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Selective transport of IgE into ovine mammary secretions

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ABSTRACT

Immunoglobulin (Ig) E is actively transported into ovine colostrum. Here we examine the degree of selectivity and the mechanism by which this transfer occurs in sheep. Results indicate that during colostrogenesis in sheep, transfer of immunoglobulins was most selective for IgG1 and IgA followed by IgE, IgM and IgG2. In milk, selectivity was greatest for IgA, followed by IgM, IgE, IgG1 and IgG2. The neonatal Fc receptor (FcRn) and poly immunoglobulin receptor (pIgR) mediate the transport of IgG1 and IgA across the ovine mammary epithelium respectively. In primates and rodents, the low-affinity IgE receptor, FcεRII, functions to transport IgE across the intestinal epithelium. We therefore investigated the expression of the low-affinity IgE receptor (CD23), pIgR and FcRn transcripts in the ovine mammary gland. The expression profiles of FcRn, pIgR and CD23 mRNA reflected concentrations of their Ig ligands in mammary secretions. These findings suggest a role for CD23 in transport of IgE across the mammary epithelium of sheep.

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1. Introduction

Ruminants are born markedly hypogammaglobulinaemic (Husband et al., 1972). As such their adaptive immune defences in early life are reliant on passive transfer of maternal antibodies via ingestion of immunoglobulin-rich colostrum. Transfer of immunoglobulin (Ig) into colostrum is an active process, highly selective for IgG1, the predominant immunoglobulin found in ruminant colostrum (Table 1). IgG1 in ruminant colostrum is almost entirely derived from maternal plasma (Pierce and Feinstein, 1965; Brandon et al., 1971). The approach of parturition induces mammary epithelial cells to preferentially (Kemler et al., 1975; Sasaki et al., 1977) transport IgG1 from blood into colostrum, a process mediated by the neonatal Fc receptor (FcRn) (Mayer et al., 2002b; Mayer et al., 2005).

It is well documented that ruminant colostrum is also enriched in IgA and IgM when compared to plasma (Gapper et al., 2007) (Table 1). Analogous to the function of the FcRn in transporting IgG1, the polymeric immunoglobulin receptor (pIgR) mediates transport of IgA across the mammary epithelium into ruminant colostrum (Kacskovics, 2004). Transport of IgA is initiated by the binding of polymeric IgA to the pIgR, expressed on the basolateral surface of mammary epithelial cells. The complex is then endocytosed and actively transported across the cell. Upon reaching the apical

membrane, the complex dissociates resulting in the proteolytic cleavage of the external domains of the pIgR, referred to as secretory component, which remain covalently bound to IgA as it is secreted into the lumen (Kacskovics, 2004). Although the pIgR functions primarily to transport IgA, this receptor can also transport IgM (Mostov, 1994; Kacskovics, 2004). There are two forms of bovine pIgR (Kulseth et al., 1995). The cDNA of the longer form is highly similar to the pIgR of other species, while the shorter form lacks the second and third extra-cellular domains. Expression of the shorter form is extremely low relative to the long form in the ovine and bovine mammary gland (Kulseth et al., 1995; Verbeet et al., 1995; Rincheval-Arnold et al., 2002).

Ruminant colostrum contains elevated concentrations of IgE relative to blood (Table 1). Transfer of maternal IgE from mother to young via colostrum was first described in cattle by Hammer et al. (1971) who detected homocytotropic antibodies in bovine colostrum and their passive transfer to the neonate. Pfeffer et al. (2005) reported that the mean concentration of IgE in colostrum was approximately three times that in plasma of parturient ewes and that within 48 h of birth, the concentration of IgE in lamb plasma, which was undetectable prior to suckling, was similar to that of adults confirming transfer of IgE to the neonate via ingested colostrum. The selectivity of this process, relative to other Ig isotypes, and the mechanism by which IgE is transported across the mammary epithelium during colostrogenesis remain unknown. Pfeffer et al. (2005) hypothesised, based on observations in other species, that IgE binds to the low-affinity IgE receptor FcεRII (CD23), on mammary epithelial cells and is transported into ruminant colostrum. An alternative hypothesis is that IgE is carried into

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Table 1
Immunoglobulin concentration in ruminant serum and mammary secretion.

