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Developmental expression of pIgR gene in sheep mammary gland and hormonal regulation

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Summary. Secretory IgA found in external secretions are constituted by polymeric IgA (pIgA) bound to the extra-cellular part of the polymeric immunoglobulin receptor (pIgR). The receptor mediates transcytosis of pIgA across epithelial cells. The aim of the present study was to analyse the evolution of pIgR expression in the sheep mammary gland during the development of the mammary gland and to analyse its hormonal regulation. Gene expression of the pIgR was analysed in sheep mammary gland during pregnancy and lactation. By Northern Blot analysis, we observed that low levels of pIgR mRNA are expressed until day 70 of pregnancy. Accumulation of pIgR mRNA started during the third part of pregnancy and intensified 3 d after parturition to reach highest levels during established lactation (day 70). *In situ* hybridization analysis was used to confirm the increase in pIgR gene expression per mammary epithelial cell. In order to examine the hormonal regulation of the pIgR expression, virgin ewes were hormonally treated. Treatment with oestradiol and progesterone increased pIgR mRNA levels slightly. Subsequent addition of glucocorticoids induced a significant accumulation of pIgR mRNA in the mammary gland of the treated animals. Immunohistochemical analysis was performed to verify that the increase of pIgR mRNA level was associated with enhancement of the pIgR protein in mammary cells. No increase of pIgR mRNA levels were observed if PRL secretion was blocked by bromocryptine injections throughout the hormonal procedure. In conclusion, the present experiments suggest that the enhancement of pIgR levels during lactation result from combined effects of both prolactin and glucocorticoids.

Keywords: Sheep, mammary gland, polymeric immunoglobulin receptor, hormones

Transmission from the mother of specific defence factors, particularly IgG and IgA, is essential for the protection of the newborn. For ungulates (pig, cow, sheep and horse), this passive immunization is only transferred postpartum via the absorption of colostrum and milk, containing essentially IgG. Depending on the animal species, isotype content (IgG and IgA) and the concentration of immunoglobulins present in the colostrum and milk are different. Concentrations of IgA are high in primates and lagomorphs but lower in ruminants.

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Polymeric IgA (pIgA), found in mammary secretions, are produced locally by plasma cells which originate from MALT (mucosa-associated-lymphoid tissue) and differentiate in the *lamina propria* of the mammary gland (Kraehenbuhl & Neutra, 1992). However, in some species like ovine, pIgA may also originate from the general circulation particularly at the time of early lactation. The pIgA binds specifically to the polymeric immunoglobulin receptor (pIgR) located at the basolateral surface of the mammary epithelial cells (reviewed in Mostov, 1994; Hunziker & Kraehenbuhl, 1998). This active transport allows transfer of the pIgA from the basolateral surface to the apical surface of epithelial cells. The secretory IgA (sIgA) released into colostrum and milk is produced after the proteolytic cleavage of the extracellular part (secretory component: SC) of the pIgR associated with pIgA. This association makes sIgA very effective in the local protection against environmental pathogens which invade the mucosal and glandular surfaces (Wold *et al.* 1990; Kaetzel *et al.* 1991; Mazanec *et al.* 1992; Bomsel *et al.* 1998). Models of mice carrying backpack tumours secreting specific-sIgA have demonstrated the importance of sIgA in the protection against lethal dose of pathogens (Burns *et al.* 1996; Ruggeri *et al.* 1998). Moreover recent studies showed that pIgR-deficient mice are defective in epithelial barrier function (Johansen *et al.* 1999).

The expression of pIgR at epithelial cells depends on the physiological conditions and on the tissue considered. It has been shown that pIgR expression increases in the rat uterus from dioestrus to proeostrus (Sullivan & Wira, 1983). In the female reproductive tract, SC levels are under the control of ovarian steroids. Thus, oestradiol injections stimulate SC accumulation in uterine secretion and pIgR mRNA accumulation in uterus (Sullivan *et al.* 1983; Kaushic *et al.* 1995). In addition, progesterone partially inhibits the effects of oestradiol in the rat uterus. However the control of pIgR expression seems to be tissue specific. Indeed oestradiol reduces the pIgR mRNA levels in the vagina (Kaushic *et al.* 1997).

Previous studies from our laboratory have shown that pIgR expression changes during pregnancy and lactation in the rabbit mammary gland and that prolactin (PRL) plays a key role in this regulation (Rosato *et al.* 1995). Several authors have proposed that, in the sheep, the availability of SC would be a limiting factor for the transport of pIgA from the serum into secretions (Sheldrake *et al.* 1984; Scicchitano *et al.* 1986). So it was interesting to investigate the mammary gland pIgR levels in species like ruminants which have much lower concentrations of IgA in colostrum and milk than the rabbit or pig. Despite the fact that the bovine pIgR cDNA was cloned several years ago (Kulseth *et al.* 1995), only limited information is available on pIgR gene expression in ruminants. The aim of the present study was to analyse the evolution of pIgR expression in the sheep mammary gland during pregnancy and lactation and to analyse its hormonal regulation. A better understanding of the regulations of pIgR levels in the mammary gland could be useful to the development of new strategies to increase the amount of sIgA in the ruminant milk. This approach could be of interest to improve passive immunization in human and animal species.

