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Ovine trophoblasts express cathelicidin host defence peptide in response to infection

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ABSTRACT

Cationic host defence peptides (CHDP; also known as antimicrobial peptides) are key components of the immune response in the female reproductive tract. The role of the placental trophoblast in ovine host defence remains poorly understood. This study characterises expression of genes for cathelicidin and defensin peptides in primary ovine placental tissues, the ovine trophoblast cell line (AH-1) and in response to the TLR-4 ligand LPS, the abortifacient organism *Waddlia chondrophila* and 1α,25-dihydroxyvitamin D_3.

Using RT-PCR, expression of the CHDP SMAP-29, sBD-1 and sBD-2 was assessed in the AH-1 cell line in response to LPS, 1α,25-dihydroxyvitamin D_3 exposure (a known stimulator of cathelicidin gene expression), or *W. chondrophila* infection. Expression of cathelicidin in the trophoblast compartment of the ovine placenta and in the ovine trophoblast cell line (AH-1) was also established. AH-1 cells did not upregulate expression of CHDP in response to LPS, but sBD-1 and sBD-2 expression was significantly increased in response to *W. chondrophila* infection. SMAP-29 expression was not altered by *in vitro* exposure to 1α,25-dihydroxyvitamin D_3.

This study demonstrates that the ovine trophoblast expresses cathelicidins, but does not upregulate expression of CHDP in response to LPS. Ovine trophoblasts are shown to differentially regulate expression of CHDP and lack a demonstrable vitamin D-mediated cathelicidin response.

1. Introduction

Cationic host defence peptides (CHDP) are a diverse group of evolutionary conserved peptides with a broad range of antimicrobial and immunomodulatory functions (Brogden et al., 2003; Zasloff, 2002; Barlow et al., 2014). Increased expression and release of CHDP is an important part of the innate immune response following detection of infection *via* recognition of pathogen-associated molecular patterns (PAMP) through pattern recognition receptors (PRR) such as the Toll-like receptors (TLR) and nucleotide-binding oligomerisation domain (NOD) receptors (NOD-like receptors or NLRs) (Ganguly et al., 2009; Lande et al., 2007; Rietdijk et al., 2008). In humans, there are two main families of CHDP, cathelicidins, and defensins (Peschel and Sahl, 2006) and these peptides have been identified in several cell types, including neutrophils, macrophages, lymphocytes, eosinophils, epithelial cells and placental trophoblasts (Bowdish et al., 2006).

The expression of CHDP within the human female reproductive tract is well characterised (Horne et al., 2008; King et al., 2003, 2007a). During pregnancy, the human placenta expresses several β-defensins, secretory leukocyte protease inhibitor (SLPI), and the sole human cathelicidin hCAP-18. Notably, it has been demonstrated that human placental trophoblasts lack expression of Toll Like Receptor-4 (TLR4) translating to a lack of inducible secretion of CHDP in this cell type in response to lipopolysaccharide stimulation (King et al., 2003; Klaffenbach et al., 2011). In contrast, it was demonstrated that ovine trophoblasts do possess intact TLR4 signalling pathways and can respond to LPS stimulation with a pro-inflammatory response characterised by TNF-α and IL-8 (CXCL8) secretion (Wheelhouse et al., 2009). However, unlike well-characterised cells and tissues such as the gut and lung (Skerlavaj et al., 1999), it is not yet known if ovine trophoblasts are capable of expressing cathelicidin peptides. Indeed, the potential for the TLR-4 signalling pathway to stimulate CHDP production in ovine trophoblast cells is not yet known.

In humans the expression of CHDP can be stimulated by 1α,25-dihydroxyvitamin D3, signalling *via* the vitamin D receptor (Gombart et al., 2005; Wang et al., 2004), and peptide concentrations can be increased rapidly at sites of infection and inflammation. The use of vitamin D_3 as a potential therapeutic is of significant clinical interest and several studies have shown that vitamin D attributed antimicrobial activity is solely mediated by the expression of cathelicidin (Liu et al., 2007). However, while cathelicidin and defensin expression in humans in response to vitamin D is well characterised, CHDP gene expression and release in key immune cells of ovine species, and indeed the role that cathelicidins and defensins can play in host defence against infections in these species, is poorly understood.

Within livestock species, *Chlamydia* and *Chlamydia*-related pathogens such as *Chlamydia abortus* and *Waddlia chondrophila* are

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al., 2006). Understanding the upregulation of expression of host defence molecules such as cathelicidins and defensins in reproductive tissues would provide key insights into host defence in these areas, particularly since the ovine cathelicidin SMAP-29, ortholog of the human cathelicidin LL-37, has been shown to have potent antimicrobial activity (Entrican et al., 2010; Longbottom et al., 2013; Brogden et al., 2001).

