was determined in EC cultures treated with a variety of inocula in an effort to further characterize the role of inflammatory mediators released during BTV infection of ECs (Table 1). The incidence of apoptosis was increased in ovine pulmonary artery and lung microvascular EC cultures treated with the supernatant of EC lysates from which the partially purified BTV inoculum was derived. In contrast, the incidence of cell death in cultured ECs...
BTV infection of endothelial cells

Fig. 2. Regression analyses of virus titre (log_{10} TCID_{50}) and the incidence of infection, apoptosis and necrosis (%) from bovine pulmonary artery (BP), ovine pulmonary artery (OP), bovine lung microvascular (BL) and ovine lung microvascular (OL) ECs infected at low multiplicity (m.o.i. = 0.05) with partially purified BTV.

Table 1. Mechanism of cell death in BTV-infected ECs

The incidence of necrosis and apoptosis were determined at 4–8 h intervals. The values reported are the maximum values recorded during a 96 h infection. The maximum incidence of necrosis and apoptosis did not occur at the same time for each EC type. —, < 25% of the cell population (equivalent to background levels); +, 25–50%; ++, 51–75%; ++++, > 75%.

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<th>Origin of EC</th>
<th>Pulmonary artery microvasculature</th>
<th>Lung microvasculature</th>
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<td>Inoculum</td>
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<td>Necrosis</td>
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<td>Partially purified BTV</td>
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<td>BTV-infected EC lysate*</td>
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<td>Partially purified BTV + IL-1β</td>
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<td>IL-1β, TNF-α and PAF†</td>
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<td>Supernatant from purification of BTV</td>
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<td>Uninfected EC lysate (mock infection)</td>
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* Lysate of BTV-infected bovine microvascular ECs.
† Recombinant interleukin-1β, recombinant tumour necrosis factor-α and platelet-activating factor; see text.

was unaltered by lysates of uninfected ECs, indicating that EC-derived mediators that caused cell death were induced by BTV infection and were not constitutively expressed by cultured ECs. The incidence of apoptosis and necrosis in uninfected EC cultures treated with IL-1β or a combination of IL-1β, TNF-α and PAF was similar to the background levels that occurred in mock-infected cultures, whereas the incidence of apoptosis was markedly increased in ovine and bovine microvascular EC cultures that were both treated with IL-1β and infected with partially purified BTV. The incidence of apoptosis in BTV-infected cultures treated with IL-1β was similar to that which occurred in cultures inoculated with the BTV-infected EC lysate, and very different from that which occurred in EC cultures infected with partially purified BTV alone. The data
Fig. 3. Regression analyses of virus titre (log_{10} TCID_{50}) and the incidence of infection, apoptosis and necrosis (%) from bovine pulmonary artery (BP), ovine pulmonary artery (OP), bovine lung microvascular (BL) and ovine lung microvascular (OL) ECs infected at low multiplicity (m.o.i. = 0.05) with a BTV-infected EC lysate that contained mediators released from the ECs.

Fig. 4. Regression analyses of prostacyclin release from ovine lung microvascular (OL), bovine lung microvascular (BL), ovine pulmonary artery (OP) and bovine pulmonary artery (BP) ECs infected at low multiplicity (m.o.i. = 0.05) with partially purified BTV.

indicate that inflammatory mediators such as IL-1β influence the mechanism of cell death in BTV-infected ECs, especially in lung microvascular EC cultures.