Materials and methods

Animals and sample collection

Procedures relating to the care and use of animals were approved by the French Ministry of Agriculture according to the French regulations for animal experimentation (guideline 19/04/1998). One-year-old nulliparous Prealpes du Sud ewes were used in this study.

Animals had their oestrus cycles synchronized prior to the beginning of the experiment. Oestrus was synchronized using intravaginal sponges containing 40 mg fluoro-progesterone (Intervet, Angers, France) for 14 days. Two days before the time of sponge withdrawal, the ewes received one intramuscular injection of 500 IU of pregnant mare serum gonadotropin (PMSG; Intervet, Angers, France). Oestrus was observed 48 h later.

Ewes were then mated twice (day 0). Animals were killed at days 15 ($n = 2$), 70 ($n = 3$), 106 ($n = 3$) and 112 ($n = 3$) of pregnancy and at days 3 ($n = 3$) and 70 ($n = 3$) of lactation.

Portions of each mammary gland were frozen until use for RNA extraction or treated for *in situ* hybridization and immunohistochemistry. For these two experiments, slices were fixed in 10 g/l paraformaldehyde for 24 h, cryoprotected by a 150 g/l sucrose solution, frozen at −45 °C in isopentane, cooled on dry ice, and stored at −80 °C until use.

For the *in vivo* experiment, four groups of three animals were constituted. Day of oestrus was the first day of the experimental treatment (day 1). Group 1 contained control ewes which received no treatment during the 20 consecutive days of the experimental protocol. In group 2, the ewes received subcutaneously 0·5 mg/kg oestradiol 17 beta (E_2) and 1·25 mg/kg progesterone (P_4) dissolved in 80 % ethanol twice daily from day 1 to 7 (Roussel-UCLAF, Romainville, France). In group 3, the ewes were treated as in group 2 and on experimental days 18 to 20 received twice daily intramuscular injection of 1 mg hydrocortisone acetate/kg (Roussel-UCLAF, Romainville, France). Ewes in group 4 received the same treatment as group 3 as well as a 2-bromo-α-ergocryptine (CB 154: Sandoz Ltd, Basel, Switzerland) treatment of 1 mg subcutaneously twice daily throughout the 20 consecutive days. On day 20, the ewes were killed. Biopsies of the mammary gland were collected and used for RNA extraction or treated for *in situ* hybridization and immunohistochemistry.

Development of a specific ovine pIgR cDNA probe

Approximately 2 μg RNA was denatured by heating and then reverse transcription (RT) was performed in a total volume of 20 μl according to the recommendations of the manufacturer (Kit for RT-PCR, Boehringer Mannheim). Among the RT products, one fragment of 942 bp was amplified using primers chosen in highly conserved regions of the rat and bovine pIgR cDNA sequence (sense: 5′-CAGCTATAAAGACAGAGCA-3′ and antisense: 5′-TTGCTCCACTTACACCAGTA-3′). DNA was subjected to 30 cycles of PCR (1 min each 94 °C, 55 °C and 72 °C). This 942 bp fragment was cloned into pGEM-T (Promega). The cDNA fragment was sequenced by automatic sequencing (Taq Dye Primer Cycle Sequencing kit, Applied Biosystems) and compared to the nucleotide sequences of the bovine cDNA as well as the cDNA of the human SC. These comparisons revealed a significant alignment of 91 % and 81 % identities, respectively. For Northern Blot analysis, a probe of 657 bp was obtained after KpnI enzymatic digestion of this cloned cDNA fragment. This probe was labelled by a random-primed DNA labelling kit (Boerhinger Mannheim, Illkirch, France) using α^{32}P-dCTP.

For *in situ* hybridization, pGEM-T containing the pIgR cDNA was linearized. Antisense RNA probe was transcribed using SP6 RNA polymerase with α^{35}S-UTP (> 1000 Ci/mmol; Amersham) as labelled ribonucleotide triphosphates. Sense RNA probe was also synthesized and used as control.

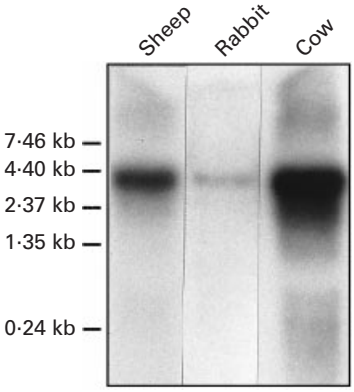

Fig. 1. Detection of pIgR mRNA in the mammary gland of sheep, cow and rabbit. Analysis by Northern Blot was performed with a gel containing 20 μg of total RNA prepared from mammary gland. After blotting, RNA was probed with the 657 bp fragment of the ovine pIgR cDNA. The size of pIgR mRNA from the different species is indicated in kilobases.

RNA extraction and Northern Blot analysis

Total RNA was extracted according to the method of Chomczynski & Sacchi (1987) modified by Puissant & Houdebine (1990).