Compartment	Stage of lactation	Immunoglobulin concentration (mg/ml)				
		IgG1	IgG2	IgA	IgM	IgE
		Cattle				Sheep ^a
Blood serum ^{A,F}	Dry	14.0	13.0	0.4	3.8	0.03 ^b
Colostrual whey ^B	Pre-parturition (–2 weeks) ^c	100.0	3.5	3.6	13.7	NA
Colostrual whey ^{C,F}	Parturition	48.0	4.0	4.7	7.1	0.1
Milk whey ^D	Early lactation (+3 weeks) ^c	0.4	<0.1	<0.1	<0.1	<0.001
Milk whey ^D	Lactation (+21 weeks) ^c	0.3	<0.1	<0.1	<0.1	NA
Milk whey ^E	Involution (+7 days) ^d	10.3	2.0	3.1	4.1	NA

^a Immunoglobulin E concentrations were measured in sheep.

^b Blood serum value measured 30 days post-partum.

^c Number in brackets indicates weeks from parturition.

^d Number in brackets indicates days from drying off.

^A Lascelles (1979).

^B Brandon et al. (1971).

^C Husband et al. (1972).

^D Guidry and Miller (1986).

^E Watson et al. (1972).

^F Pfeffer et al. (2005).

mammary secretion bound to CD23 receptors on the surface of somatic cells such as leukocytes and is subsequently released within the lumen of the mammary gland.

Two structurally unrelated IgE Fc receptors exist: the high affinity Fcε receptor (FcεRI), a member of the Ig superfamily; and CD23, a C-type lectin. The FcεRI receptor is expressed on mast cells, basophils and activated eosinophils, where upon being cross-linked by a monomeric IgE/antigen complex, it induces release of allergic and inflammatory mediators (Kacskovics, 2004; Tu et al., 2005). In contrast, CD23 is expressed on many cell types including B cells, activated T cells, monocytes, eosinophils, platelets, follicular dendritic cells and some epithelial cells (Kacskovics, 2004) where it functions to enhance antibody responses by regulating IgE synthesis, presenting IgE/antigen complexes to immune cells and enhancing mediator release from activated basophils (Zhang et al., 2007). Although considered a low-affinity receptor, CD23 binds its ligand, monomeric IgE, with greater affinity than do other low-affinity Fc receptors (Dierks et al., 1993). A role for CD23 in the apical to basolateral transport of IgE/antigen complexes across the intestinal epithelium of rats has been described by Yang et al. (2000).

CD23 is selectively expressed on the apical membrane of the intestine in rodents but is found on both the apical and basolateral membranes in humans (Montagnac et al., 2005). Expression of CD23 on basolateral membranes appears to be species specific, suggesting it may play a role in the basolateral uptake and transport of free IgE across the epithelium in some species including humans (Montagnac et al., 2005). The possible involvement of CD23 in the transport of IgE across epithelial surfaces in ruminants has not been reported. Therefore our objective was to investigate the selectivity of IgE transport into ovine colostrum and milk and the possible lactation-stage dependent expression of CD23 in the ovine mammary gland.

2. Materials and methods

2.1. Animals and sampling

Blood, mammary secretion and mammary tissue samples were collected from adult Merino ewes aged 3–5 years. Ewes were run outdoors and grazed improved pasture *ad libitum* throughout the experiment. Blood and mammary secretion samples were collected for ELISA within twelve hours of parturition (representing colostrum, *n* = 24) and at 6–8 weeks post-partum (representing lactation, *n* = 24) for ELISA. Blood was collected via jugular