This study characterises the expression of CHDP within the ovine placenta, using both primary ovine tissue and the ovine trophoblast AH-1 cell line. We characterise the expression of genes associated with CHDP production in trophoblasts following stimulation with the TLR4 ligand, LPS, and during infection of AH-1 cells with the invasive clinically relevant *Chlamydia*-related pathogen of emerging pathological significance *Waddlia chondrophila*.

2. Materials and methods

2.1. Cell culture

The SV40 large T antigen transformed ovine trophoblast cell line AH-1, developed as previously described (Haldorson et al., 2006) (a kind gift from Professor T. Baszler, Washington State University, USA), was grown and maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 5% heat inactivated fetal bovine serum (PAA Laboratories Ltd., Yeovil, Somerset, UK) in a heated, humidified incubator at 37 °C and 5% CO_2. Cells were passaged at sub-confluency with 1× Trypsin-EDTA.

2.2. Propagation of W.chondrophila

Waddlia chondrophila strain ATCC VR-1470 was grown at 37 °C in McCoy cells with RPMI-1640 medium that was supplemented with 2% heat inactivated fetal bovine serum (PAA Laboratories Ltd., Yeovil, Somerset, UK). After 72 h growth, cell monolayers were disrupted with glass beads, and medium containing cell debris aspirated then centrifuged at 50*g* for 5 min at 4 °C to remove intact cells. Supernatant was aspirated and subsequently centrifuged at 20,000*g* using a J-LITE JLA-16.250 rotor (Beckman Coulter Ltd. High Wycombe, UK). The pellet was resuspended in ice-cold sucrose-phosphate-glutamic acid (SPG) buffer (10 mM sodium phosphate [8 mM Na_2HPO_4–2 mM NaH_2PO_4], 220 mM sucrose, 0.5 mM L-glutamic acid pH7.4), aliquoted into microcentrifuge tubes and stored at −80 °C. To quantify viable organisms, aliquots were thawed at room temperature and titrated on McCoy cells. Serial dilutions of the inoculum were added to confluent cell monolayers in 8-well chamber slides (BD Falcon, Becton Dickinson, Bedford, UK). After 24 h, medium was removed, cells were fixed in ice-cold acetone, air-dried, and slides were frozen at −20 °C prior to analysis by fluorescent immunocytochemistry as previously described (Wheelhouse et al., 2014) (polyclonal sera kindly supplied by Prof Gilbert Greub, University of Lausanne).

2.3. Expression of cationic host defence peptides by AH-1 ovine trophoblast cells

To assess CHDP expression in response to infection, an *in vitro* ovine placental model was employed. AH-1 trophoblast cells were grown to 80% confluency overnight in a 24 well plate, and subsequently exposed to LPS or infected with live *W. chondrophila*. For LPS exposures, AH-1 cells were treated in duplicate with LPS (500 ng/mL, lipopolysaccharide from *E.coli* O111:B4, Sigma, L2630–10 mg) which was dissolved in ultrapure water and stored at −80 °C (n = 4). AH-1 cells were also infected with *W. chondrophila* at a multiplicity of infection (MOI) of 0.1, 1 and 10 (equivalent of 1 inclusion forming unit (IFU) per 10 cells, 1 IFU per cell and 10 IFU per cell, respectively) or exposed to UV irradiated organisms at MOI 10 (n = 3). Cells were maintained in a heated, humidified incubator at 37 °C with 5% CO_2 for 48 h during treatment. For Vitamin D_3 exposures, 1α,25-dihydroxyvitamin D_3 (calcitriol, Enzo Life Sciences, Exeter, United Kingdom) was dissolved in absolute alcohol at a stock concentration of 1 mM and stored at −80 °C. AH-1 cells were exposed to Vitamin D_3 in duplicate at concentrations of 1 nM, 10 nM and 100 nM for 24 h and in all treatments, a vehicle control was performed (1 μl/mL EtOH) (n = 3).

2.4. Expression of cationic host defence peptides in the ovine placenta

All animal studies were approved by the UK Home Office (conducted under approved Project Licence PL 60/3744) after review by the University of Edinburgh Animal Research Ethics Committee. Scottish Greyface ewes were fed to achieve comparable body condition prior to estrous cycle synchronization. Estrous cycles were synchronised *via* intravaginal Chronogest sponge pessaries (20 flugestone acetate, synthetic progesterone analogue) (Intervet UK Ltd.). Sponges were removed after 12 days, and 0.5 ml Prostaglandin estrumate injected intramuscularly to terminate luteal phase prior to mating with Texel rams 48 h later. On day 90 of gestation (term is 147 days), ewes were euthanized by barbiturate overdose and a placentome was collected from the ewe (as described in (Rae et al., 2013)). Lung samples were also obtained from the foetus as a positive tissue control. All tissues were snap frozen and stored at −80 °C for mRNA expression analysis.