Total RNA (20 μg) was denatured with formamide and size separated by electrophoresis in 1·5% agarose −1·2 M-formaldehyde gel in 10 mM-phosphate buffer, pH 7·5. RNA samples were blotted onto a Zeta-probe membrane (Bio-Rad, Ivry-sur-Seine, France) by capillarity in 10-strength sodium saline citrate (SSC: 1·5 M-sodium chloride, 0·15 M-sodium citrate, 5 g SDS/l). After (UV) fixation, the membranes were prehybridized in a 0·5 M-sodium dihydrogenophosphate, pH 7·2 containing 70 g SDS/l, 1 mM-EDTA and 5 g non-fat dry milk/l at 65 °C for 3 h. Hybridization was performed overnight at 65 °C in the presence of the α-^{32}P-labelled probe (2×10^6 cpm/ml) in the same medium.

Membranes were washed in 4-strength SSC and 5 g SDS/l at 65 °C for various durations. Autoradiographs were obtained by exposure to Amersham Hyperfilm between two intensifying screens at −80 °C. Signals on the filters were scanned with a STORM-860 machine (Molecular Dynamics, Bondoufle, France) and quantified with ImageQuaNT software (IQNT-130; Molecular Dynamics). Values were corrected using the ovine GAPDH hybridisation signal as a control for the RNA loading.

In situ *hybridisation*

Slices, 10 μm-thick, were cut, mounted onto Superfrost/Plus slides (Menzel-Glaser, Prolabo, Bondoufle, France), postfixed in 40 g paraformaldehyde/l, washed in 4-strength SSC solution pH 8·0 containing 2·5 g acetic anhydride/l and 0·1 M-triethanolamine, serially dehydrated in ethanol, and air dried. 50 μl of the hybridisation mixture (20 mM-Tris-HCl, pH 7·4, 300 mM-NaCl, 1 mM-EDTA, 500 g deionized formamide/l, 100 g dextran sulphate/l, 0·2 g BSA/l, 0·2 g Ficoll/l, 0·2 g polyvinylpyrrolidone/l, 100 mM-dithiotreithol, 1 g SDS/l, 1 g sodium thiosulphate/l, 0·1 mg/ml salmon sperm DNA, and 0·4 mg/ml yeast tRNA) containing approximately 10^6 cpm/ml sense or antisense RNA probe was deposited onto each slide. Sections were overlaid with coverslips and incubated overnight at 55 °C in a moist chamber. The slides were then treated with RNase A (20 μg/ml in 4-strength SSC) at 37 °C for 30 min, washed in decreasing concentrations of SSC containing

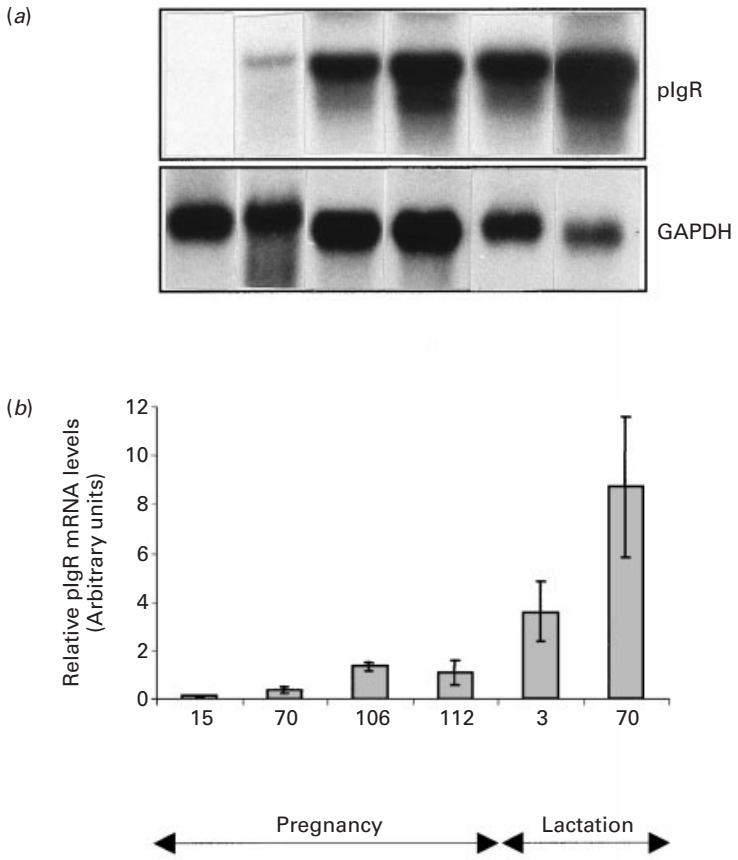

Fig. 2. Evolution of pIgR mRNA in the sheep mammary gland at different stages of pregnancy and lactation. (*a*) Northern Blot analysis of total RNA prepared from mammary gland of animals killed during pregnancy at days 15, 70, 106 and 112 and during lactation at days 3 and 70. A representative pattern of the evolution of RNA levels at different stages is shown. After blotting, RNA was probed with the 657 fragment of the ovine pIgR cDNA. The same blot was rehybridised with ovine GAPDH probe to normalise the amount of total RNA in each lane. (*b*) After each hybridisation, the relative intensities of hybridisation signals were measured using a STORM machine and ImageQuaNT software. Data are presented for each time as the mean ± SEM ($n = 2$–3) of relative amount of pIgR mRNA normalised with GAPDH.