venipuncture into potassium-EDTA Vacutainers (Becton Dickinson & Company, USA). Mammary secretions were collected aseptically by hand milking. Briefly, teats were swabbed with 70% ethanol and colostrum (or milk) stripped using a gloved hand into a sterile collection tube. Plasma, skimmed colostrum and skimmed milk was prepared by centrifuging samples at 1750 g for 20 min at 4 °C. Samples were stored at –20 °C. Tissue samples were collected for quantitative real-time PCR (qRT-PCR) at approximately 14 days prepartum (representing pregnancy, *n* = 4), within 12 h of parturition (representing colostrogenesis, *n* = 8), approximately 14 days post-partum (representing early lactation, *n* = 4), 6–8 weeks post-partum (representing lactation, *n* = 4) and from dry ewes (*n* = 4) which were non-pregnant and non-lactating. Mammary tissue samples were collected from euthanased animals for biopsy at the junction of the teat canal and teat cistern, as 1–2 g (or around 1 cm³) of the glandular secretory tissue. Tissue samples were placed directly in RNAlater (Ambion, USA), kept at 4 °C overnight and then stored at –20 °C. All animals used in the study had sound udders and did not have clinical signs of mastitis. All animal experiments were approved by the F.D. McMaster Laboratory Animal Ethics Committee.

2.2. Elisa

The selectivity of IgG1, IgG2, IgM, IgA and IgE transfer into colostrum and milk was assessed by calculating the ratio of immunoglobulin, of each respective isotype, in mammary secretion relative to plasma at concurrent times. For each immunoglobulin isotype, a ratio termed the compartment ratio was calculated as: (immunoglobulin titre in mammary secretion)/(immunoglobulin titre in mammary secretion + immunoglobulin titre in plasma). IgG1, IgG2, IgM, IgA and IgE titres were measured in ovine plasma, skimmed colostrum and skimmed milk samples using a sandwich ELISA as modified from Shaw et al. (1997). IgE titres were determined as described with the exception that the sample diluent used was PBS + 0.05% Tween20 (PBST) containing 1% sodium casein. To determine IgE titres, plates were coated with mouse anti-ovine IgE (2.0 µg/ml) (clone YD3; Shaw et al., 1997) capture antibody and bound IgE was detected using biotin-conjugated mouse anti-ovine IgE (3.0 µg/ml) (clone XB6; Shaw et al., 1997).

To determine IgG1, IgG2, IgA and IgM titres the following additional modifications were made. Plates (Maxisorp, NUNC, USA) were coated with capture antibodies, mouse anti-ovine IgG1 (1.0 µg/ml) (clone MCM1; Beh, 1987), mouse anti-ovine IgG2

(1.0 µg/ml) (clone MCM4; Beh, 1987), mouse anti-bovine IgA (1.0 µg/ml) (clone BIG312D3, VMRD, Inc., USA) and mouse anti-porcine IgM (1.5 µg/ml) (clone PIG45A, VMRD, Inc.) respectively and incubated overnight at 4 °C. Cross-reactivity of mouse anti-bovine IgA and mouse anti-porcine IgM with ovine Igs was confirmed by the manufacturers. Following addition of test and control samples, plates were incubated for 1 h at RT. Detection antibodies used were rabbit anti-ovine IgG (H + L) (0.2 µg/ml) (5184–2104, AbD Serotec, UK) for IgG1 and IgG2 determinations, rabbit anti-ovine IgA (1.0 µg/ml) (AHP949, AbD Serotec) for IgA and rabbit anti-ovine IgM (AHP950, AbD Serotec) (1.0 µg/ml) for IgM. Following incubation with detection antibodies, mouse anti-rabbit IgG-biotin conjugate (clone RG-96, Sigma, USA) (100 µl/well diluted 12,500-fold in diluent) was added and plates were incubated for 1 h at RT. HRP-streptavidin conjugate (Zymed Laboratories, USA) was used at a concentration of 0.125 µg/ml.

Test and control samples were serially diluted across the plate 11 times starting at an appropriate starting dilution. Skimmed colostrum samples from individual animals were pooled to produce a positive control sample which was run on all plates. Mammary secretion and plasma samples collected from individual animals during colostrogenesis and lactation were assayed (for each respective isotype) on the same plate. Wells in the last column of each plate received only diluent and were considered as assay blanks. Optical density (OD) values of blank wells were subtracted from those of corresponding test and control sample wells. For IgG1 and IgG2 determinations, samples were assayed on duplicate plates, one coated with PBS only (PBS-control plate) and the other coated with the appropriate anti-IgG1 or IgG2 monoclonal (test plate). Individual well OD values obtained from PBS-control plates were subtracted from their corresponding test plate OD values prior to calculating immunoglobulin titre values. Titres were calculated by fitting a linear regression equation to the linear portion of the sample titration curve (using a minimum of four points). Titre values represent the inverse of the sample dilution factor as calculated from the regression equation using a fixed absorbance corresponding to the midpoint of the regression line. Titre values for all isotypes were normalized based on the titre of the positive control sample run on every plate. The same positive control sample was used to normalize both mammary secretion and plasma sample data.