Discussion

ECs cultured from the pulmonary vasculature of sheep and cattle provide a convenient and relevant in vitro system with which to evaluate the comparative pathogenesis of BTV infection of ruminants, and the occurrence of direct virus-mediated endothelial injury in BTV-infected sheep but not cattle in particular. Although BTV caused a lytic infection in all four types of ECs, the kinetics of infection varied significantly in the various EC cultures infected with partially purified BTV at low m.o.i. Differences in virus yield likely reflect inherent differences in the susceptibility of each EC type to BTV infection as ECs cultured from different regions of the pulmonary vasculature retain their phenotype in culture (Meyrick et al., 1991). Thus, although ovine and bovine microvascular ECs infected at low m.o.i. with partially purified BTV were equally susceptible to BTV-induced cell death, ovine microvascular ECs had a lower incidence of infection and produced significantly less virus than did bovine microvascular ECs. The enhanced susceptibility of ovine microvascular ECs to BTV-induced cell death is consistent with the acute disease subsequent to endothelial injury in small vessels that occurs in BTV-infected sheep but not cattle. In distinct contrast, BTV-infected ovine umbilical vein ECs produced more virus but exhibited less cell death than did BTV-infected bovine umbilical vein ECs (Russell et al., 1996), emphasizing the critical need to use ECs derived from target tissues in the in vitro characterization of the pathogenesis of endotheliotropic virus infections such as that caused by BTV.

Mediators released by ECs clearly modulated the course of BTV infection in ECs, as previously described for BTV infection of ovine and bovine umbilical vein ECs and a bovine cardiopulmonary EC line (Coen et al., 1991; Russell et al., 1996). EC-derived mediators that modulate thrombosis and inflammation include IL-1, IL-6, interferons, prostacyclin and chemokines such as IL-8 (Mantovani et al., 1992), and likely were present in the BTV-infected EC lysate but not in the partially purified BTV inoculum used in our studies. Thus, virus yields consistently were lower in ECs infected with the BTV-infected EC lysate as compared to those infected with partially purified BTV. Furthermore, necrosis was the major cause of cell death in all ECs infected with partially purified BTV except ovine pulmonary artery ECs, whereas apoptosis predominated in all ECs infected with the BTV-infected cell lysate. Similarly, addition of IL-1β to the partially purified BTV inoculum caused apoptosis of virus-infected ovine and bovine microvascular ECs, whereas infection of these cultures with partially purified
BTV alone resulted only in necrosis of virus-infected ECs. Apoptosis of ECs is linked to paracrine release of IL-1, and genes such as that encoding IL-1-converting enzyme (caspase-1) also regulate release of inflammatory cytokines (Steller, 1995; Henkart, 1996; Vaux & Strasser, 1996; Hebert et al., 1998). The expression of clinical bluetongue disease in sheep but not cattle might, therefore, reflect inherent differences in the susceptibility of ovine and bovine pulmonary microvascular ECs to induction of apoptosis by IL-1 or other virus-induced EC-derived mediators.

Prostacyclin is a potent antithrombotic agent that is produced primarily by ECs, and there were significant differences in production of prostacyclin following BTV infection of the four types of ECs. BTV-infected ovine lung microvascular ECs consistently and reproducibly produced significantly lower levels of prostacyclin than did the other types of ECs. Similarly, ovine and bovine pulmonary artery and microvascular ECs produced different amounts of prostacyclin after activation with endotoxin (Meyrick et al., 1989). The very low levels of prostacyclin produced in BTV-infected ovine microvascular ECs suggest that sheep might be less able than cattle to regulate platelet aggregation subsequent to BTV-induced endothelial injury, consistent with the consumptive coagulopathy, haemorrhagic diathesis and severe pulmonary oedema that are characteristic of bluetongue disease of sheep but which rarely, if ever, occur in BTV-infected cattle.

In summary, our studies indicate that the site and species of EC origin can markedly influence the outcome of BTV infection in vitro. Thus, endothelial injury in BTV-infected sheep but not cattle could reflect differences in either the direct pathogenic effects of BTV infection of ECs, or their response to inflammatory mediators released by virus-infected ECs and, perhaps, other cell types such as monocytes (Whetter et al., 1989). Although precise characterization clearly will require additional studies, data from these in vitro studies strongly suggest that inherent differences in the susceptibility of ruminant microvascular ECs to BTV infection, and their production and response to mediators such as prostacyclin and IL-1, contribute to endothelial injury and subsequent expression of bluetongue disease in sheep but not cattle.

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