1 mM-DTT at room temperature, and finally incubated in two successive baths of 0·1-strength SSC, with 1 mM-DTT added, at 65 °C for 30 min each. Slides were then serially dehydrated in ethanol, air-dried, processed for autoradiography using NTB2 photographic emulsion (Eastman Kodak, Poly Labo, Strasbourg, France), and exposed for 2–4 weeks at 4 °C. Sections were then stained with toluidine blue and examined with a microscope equipped with an epi-illumination system and a polarizing filter (Reichert, Paris, France).

Immunohistochemistry

For permeabilisation, each section was pretreated in PBS containing 20 g BSA/l, 0·5 g saponin/l (Sigma) and 0·5 g sodium azide/l (Prolabo) for 1 h. To detect pIgR protein, sections were stained with a rabbit serum anti-human SC[1], at 1/100 in the same buffer. After washing (3 times, 10 min) with PBS-(2 g BSA/l, 0·5 g saponin/l,

[1] anti-human SC (kindly provided by J.P. Kraehenbuhl, Lausanne, Switzerland).

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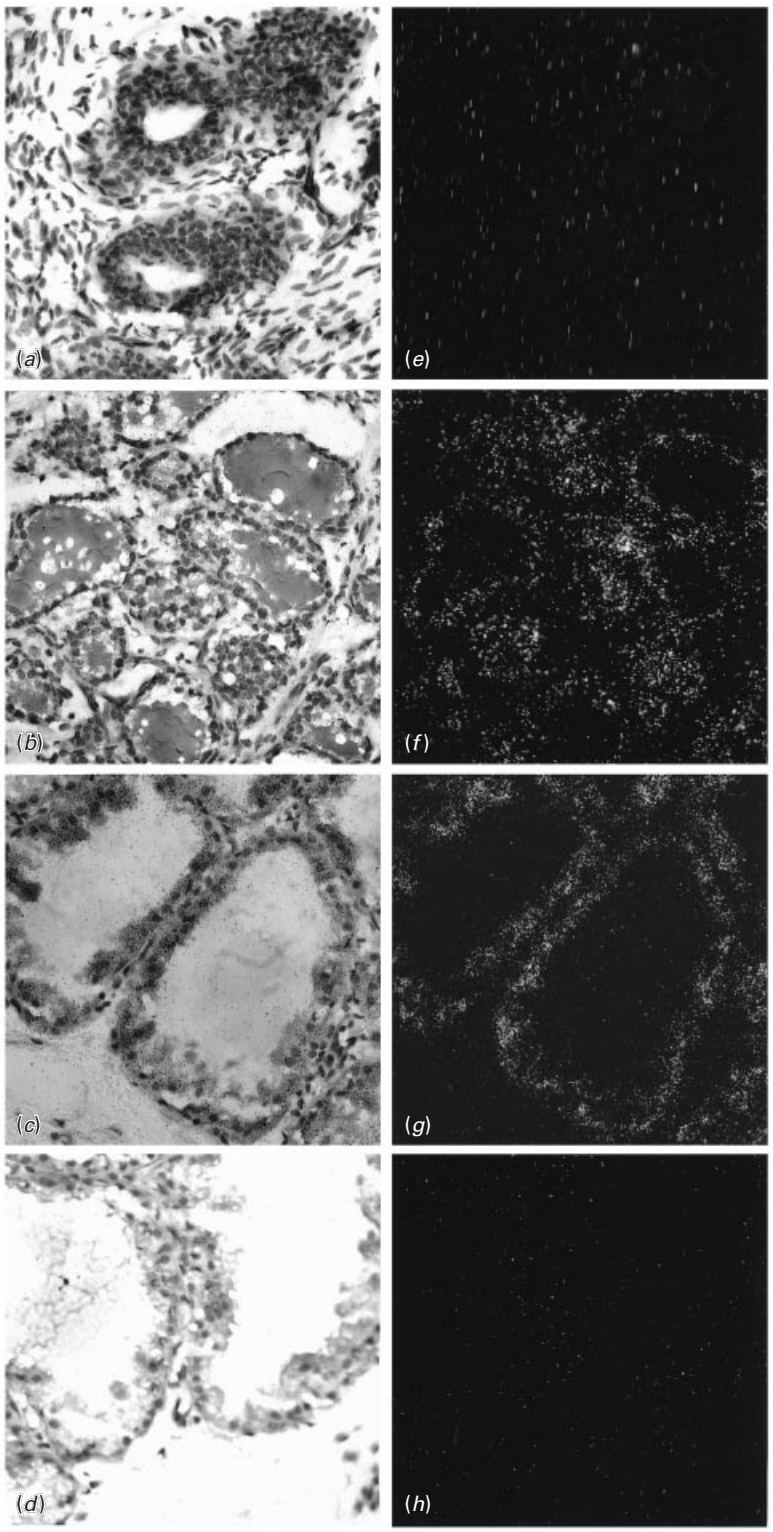

Fig. 3. For legend see facing page.