2.3. Quantitative real-time PCR (qRT-PCR)

qRT-PCR was used to determine the relative expression of CD23, plgR and FcRn mRNA in ovine mammary tissue during the lactation cycle. Total RNA was extracted from mammary tissue samples using an RNeasy Midi Kit (Qiagen, Germany), as per the manufacturer's protocol. Following extraction, RNA was treated for genomic DNA contamination using the TURBO DNA-free kit (Ambion, USA). Yield and quality of extracted RNA was determined by measuring absorbance at 260 and 280 nm and RNA integrity assessed using agarose gel electrophoresis.

PCR reactions were performed on a Rotor-Gene 3000 (Corbett Research, Australia) using an one-step QuantiTect SYBR Green RT-PCR Kit (Qiagen). Total reaction volumes were 25.0 µl, consisting of 12.5 µl of 2× SYBR green master mix, 0.5 µl of 25 µM forward and reverse primers, 0.25 µl of reverse-transcriptase (RT) mix, 5.0 µl of RNA template and 6.25 µl of nuclease free water. The following primer pairs were used to amplify target ovine genes: CD23 (Fwd: 5'-tcttctggcactgggaaat-3'; Rev: 5'-tccatgtctgcatcatctg-3'), plgR (Fwd: 5'taccagcctgaggaagagg3'; Rev: 5'-gtaggggaagatacggta-3') and FcRn (Fwd: 5'-gcggtcaacaaggagaa-gac-3'; Rev: 5'-ctgcaggtgagcacagag-3'). Primer design was based on mRNA sequences with accession numbers XM_592551 (CD23), NM_174143 (plgR), NM_001123403 (FcRn) along with ovine

expressed sequence tag data (where available). Primers were designed to flank a minimum of one intron. Primers used to evaluate FcRn expression were designed to amplify a fragment of the FcRn α-chain spanning exon 4 and exon 5, the α2 and α3 extra-cellular domains respectively, and are expected to amplify both forms of FcRn (Kacskovics et al., 2000) should they exist in sheep as they do in the cow. Primer sequences for the housekeeping genes ribosomal subunit 18s (18s) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described previously (Menzies and Ingham, 2006; White et al., 2008). The suitability of 18s and GAPDH as housekeeping genes were assessed using BestKeeper software (Pfaffl et al., 2004). The standard deviation of 18s, GAPDH and a combined index of 18s and GAPDH expression values were 0.33, 0.89 and 0.44 respectively. Based on these results, gene expression ratios were normalized to 18s expression. PCR cycling conditions were 50 °C for 30 min, 95 °C for 15 min, followed by 45 cycles of 94 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s and 78 °C for 15 s. Upon completion of cycling, reactions were held at 72 °C for 10 min and exposed to melt-curve analysis. Data were acquired at 78 °C. Control samples containing no template or no RT were evaluated for each primer set. For individual animals, expression of target and housekeeping genes were determined (in duplicate) in a single PCR run. Specificity of all primer sets were assessed by sequencing, melt-curve analysis and by assessing the size of amplified products on agarose gels. Serial dilutions of a pooled RNA sample were used to determine the efficiency of target and housekeeper gene amplification reactions.

2.4. Statistical analysis

Gene expression ratios, normalized to 18s expression and corrected for the efficiencies of both the housekeeping and target gene amplification reactions, were determined using GenEx (Biorad). Data were then analysed using non-parametric permutation tests conducted on raw data scores using proc npar1way (SAS, Version 9.1). No adjustments were made for multiple comparisons. For descriptive purposes, data are presented as means ± SEM (of log (base 2) transformed values).