2.5. RNA extraction, DNase treatment and cDNA synthesis

For all RNA extractions, RLT buffer from RNeasy® mini kits with 2-mercaptoenthanol, 1% v/v (Qiagen, Crawley, UK) were used for lysis of *in vitro* and *ex vivo* samples. For frozen tissues, 30 mg tissue was weighed and lysed in RLT buffer using magnetic beads and a Qiagen TissueLyser. *In vitro* samples were lysed *in situ* with RLT buffer and collected into sterile DNase/RNase free tubes and stored at −80 °C until extraction. Once samples were lysed, RNA was extracted from the lysates using the RNeasy® mini kit system following manufacturers protocols. All samples were analysed using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Loughborough, UK) to ascertain RNA quantity, and RNA quality was determined using an Agilent Bioanalyser (Agilent, UK), where RIN ≥ 8 was used as a quality filter for further downstream analyses. Genomic DNA (gDNA) was removed from extracted RNA using PrimerDesign Precision DNase Kits (PrimerDesign, Southampton, UK) as per manufacturer's protocol. Complimentary DNA (cDNA) was synthesised from the extracted RNA using Precision nanoScript reverse transcription kits as per manufacturers protocol (PrimerDesign) and stored at −20 °C until analysis. Negative control reactions containing no reverse transcriptase (no RT) in the reaction were performed in parallel.

2.6. Quantitative real time – polymerase chain reaction (q-PCR)

Quantitative real-time polymerase chain reaction (q-PCR) was performed using an Applied Biosystems StepOne Real-Time PCR machine with SYBR green detection. q-PCR primers for the ovine

genes; SMAP-29, sBD1, and sBD2 (Table 1), were designed and optimised by PrimerDesign Ltd. Melt curve analysis was carried out with every GOI (gene of interest) to determine specificity of the primers alongside a no-template (PCR H_2O) control and RT-ve controls. Housekeeper genes were identified using a GeNorm Kit to establish the most stable housekeeping genes (PrimerDesign GeNorm Ovine 12 reference gene kit). The geometric mean of a panel of stable housekeeper genes was used in calculations of relative gene expression (RPS2, RPL19 and YWHAZ in ovine tissue samples, β-Actin and GAPDH in AH-1 cells). All samples were analysed using $2^{-(\Delta\Delta Ct)}$ quantification method relative to a mixed (pooled cDNA from placentomes) reference sample. Each 10 µl reaction consisted of 1 µl (40 ng) cDNA template, 0.5 µl (300 nM) gene specific primers, 5 µl Precision 2X qMasterMix with SYBR Green, PrimerDesign and the reaction adjusted to 10 µl with DNase/RNase free H_2O. RT- H_2O controls were performed on each plate to act as negative controls.

2.7. Statistical analyses

Statistical analysis was performed using GraphPad Prism software. Expression of CHDP in ovine lungs and placenta, and expression of CHDP and CXCL8 in AH-1 cells was assessed by unpaired *t*-test. Expression of CHDP in response to infection with *W. chondrophila* and in response to 1α,25-dihydroxyvitamin D_3 exposure was assessed by one-way ANOVA with Tukey's *post-hoc* test.

3. Results

3.1. CHDP are expressed by the ovine trophoblast cell line AH-1, but are not altered by LPS stimulation despite an intact TLR-4 response

We performed RT-PCR on the ovine trophoblast cell line AH-1 to investigate if the trophoblast compartment of the sheep placentome specifically expressed genes for CHDP, Quantitative RNA analysis demonstrated that trophoblasts could indeed express the cathelicidin SMAP-29 (Fig. 1A), together with the ovine defensins sBD-1 (Fig. 1B) and sBD-2 (Fig. 1C). Interestingly, the expression of these CHDP were not altered following exposure of the AH-1 cell line to the TLR-4 agonist LPS. This was despite an intact and robust TLR-4 response in the cells as indicated by significantly increased expression ($P < 0.001$) of ovine CXCL8 (Fig. 1D).

3.2. Increased expression of CHDP by ovine trophoblast AH-1 cells in response to infection with Waddlia chondrophila

To establish that ovine trophoblast cells were capable of upregulating CHDP gene expression during the innate response to a live pathogen, we characterised the innate response of AH-1 cells to *W. chondrophila*, an invasive intracellular pathogen. In response to varying multiplicities of infection (MOI) of *W. chondrophila*, mRNA abundance for the cathelicidin SMAP-29 was not altered at any MOI tested (Fig. 2A). In contrast, mRNA for both sBD-1 (Fig. 2B) and sBD-2 (Fig. 2C) increased in abundance as the severity of the infection increased from an MOI of 0.1-10 ($P < 0.001$). Supporting our observations with AH-1 cells and LPS treatment, we found that UV-killed *W. chondrophila* did not elicit increases in mRNA abundance for either SMAP-29, sBD-1 or sBD-2. Fig. 2D shows a representative image of *W. chondrophila* infection of AH-1 cells at MOI 1.