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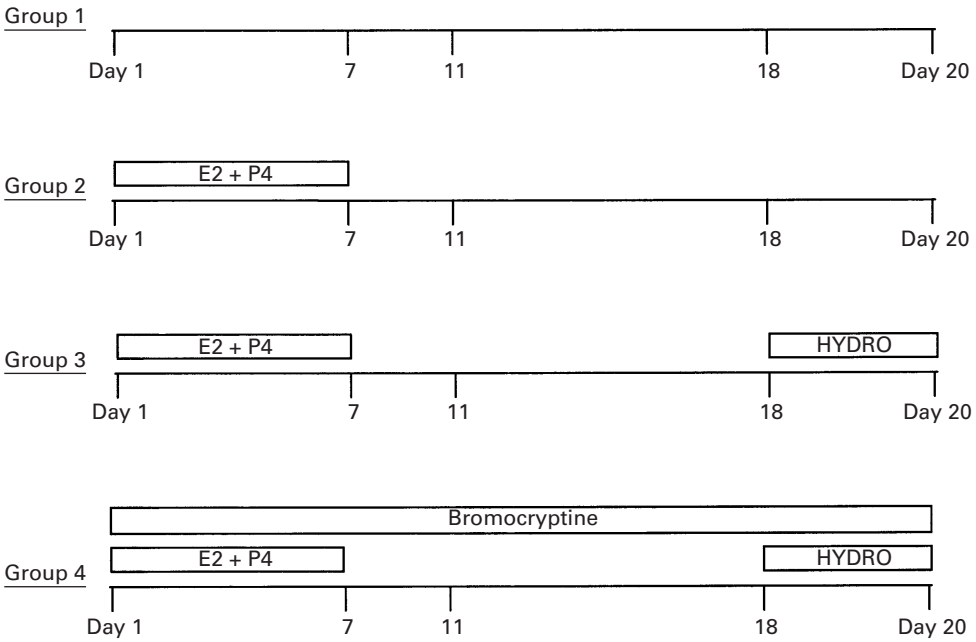

Fig. 4. Schematic representation of the experimental protocol showing the hormonal treatments of virgin ewes in the 4 experimental groups. E2 = oestradiol; P4 = progesterone; Hydro = hydrocortisone acetate. Day 1 = day of progesterone + PMSG induced oestrus in virgin sheep.

Table 1. *Relative amount of pIgR mRNA (arbitrary units) in the mammary gland of each sheep of the four experimental groups. The results are expressed as the ratio of the pIgR mRNA to the GAPDH mRNA. Gene expression was quantified with ImageQuaNT software. Values for 3 animals of each group are presented together with means* ± *SEM*

Treatment			
Group 1	Group 2	Group 3	Group 4
Control	E2 + P4	E2 + P4 + Hydro	E2 + P4 + Hydro + CB
0·01	0·24	0·48	0·06
0·03	0·53	0·82	0·47
0·06	0·05	1·13	0·34
0·03 ± 0·02[a]	0·27 ± 0·17[a,b]	0·81 ± 0·23[b]	0·29 ± 0·15[a,b]

E2, oestradiol; P4, progesterone; Hydro, hydrocortisone acetate.
[a,b] Values without a common superscript letter were significantly different, $P = 0·055$.

0·5 g sodium azide/l) each section was incubated with FITC conjugated-anti rabbit IgG (H + L) (Sanofi Diagnostics Pasteur, France) diluted at 1/200 in PBS-(BSA, saponin, azide) 1 h. After 10 min washing, nuclei were stained in washing buffer with 2 mg/ml Hoechst. Slices were washed twice and then mounted in Vectashield (Vector Laboratories, H-100). Negative control was incubated with normal rabbit serum.

Fig. 3. Detection by *in situ* hybridisation of pIgR mRNA in the mammary gland at different stages of pregnancy and lactation. Sections of sheep mammary glands at day 15 (*a*, *e*), at day 106 (*b*, *f*) of pregnancy and at day 70 of lactation (*c*, *g*) were hybridised with antisense RNA probe for ovine pIgR (see Materials and Methods). Sections of sheep mammary gland at 70 d of lactation were hybridised with sense RNA probe for ovine pIgR and used as control of specificity (*d*, *h*). Results are shown as bright field (*a*, *b*, *c* and *d*) and as epi-illuminated (*e*, *f*, *g* and *h*) photomicrographs. Bar = 50 μm.