A logit transformation was applied to compartment ratio values to improve data normality prior to analysis. Data were then analysed using a general linear model with the random effect of animal using proc mixed (SAS). Tukey adjustments were made for multiple comparisons. To test the assumptions of the ANOVA procedure, comprehensive residual analyses were conducted to test for normality including the Anderson–Darling and Shapiro–Wilk tests and residuals were plotted against predicted and explanatory variable values. For descriptive purposes data are presented as means ± SEM (of raw values). A *P* value of less than 0.05 was considered significant.

3. Results

3.1. Selectivity of immunoglobulin transfer into ovine mammary secretion during colostrogenesis and lactation

Compartment ratios were calculated as immunoglobulin titre in mammary secretion/(immunoglobulin titre in mammary secretion + immunoglobulin titre in plasma) for each isotype during colostrogenesis (Fig. 1) and lactation (Fig. 2). Significant differences between ratios for isotypes suggest immunoglobulin transport from the circulation into mammary secretion is a selective process. On this basis, the transfer process in sheep during colostrogenesis was found to be most selective for IgG1 and IgA followed by IgE, IgM and IgG2 (Fig. 1). During colostrogenesis, compartment ratios for IgG1 and IgA were not significantly different (*P* = 0.999) but

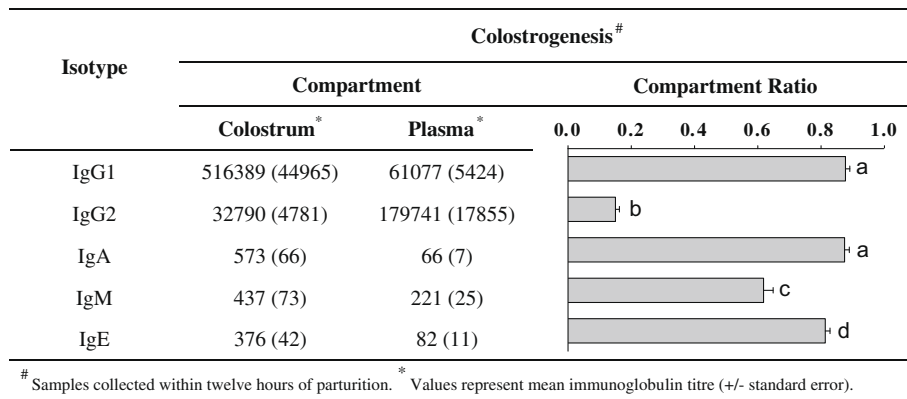


Fig. 1. Immunoglobulin isotype titres and compartment ratios in ovine plasma and mammary secretion during colostrogenesis. Compartment ratios were calculated for each animal as immunoglobulin titre in mammary secretion/(immunoglobulin titre in mammary secretion + immunoglobulin titre in plasma) for each isotype. Significant differences between ratios for isotypes suggest immunoglobulin transport from the blood into mammary secretion is a selective process. Means for each isotype that differ significantly are depicted using different superscripts ($P < 0.05$).

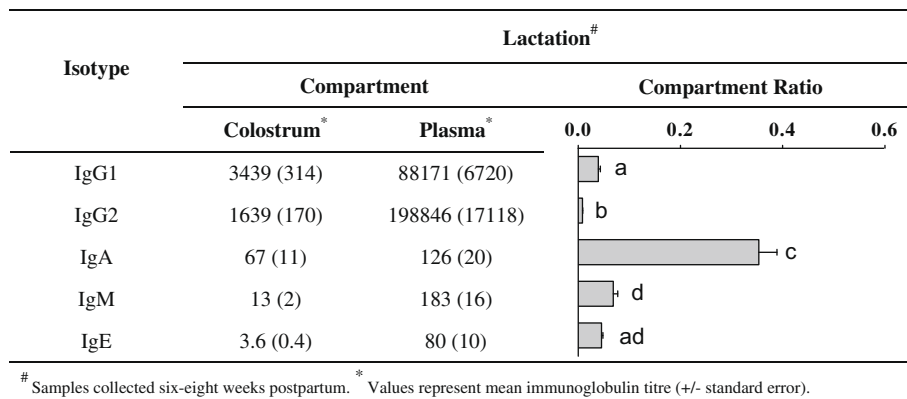


Fig. 2. Immunoglobulin isotype titres and compartment ratios in ovine plasma and mammary secretion during lactation. Compartment ratios were calculated for each animal as immunoglobulin titre in mammary secretion/(immunoglobulin titre in mammary secretion + immunoglobulin titre in plasma) for each isotype. Significant differences between ratios for isotypes suggest immunoglobulin transport from the blood into mammary secretion is a selective process. Means for each isotype that differ significantly are depicted using different superscripts ($P < 0.05$).