3.3. Exposure of AH-1 ovine trophoblast cells to vitamin D_3 does not alter expression of the cathelicidin SMAP-29

To assess whether ovine trophoblast cells would upregulate SMAP-29 in response to Vitamin D_3 treatment, we exposed AH-1 cells to varying concentrations of 1α,25-dihydroxyvitamin D_3 for 24 h. Following quantitation of mRNA abundance, we determined that expression of the ovine cathelicidin, SMAP-29, was not altered by Vitamin D_3 treatment at any of the concentrations tested (Fig. 3).

3.4. Confirmation of CHDP mRNA expression in the placentome and trophoblast in primary ovine tissue

To validate expression of sheep antimicrobial peptide-29 (SMAP-29), sheep beta defensin-1 (sBD1) and sheep beta defensin-2 (sBD2) in ovine placentomes, qRT-PCR was performed (Fig. 4) on tissue from the placenta of animals involved in the study. Placental expression was compared to fetal lung expression of CHDP as a positive tissue control. Expression of the sheep cathelicidin, SMAP-29, was detected at comparable mRNA abundance levels in both lung and placentome tissues (Fig. 4A). sBD-1 and sBD-2 expression was also identified in the ovine placentomes (Fig. 4B and C), albeit with mRNA abundance that was approximately 50% of that observed in the lung tissue ($P < 0.001$).

4. Discussion

The human placenta is known to express a broad repertoire of CHDP (Klaffenbach et al., 2011; King et al., 2007b), and it is thought that this comprehensive innate barrier protects the developing fetus against the wide variety of pathogens that could result in adverse fetal development or maternal complications during pregnancy. Expression of the human cathelicidin LL-37 has been detected in several female reproductive tissues and fluids, including the cervix, vagina and cervicovaginal secretions (Frohm Nilsson et al., 1999; Levinson et al., 2012; Klaffenbach et al., 2011). In contrast to the well-characterised expression of Cationic Host Defence Peptides within the human placenta, expression of CHDP within the placental trophoblasts of sheep is poorly understood. Here, we demonstrate for the first time that the ovine cathelicidin SMAP-29 is expressed by trophoblast cells of the ovine placenta. While expression of the ovine cathelicidin SMAP-29 was originally identified in sheep leukocytes (Skerlavaj et al., 1999), until now it was not known if ovine trophoblasts shared the same expression pattern of their cathelicidin analogue with that of the human cathelicidin LL-37. Here we show that, in addition to the ovine defensins sBD-1 and sBD-2, the ovine cathelicidin SMAP-29 is indeed expressed in the placenta, and our *in vitro* findings could suggest that the trophoblast compartment, rather than resident immune

Table 1
Primer sequences of genes of interest.

	Forward Primer	Reverse Primer
SMAP-29	TAGACCCGCCTCCCAAGC	CCATTCTCCTTGAAGTCACACT
sBD1	GGCTGTCTAATGTTCATCTTACC	TCTCCGCATCCTCTCTCTC
sBD2	CTCCATCACCTGCTCCTC	GCTTAGACTATCTGTTACTCCAT
CXCL8	AAGCTGGCTGTTGCTCTC	GGCATCGAAGTTCTGTACTC