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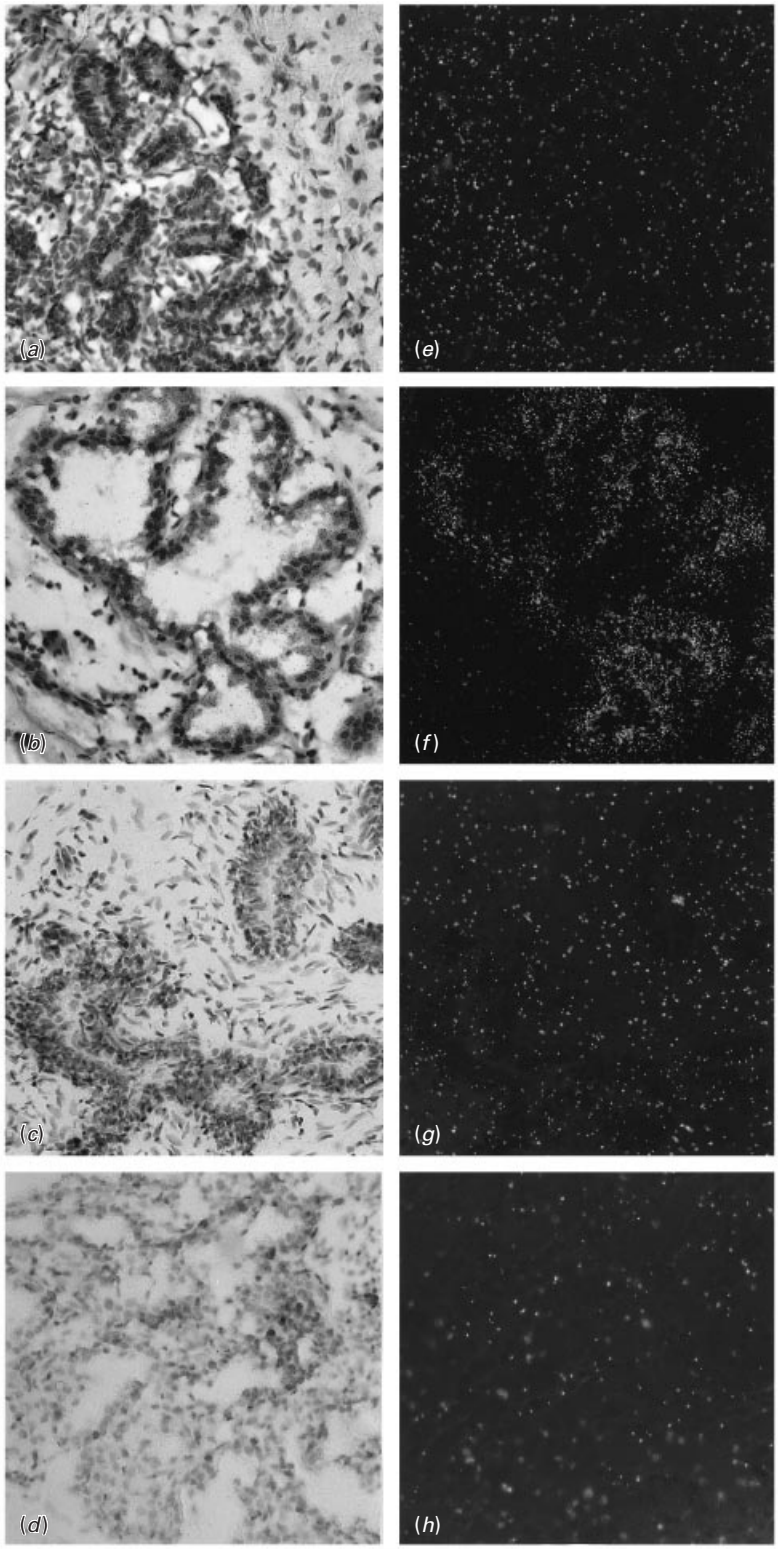

Fig. 5. For legend see facing page.

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The preparations were observed with a Reichert microscope equipped for ultra-violet illumination.

Statistical analysis

Data were analysed by ANOVA. The model used to analyse the data accounted for the effects associated with the treatment. Differences between each treatment and control were analysed by Student's t test.

RESULTS

Development of a specific ovine pIgR probe

In order to obtain an ovine homologous cDNA probe, we isolated a 950 bp pIgR cDNA fragment by RT-PCR using total RNA extracted from sheep mammary gland. A fragment of 657 bp was obtained after KpnI enzymatic digestion and used for Northern Blot hybridisation. This probe corresponds to the major part (447 bp) of the exon 3 of the putative sequence of the pIgR gene. As shown in Fig. 1, the probe revealed in the sheep mammary gland one type of transcript the size of which was approximately 3·6 kb as found by Kulseth *et al.* (1995) for the bovine mRNA pIgR. Moreover we verified that this probe hybridises with transcript obtained from rabbit and cow mammary gland collected respectively at the end of pregnancy and during lactation. This experiment revealed in both cases the presence of one type of transcript of about 3·6 kb.

Regulation of pIgR mRNA levels during pregnancy and lactation

We have examined the expression of pIgR in sheep mammary gland during pregnancy and lactation by Northern Blot analysis with the 657 bp pIgR probe. A representative pattern of RNA levels at different stages is shown in Fig. 2*a*. In order to check the standardisation of the amount of RNA in each sample, the same blot was stripped and hybridised with a probe for ovine GAPDH mRNA. Fig. 2*b* shows the mean for each time normalised with GAPDH. The levels of GAPDH mRNA showed differences in RNA loading between each group reflecting differences in total RNA concentrations. Poly IgR mRNA is poorly expressed during a first period of pregnancy from day 15 to 70. Accumulation of mRNA starts to increase from day 106 during the last third part of pregnancy and intensified 3 d after parturition to reach highest levels of pIgR mRNA during lactation (day 70).

In order to confirm these data at a cellular level, we performed *in situ* hybridisation in sheep mammary gland sections. The antisense probe revealed the same pattern as in Northern Blot analysis. As shown in Fig. 3, specific hybridisation appeared at day 106 of pregnancy (*b* and *f*) compared with day 15 of pregnancy (*a* and *e*). Hybridisation became very strong at day 70 of lactation (*c* and *g*). The expression was observed exclusively in mammary epithelial cells. These data clearly indicate that the increase in mRNA level was not the consequence of an increase in the numbers of epithelial cells but was truly the consequence of an increase in pIgR gene expression per mammary epithelial cell.