were significantly greater than were observed for IgE, IgM and IgG2 ($P < 0.0001$ for all pair-wise comparisons). The compartment ratio for IgE was significantly greater than was observed for IgM ($P < 0.0001$) which in-turn was significantly greater than was observed for IgG2 ($P < 0.0001$). Immunoglobulin titres were higher in colostrum than in plasma for all isotypes, except IgG2.

In milk, selectivity was greatest for IgA, followed by IgM, IgE, IgG1 and IgG2 (Fig. 2). During lactation, the compartment ratio for IgA was significantly greater than was observed for any other isotype ($P < 0.0001$ for all pair-wise comparisons). The compartment ratio for IgM was not significantly different to that observed for IgE ($P = 0.078$) but was significantly greater than that observed for IgG1 and IgG2 ($P = 0.001$ and $P < 0.0001$ respectively). During both colostrogenesis and lactation, the compartment ratio for IgG2 was significantly lower than that observed for any other isotype ($P < 0.0001$).

3.2. Fc receptor mRNA expression in the ovine mammary gland during the various stages of the lactation cycle

Expression of CD23, FcRn and pIgR mRNA were investigated in the ovine mammary gland during the lactation cycle. FcRn expression was highest in the dry and pregnancy periods, declined significantly during colostrogenesis and early lactation and was lowest during the lactation period. FcRn expression did not differ significantly

during the dry and pregnancy periods ($P = 0.229$). During colostrogenesis, expression was significantly lower than was observed during the dry ($P = 0.006$) and pregnancy periods ($P = 0.033$) and significantly higher than was observed during early lactation ($P = 0.006$) and lactation ($P = 0.003$). Expression during early lactation was significantly higher than was observed during the lactation period ($P = 0.029$).

A somewhat similar pattern was observed for CD23 expression with the exception that expression was higher during colostrogenesis than earlier during pregnancy. CD23 expression was significantly higher during the dry period as compared to all other stages of lactation ($P \leq 0.029$ for all pair-wise comparisons). Although expression was higher during colostrogenesis than was observed during pregnancy, this difference was not significant ($P = 0.075$). Expression during colostrogenesis was significantly higher than was observed during early lactation ($P = 0.034$) and lactation ($P = 0.032$).

In contrast to FcRn and CD23 expression, pIgR expression was lowest in the dry mammary gland, increased significantly during pregnancy and remained constant through colostrogenesis, early lactation and into mid lactation (Fig. 3). pIgR expression was significantly lower during the dry period as compared to all other stages of lactation ($P \leq 0.032$ for all pair-wise comparisons). No significant differences in expression were observed between other stages of the lactation cycle ($P \geq 0.135$ for all pair-wise comparisons).