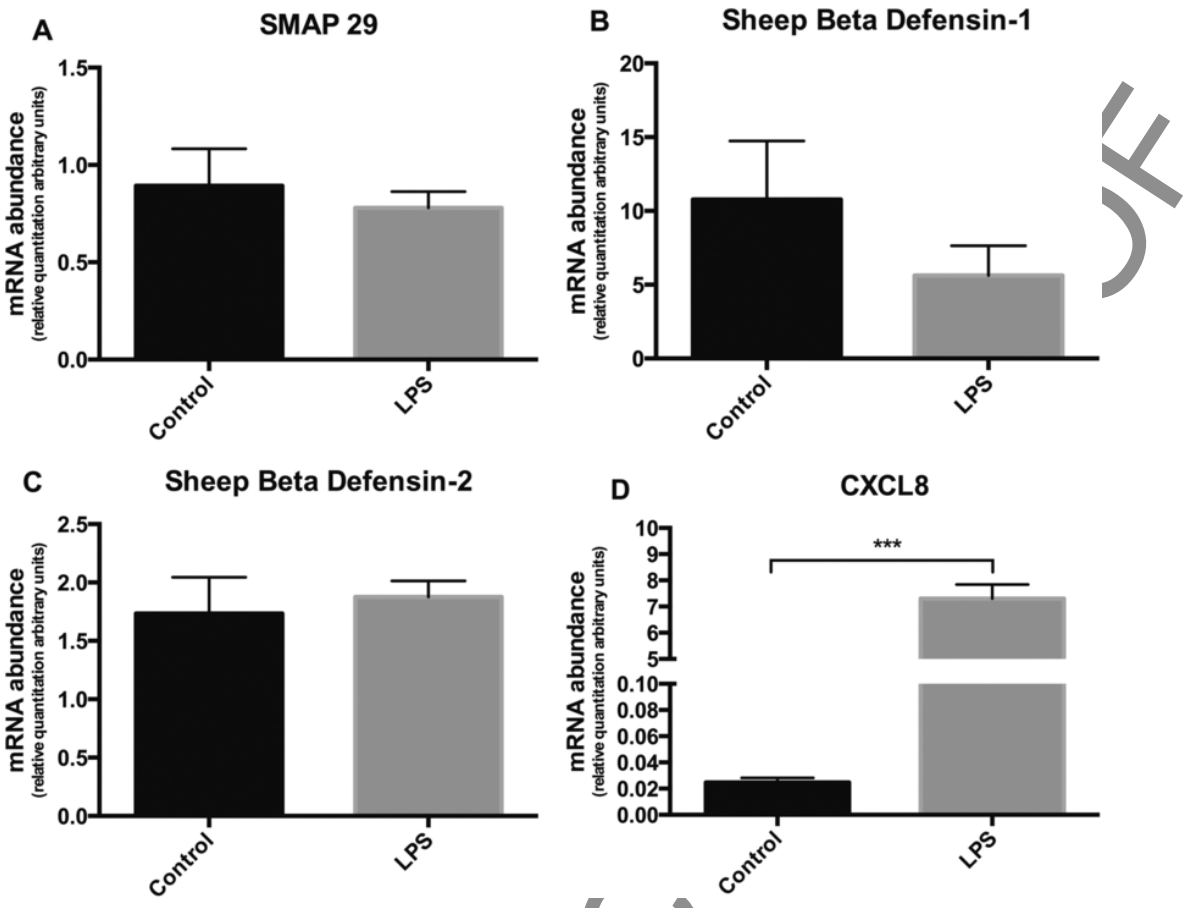

Fig. 1. Expression of cationic host defence peptides and Interleukin-8 by AH-1 ovine trophoblast cells in response to LPS exposure. Expression of mRNA for cathelicidin (SMAP-29) and defensin (sBD-1, sBD-2) peptides and CXCL8 by AH-1 trophoblast cells in the presence and absence of LPS (*E. coli*) was analysed by qRT-PCR after 24 h of treatment. Data represents mRNA abundance for SMAP-29 (1A), sBD-1 (1B), sBD-2 (1C) or CXCL8 (1D) in relatively quantified arbitrary units relative to geometric mean of two housekeeper genes identified by GeNorm analysis (β-actin and GAPDH) ± SEM (n = 3). Statistical significance was assessed by unpaired *t*-test comparing LPS treated samples to control, and is denoted by *** $P < 0.001$.

cells, is responsible for production. We also conducted comparative analysis in lung tissue as a positive control showing that SMAP-29, sBD-1 and sBD-2 was constitutively expressed in the fetal sheep lung, as SMAP-29 has previously been described to have potent antimicrobial activity in an *in vivo* model of respiratory tract infection (Brogden et al., 2001).

We used lipopolysaccharide (LPS) exposure in AH-1 cells, as a positive ligand for Toll-like receptor 4 (TLR4) mediated stimulation of CHDP. CXCL8 mRNA message was measured as an indirect indicator of intact TLR-4 signalling. Our data indicate that LPS treatment does not induce expression of either SMAP-29, sBD-1 or sBD-2, despite inducing a robust increase in CXCL8 expression (Fig. 1A–D). This is consistent with previous findings, which demonstrated that in human trophoblasts, LPS treatment did not induce expression of CHDP and related molecules (Klaffenbach et al., 2011). While this has been shown in previous studies(Abrahams et al., 2005; Klaffenbach et al., 2011), latterly being attributed to contaminating leukocytes within an isolated trophoblast preparation, this is not the case in the AH-1 trophoblast cell line. In addition, the AH-1 cell line cytokine response has already been well characterised during infection, which showed that the TLR-4 signalling pathway was indeed intact *via* LPS-mediated CXCL8 mRNA expression (Wheelhouse et al., 2009). Therefore, the mechanism responsible here could be a modulated innate immune response within the trophoblast, where recruitment of specialised immune cells (uterine NK cells for example) is preferred over a classical inflammatory response.