Fig. 5. Detection by *in situ* hybridisation of pIgR mRNA in the mammary gland of hormonally treated sheep. Sections of mammary gland of sheep from group 1 (*a*, *e*), group 3 (*b*, *f*) and group 4 (*c*, *g*) were hybridised with antisense cRNA probe for ovine pIgR. Sections of mammary gland from sheep belonging to group 3 were hybridised with sense cRNA probe for ovine pIgR and used as control of specificity (*d*, *h*). Results are shown as bright field (*a*, *b*, *c* and *d*) and as epi-illuminated (*e*, *f*, *g* and *h*) photomicrographs. Bar = 50 μm.

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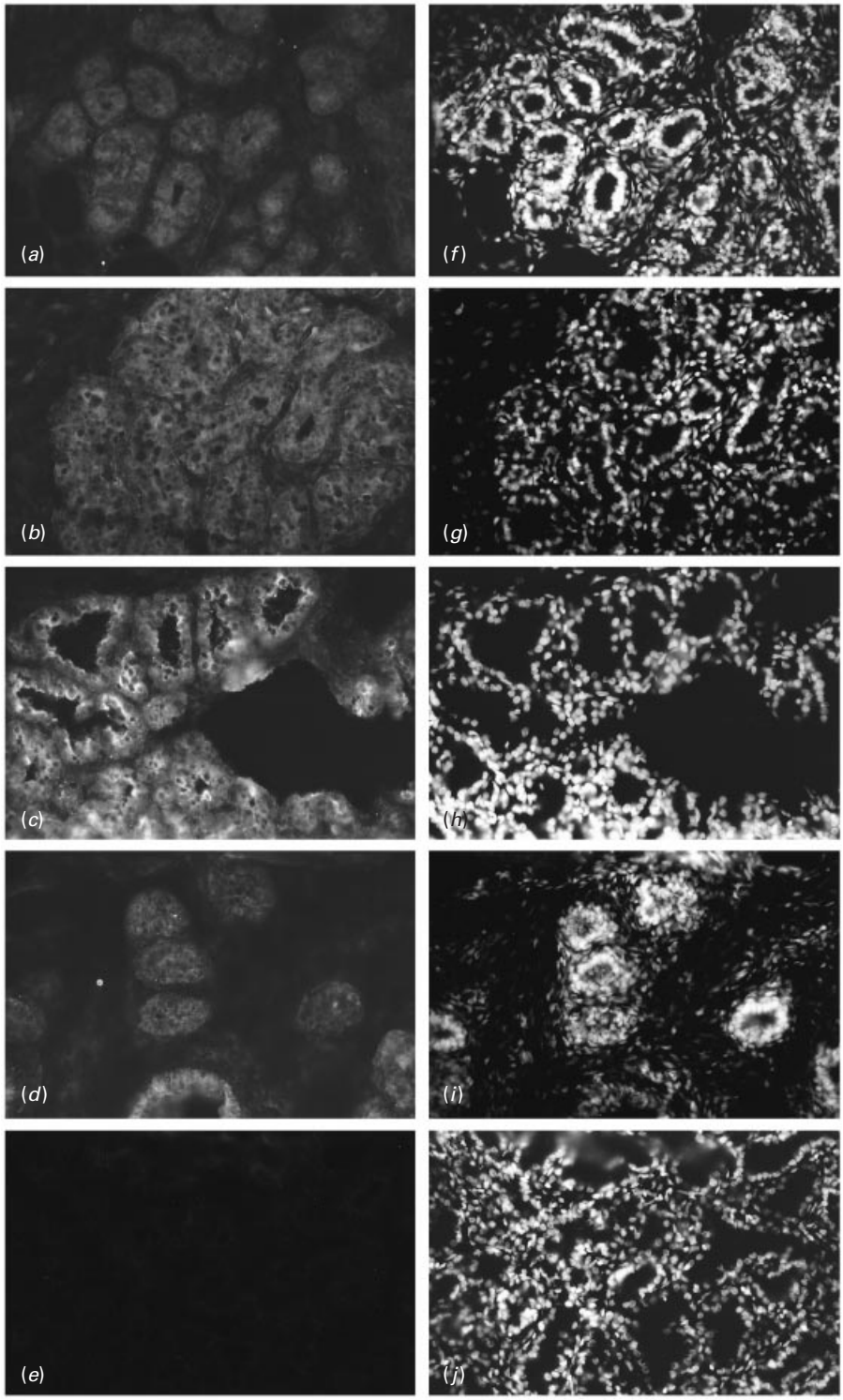

Fig. 6. For legend see facing page.

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Hormonal control of pIgR gene expression

During pregnancy and lactation, growth and differentiation of the mammary gland are controlled by numerous hormones. Following a standard hormonal treatment, it is possible to reproduce an endocrine environment resulting in an artificial growth and differentiation of the mammary gland. In order to better understand the role of these hormones on the pIgR levels, we have treated virgin ewes with oestradiol and progesterone (group 2 to 4), followed 10 d later (group 3 and 4) by a 2-d treatment with glucocorticoids. In addition, several animals (group 4) were treated with bromocryptine to suppress PRL secretion. Fig. 4 is a schematic representation of the hormonal treatments of sheep belonging to the four experimental groups.