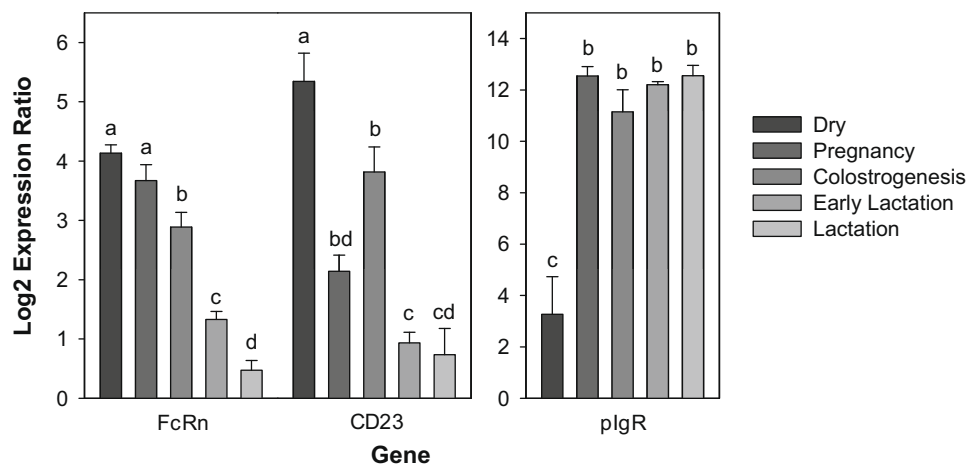


Fig. 3. Immunoglobulin Fc receptor gene expression (relative to 18s) in ovine mammary tissue at various stages of the lactation cycle. For each gene, group means that differ significantly are depicted using different superscripts ($P < 0.05$).

4. Discussion and conclusions

The compartment ratio of IgE in ovine colostrum and milk was significantly higher than IgG2 suggesting that in the sheep, low level selective transport of plasma-derived IgE into mammary secretion occurs. The expression profile of CD23 mRNA in the ovine mammary gland supports the hypothesis that CD23 plays a role in the transport of IgE across the mammary epithelium of sheep. However, functional assays will be required to confirm such a role.

Pfeffer et al. (2005) reported that the concentration of total IgE in ovine colostrum was around three times that observed in plasma during colostrogenesis, while only trace amounts of IgE were detectable in milk collected 30 days post-partum. In the current study, IgE titres in colostrum were approximately four fold higher than in plasma, whereas, IgE titres in milk were only 5% of those observed in plasma (Fig. 2). Selective transport of IgA, IgG1 and IgM relative to IgG2 into ruminant colostrum has been well documented (Watson, 1980). However, the selectivity of IgE transport, relative to other immunoglobulin isotypes, has not been previously reported.

It is generally accepted that the immunoglobulins found in ruminant colostrum are almost entirely derived from blood, rather than produced locally (Lascelles et al., 1981), whereas, a significant proportion of IgA and IgM found in ruminant milk during late lactation is thought to be synthesised locally by plasma cells resident in the mammary gland (Newby and Bourne, 1977; Sheldrake et al., 1984). In contrast to transport of Igs across the mammary epithelium, the transudation of Igs from the intravascular space into interstitial fluid that bathes the mammary epithelium is not thought to be facilitated by a receptor-mediated transport mechanism. Differences between isotypes in the compartment ratio could therefore arise due to (1) differential filtration through microvascular endothelium due for instance to differences between isotypes in molecular sizes, (2) differences in the extent of local production within the mammary gland, (3) differences in activity of the receptors mediating transport for each isotype across mammary epithelium, or in the case of IgA and IgM which are both transported by pIgR, (4) differences in affinity for the receptor. A final reason (5) for differences in the compartment ratio could arise if transport is not mediated by receptors on mammary epithelium but results from transport of Igs bound to leukocytes migrating into mammary secretions. It is noteworthy that the compartment ratio of IgA was higher than IgM both in colostrum and in milk (Figs. 1 and 2). From the current study we cannot conclude whether this was due to mechanism 1, 2, 4 or 5 above. Importantly, the compartment ratio

of IgE in colostrum and milk was significantly higher than IgG2, but lower than IgA, suggesting low level selective transport of plasma-derived IgE into mammary secretion which may be augmented by low level local production of IgE in the gland.

A molecular basis for active transport of IgE was therefore investigated. RNA transcripts encoding CD23 were detected in ovine mammary tissue during all stages of the lactation cycle examined. Primers used to evaluate CD23 mRNA expression were expected to amplify both the CD23a and b isoforms (but not the bDelta6 splice form) and to further identify bDelta5 splice forms (based on reduced amplicon size) should they exist in the sheep. Similar to FcRn expression, CD23 expression in the ovine mammary gland was found to be highly dependent on stage of lactation (Fig. 3). CD23 expression was significantly up-regulated during late pregnancy (relative to lactation), peaked during colostrogenesis and was then significantly down-regulated during lactation, consistent with the pattern of IgE detected in mammary secretion. CD23 expression was at its highest levels during the dry period. Amplicons, generated from the putative bDelta5 splice form of CD23 could not be detected in any test samples using melt-curve analysis.