Enzootic abortion resulting from infection with the intracellular pathogen *Chlamydia abortus* is common amongst livestock species. An emerging *Chlamydia*-related abortifacient pathogen *W. chondrophila* has been characterised to have similar *in vitro* pathogenesis to *C.abortus*, infecting and destroying the trophoblast compartment of the placenta, eventually translating to severe placental necrosis and premature birth of the offspring, which is often fatal (Blumer et al., 2011; Koschwanez et al., 2012). We have recently demonstrated that *W. chondrophila* can infect and stimulate an inflammatory response in ruminant trophoblasts, and also in HEp2 cells, *in vitro* (Wheelhouse et al., 2014; Storrie et al., 2016), thus providing a trophoblast infection model of high clinical relevance. Here we demonstrate that, in response to *W. chondrophila* infection of AH-1 trophoblasts, both defensins are upregulated in response to this infection, but that SMAP-29 expression remains unaffected. This indicates that, in the case of an infection with live pathogen that infects trophoblastic cell types, these CHDP are differentially stimulated and are not arbitrarily increased upon innate immune activation. The selectivity towards defensin expression by trophoblasts could be an advantageous adaptation that the infectious organism is insensitive to SMAP-29 as *Waddlia* species do not appear to be sensitive to the an-

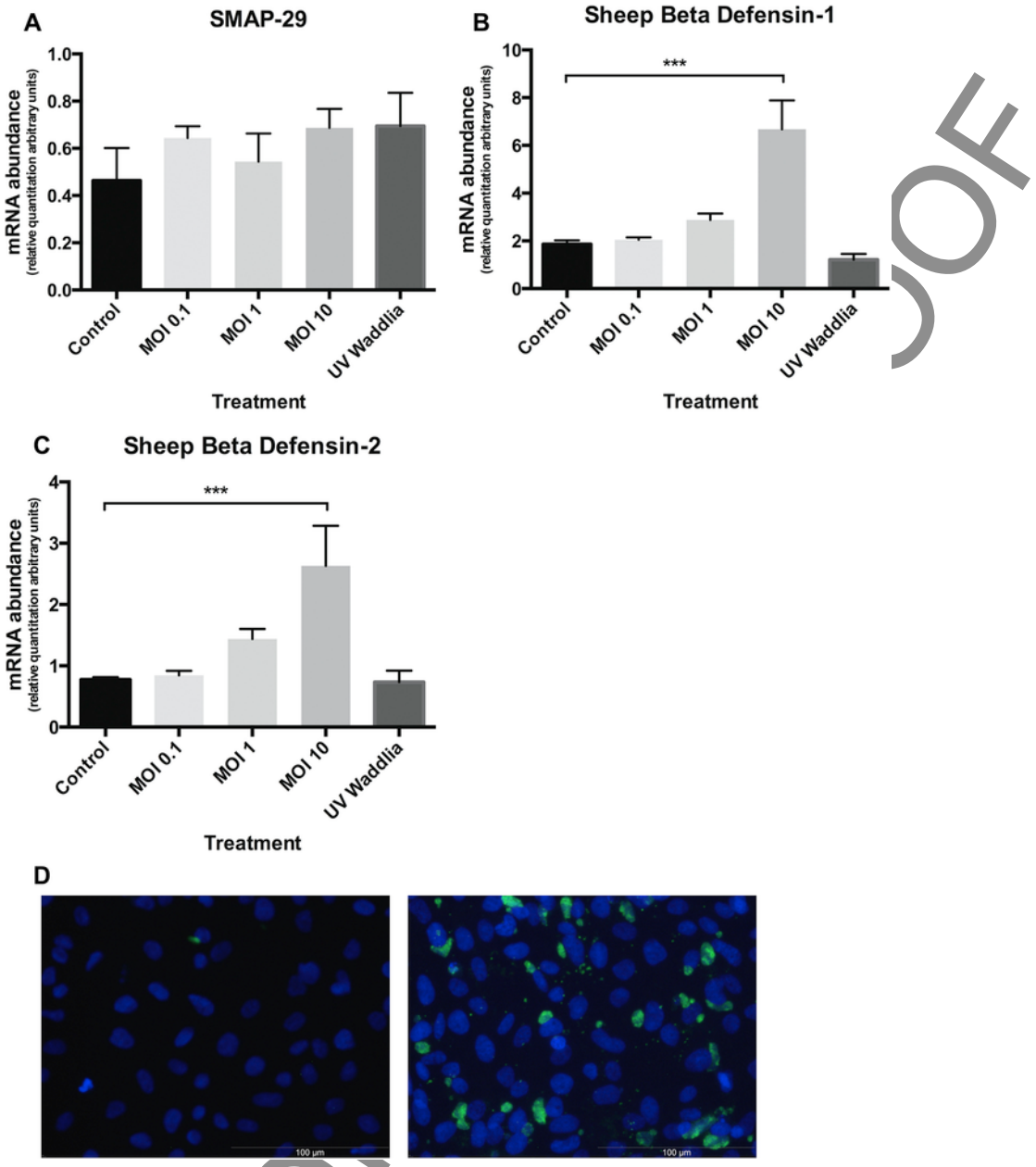

Fig. 2. Expression of cationic host defence peptides by AH-1 ovine trophoblast cells in response to infection with *Waddlia chondrophila.* Expression of mRNA for cathelicidin (SMAP-29) (2A) and defensin (sBD-1, sBD-2) (2B, 2C) peptides by AH-1 trophoblast cells in the presence and absence of either live *W. chondrophila* (MOI 0.1-10) or UV-killed *W. chondrophila* (MOI 10) was analysed by qRT-PCR after 48 h. Data represents mRNA abundance for SMAP-29 (2A), sBD-1 (2B) or sBD-2 (2C) in relatively quantified arbitrary units relative to geometric mean of two housekeeper genes identified by GeNorm analysis (β-actin and GAPDH) ± SEM (n = 4). Statistical significance was assessed by one-way ANOVA with Tukey's *post-hoc* test comparing *W. chondrophila* treated samples to control and is denoted by *** $P < 0.001$. AH-1 cells are shown as uninfected (left panel) and 24 h after infection (right panel) with *W. chondrophila* (MOI 1) (2D).

timicrobial action of SMAP-29 (P.B. C.C. M.R. J.P, and N.W unpublished data).