The expression profile of CD23 in the ovine mammary gland appears to correlate with the concentration of IgE detected in colostrum and milk, supporting a role for this receptor in the transfer of IgE across the mammary epithelium of sheep. However, in contrast to the expression of pIgR and FcRn, which, in the mammary gland has been shown to occur exclusively in epithelial cells (Rincheval-Arnold et al., 2002; Mayer et al., 2002b), CD23 is expected to be expressed on a variety of cell types in the mammary gland including B cells, activated T cells, monocytes, eosinophils and platelets (Kacskovics, 2004). Cell population differences in the mammary gland during the various stages of lactation are therefore expected to influence the patterns of CD23 expression in the mammary gland; however, the extent of such an influence has yet to be determined. The high expression of CD23 observed in the dry mammary gland may be a consequence of CD23 expression by lymphoid cells which are present at high concentrations in the mammary gland of dry as compared to pregnant or lactating ewes (Lee and Lascelles, 1969; Lee et al., 1989).

FcRn expression in both the ovine and bovine mammary gland has been reported previously (Adamski et al., 2000; Mayer et al., 2002a; Mayer et al., 2005). In the current study, FcRn expression in mammary tissue was up-regulated significantly in dry ewes and ewes in late pregnancy, down-regulated significantly during colostrogenesis and then further down-regulated through the

lactation period (Fig. 3). FcRn expression has been reported in the non-lactating ruminant mammary gland previously (Mayer et al., 2005); however, the biological significance of up-regulated FcRn expression in the dry gland remains unknown.

It is well documented that transfer of maternal IgG1 into mammary secretion peaks just prior to parturition and rapidly declines at the onset of lactation (Watson, 1980). FcRn expression and the accumulation of IgG1 in mammary secretion observed in the current study, supports the hypothesis that the FcRn plays a role in the transport of IgG1 into ruminant colostrum. Differential expression of FcRn during the lactation period has been investigated previously (Adamski et al., 2000; Mayer et al., 2002a,b). These reports suggest that the expression of the FcRn protein is substantially down-regulated by day 14 post-partum; however, the expression of transcripts encoding the FcRn α -chain remains relatively constant throughout lactation. Based on these findings, Adamski et al. (2000) proposed that the stable expression of FcRn requires the co-expression of both α -chain and β_2 -microglobulin mRNA and furthermore that the expression of functional FcRn in the ruminant mammary gland (during periods of peak IgG1 transfer) is largely controlled by expression of β_2 -microglobulin mRNA rather than α -chain mRNA. In contrast, results from the current study demonstrate that FcRn α -chain expression is significantly down-regulated in ovine mammary tissue as lactation progresses. While acknowledging that gene expression does not equate to expression of functional protein, results from the current study suggest that FcRn expression is dependent on stage of lactation. It is therefore hypothesised that the combined expression of both FcRn and β_2 -microglobulin transcripts control expression of functional FcRn in the ruminant mammary gland.

The expression of pIgR in the ovine mammary gland has also been reported previously (Rincheval-Arnold et al., 2002). In the current study, pIgR gene expression was significantly up-regulated in the mammary gland during late pregnancy, colostragenesis, early lactation and lactation relative to the dry period (Fig. 3). No significant changes in expression were observed from late pregnancy through to lactation. Rincheval-Arnold et al. (2002) reported up-regulation of pIgR transcript expression in the mammary gland during pregnancy and into lactation to peak by mid lactation. This pattern of expression was confirmed at the protein level where expression was exclusively detected on mammary epithelial cells. This observation is inconsistent with the enhanced pIgR expression in the mammary gland during late pregnancy observed in the current study. This may be a consequence of sampling time, as the Rincheval-Arnold et al. samples were collected up to day 112 of gestation whereas in the current study, samples representing the pregnancy period were collected at around day 135 of gestation in sheep.

In summary, the results support the conclusion that IgE is selectively transported into colostrum and milk. Further studies will be required to confirm the role of CD23 in mediating this transport and to gain an understanding of the factors which regulate expression of CD23 in the mammary gland at both transcriptional and translational levels.

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