It has been shown that expression of the human cathelicidin, LL-37, can be induced by vitamin D_3 (1α,25-dihydroxyvitamin D3, calcitriol) *via* vitamin D receptor signalling (VDR). In humans and higher primates, a short interspersed nuclear element (SINE) carrying a vitamin D receptor response element (VDRE) has been transposed upstream of the human cathelicidin gene (*CAMP*) rendering it vitamin-D sensitive (Gombart et al., 2005). This signalling mechanism was discovered in a range of cell lines, and has since been shown in neutrophils, monocytes, and the trophoblast of the placenta (Wang et al., 2004). Very recently it was shown that vitamin D_3 can stimulate matrix metalloproteinase (MMP-2 and -9) expression in the invading extravillous trophoblast *in vitro*, implicating vitamin D in placental implantation (Chan et al., 2015).

Exploitation of vitamin D_3-inducible expression of LL-37 as a therapeutic target has recently been an area of intense investigation. Several studies have shown that vitamin D attributed antimicrobial activity is solely mediated by the expression of cathelicidin (Liu et al., 2007). Recent work has shown that obligate intracellular pathogens such as *Mycobacterium tuberculosis* and *Mycobacterium marinarum* can be treated with vitamin D_3 supplementation (Sato et

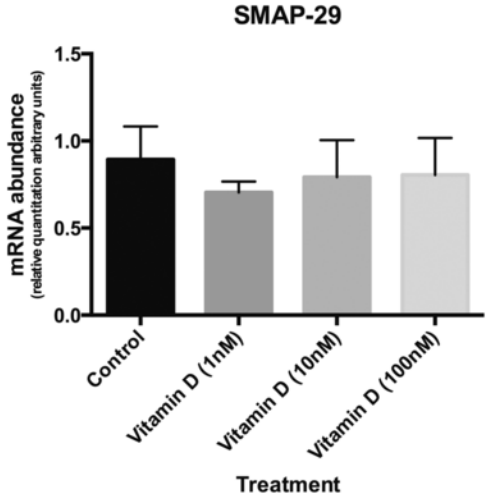

Fig. 3. Expression of cationic host defence peptides by AH-1 ovine trophoblast cells in response to treatment with 1α,25-dihydroxyvitamin D_3 (calcitriol). The expression of mRNA for cathelicidin (SMAP-29) peptide by AH-1 trophoblast cells in the presence and absence of 1α,25-dihydroxyvitamin D_3 (calcitriol; 1nM–100 nM) was analysed by qRT-PCR after 24 h treatment. Data represents mRNA abundance for SMAP-29 in relatively quantified arbitrary units relative to geometric mean of two housekeeper genes identified by GeNorm analysis (β-actin and GAPDH) ± SEM (n = 3). Statistical significance was assessed by one-way ANOVA with Tukey's *post-hoc* test comparing Vitamin D3-treated samples to control.

al., 2013). Notably, together with Vitamin D_3 stimulating expression of LL-37, it is known to induce autophagy synergistically with LL-37 once an infection has been resolved to rapidly reduce inflammation (Sato et al., 2013; Yuk et al., 2009). Importantly, it has been demonstrated that 1α,25-dihydroxyvitaminD_3 can induce cathelicidin expression in human trophoblast cells, enhancing antibacterial responses to pathogens such as *Escherichia coli* (Liu et al., 2009). However, here we demonstrate that ovine species do not increase expression of cathelicidin SMAP-29 in response to the 1α,25-dihydroxyvitamin D3 form of vitamin D_3 (Fig. 3). Vitamin D supplementation is of significant interest as an inducer in cathelicidin-mediated therapeutic strategies in humans, and these results indicate that, in the context of trophoblast mediated CHDP expression, it could not be used as an inducing agent of cathelicidins in sheep.

In summary, we demonstrate that the ovine trophoblast can express the cathelicidin SMAP-29 and that, in response to LPS stimulation, these cells do not upregulate expression of SMAP-29, sBD-1 or sBD-2, but do respond with robust CXCL8 expression, a response that has previously been associated with contaminating leukocytes in primary placental isolates. Furthermore, we show that in *in vitro* infection models with the emerging abortifacient organism *W. chondrophila*, trophoblasts are capable of differentially regulating their innate immune response *via* specific expression of defensins, rather than inducing a cathelicidin-mediated response. Finally, we demonstrate that ovine trophoblasts lack the vitamin D response required to upregulate cathelicidin expression in these cells, indicating that increased cathelicidin expression is mediated through another, as yet undetermined pathway.

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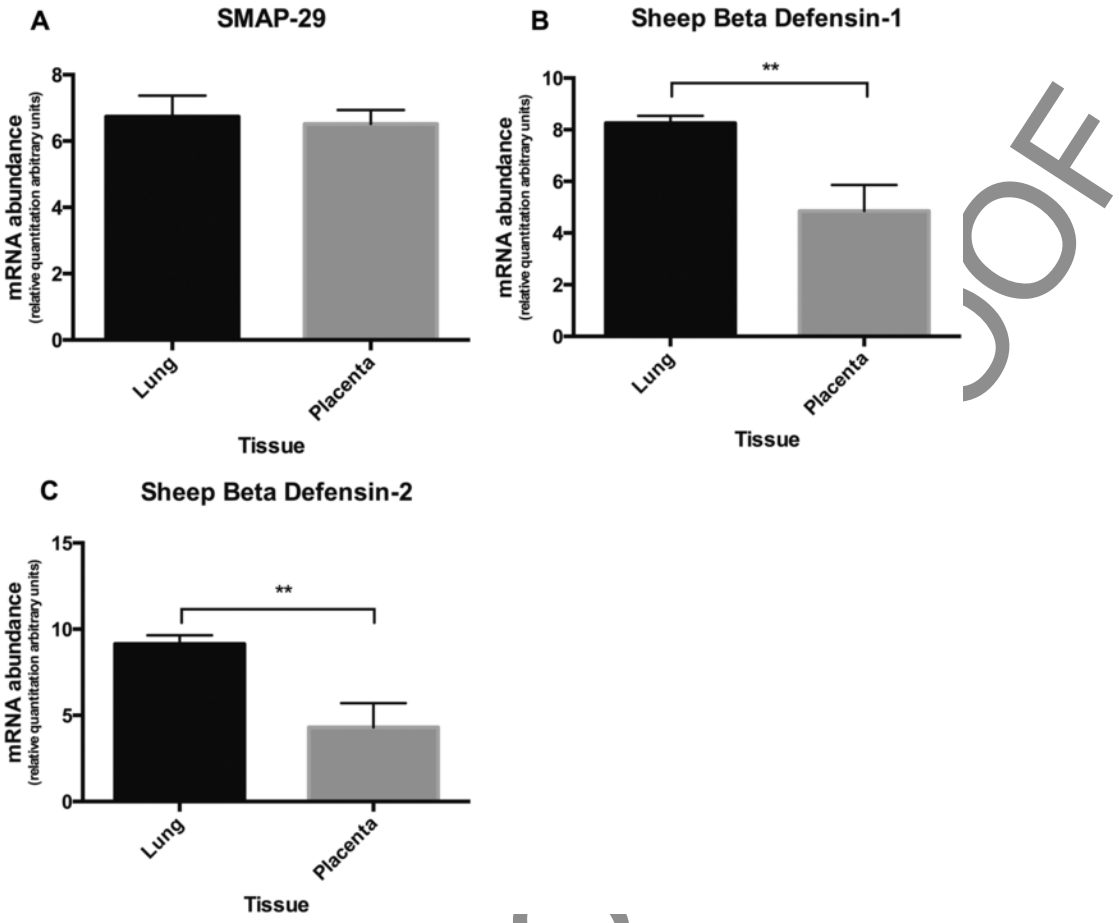

Fig. 4. Expression of cationic host defence peptides in the ovine placenta and lung. Expression of mRNA cathelicidin (SMAP-29) and defensin (sBD-1, sBD-2) peptides was analysed by qRT-PCR in primary ovine placental tissue. Expression of mRNA in fetal lung tissue was determined as a tissue positive control. Data represents mRNA abundance for SMAP-29 (4A), sBD-1 (4B) or sBD-2 (4C) in relatively quantified arbitrary units relative to geometric mean of two housekeeper genes identified by GeNorm analysis (β-actin and GAPDH) ± SEM (n = 6). Statistical significance was assessed by unpaired *t*-test and is denoted by ** $P < 0.01$.

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