Total mRNA from the mammary gland of each animal (3 animals per group) was analysed by Northern Blot. In order to normalise the amount of RNA in each sample, the same blot was stripped and hybridised with a probe for GAPDH mRNA. Ratio of pIgR mRNA to GAPDH mRNA is shown in Table 1. ANOVA analyses showed that treatments have a significant effect on pIgR mRNA level. Treatment with oestradiol and progesterone (group 2) increased pIgR mRNA levels compared with the control animals (group 1) but this was not significant, probably because of the variability between animals ($P = 0{\cdot}23$). The same treatment followed by glucocorticoid injections induced a more considerable and significant accumulation of pIgR mRNA in the mammary gland of the treated animals (group 3) compared with the control animals ($P = 0{\cdot}055$). It is interesting to note that no significant differences were observed in the group which was injected with bromocryptine throughout the hormonal procedure (group 4) compared with animals from group 1 and group 2. This result clearly indicates that hormonal steroids are not sufficient to stimulate pIgR gene expression and that PRL plays a crucial role.

In situ hybridisation was performed on sections of mammary glands of sheep belonging to group 1, group 3 and group 4. As shown in Fig. 5, we did not observe specific hybridisation in the case of group 1 (*a* and *e*), and group 4 (*c* and *g*). A strong specific hybridisation was only observed in the case of group 3 (*b* and *f*). We confirmed by this technique the increase of pIgR mRNA per mammary epithelial cell in animals treated with oestradiol, progesterone and glucocorticoids (*b* and *f*). We observed in the bright field photomicrographs showing histological aspect that the lumen of the acini became very enlarged. These changes in tissue organisation were parallel to the differentiation of the epithelial cells.

We undertook immunohistochemical localisation in order to verify whether the hormonal regulation of mRNA levels, identified by Northern Blot and *in situ* hybridisation analysis, were also effective at the pIgR protein level. This was performed on the same mammary gland sections used for *in situ* hybridisation. As seen in Fig. 6, we observed a similar pattern between pIgR mRNA and pIgR protein levels. Oestradiol, progesterone and glucocorticoids induced strong positive staining on mammary gland sections (*c* and *h*) compared with the sections of the control group (*a* and *f*) and the group of animals only treated with oestradiol and progesterone

Fig. 6. Detection by immunohistochemistry of pIgR in mammary gland from hormonally treated sheep. Sections of mammary gland of sheep from group 1 (*a*, *f*), group 2 (*b*, *g*), group 3 (*c*, *h*) and group 4 (*d*, *i*) were incubated with rabbit serum anti-hSC (left panel) and counterstained with Hoechst (right panel). For negative controls, section of mammary gland of sheep from group 3 (*e*, *j*) was incubated with non-immune rabbit serum instead of rabbit serum anti-hSC. Bar = 25 μm.

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(*b* and *g*). No immunostaining was found when animals were treated with bromocryptine (*d* and *i*).

discussion

Recently published data support the idea that the concentration of pIgA receptor may be a limiting factor which determines the amount of dIgA transcytosed by the epithelial barrier. In this line of evidence it has been demonstrated, using pIgR deficient animals, that the mice showed reduced pIgR levels in biological fluids and accumulation of IgA in the blood (Johansen *et al.* 1999; Shimada *et al.* (1999). Moreover, dIgA concentrations found in heterozygous pIgR KO mice were intermediate between those of pIgR +/+ and pIgR −/−. As illustrated by the present study, pIgR expression is tightly regulated during the development of the mammary gland. The use of the ovine species, as a model, was very suitable because mammary development occurs progressively during pregnancy and the different steps in growth and differentiation of the mammary gland have been well defined (Houdebine *et al.* 1985). The first increase in pIgR mRNA concentration occurs during the last third of pregnancy concomitantly with active lobuloalveolar development and the beginning of the differentiation of mammary epithelial cells. The most important increase in pIgR gene expression takes place during lactation when mammary epithelial cells are fully differentiated, polarised and actively secrete milk. It is interesting to note that even if IgA concentrations are very high in colostrum, the mammary epithelial cells continue the transfer of pIgA in milk all through the lactation period (Griebel, 1998) suggesting that the expression of pIgA receptor at a high level throughout lactation, as shown in this study, may be necessary for the protection of the new-born against gastrointestinal diseases.

Mammary gland development is controlled by a combination of many systemic hormones and locally produced growth factors acting in a well-coordinated equilibrium. In addition, several signalling molecules coming from the extracellular matrix also play an important role. Hormonal injection procedures have been established, particularly in ruminants, to experimentally reproduce mammary gland development and lactogenesis in non-pregnant animals (Head *et al.* 1980; Kann *et al.* 1999). We have used, in a second part of our study, one of these procedures in virgin ewes to better understand the hormonal regulation of pIgA receptor gene expression. Results indicate that a combination of oestradiol, progesterone, glucocorticoids and prolactin are necessary to induce both mammary cell differentiation and pIgA receptor expression. In addition, it appears that maximal induction of pIgA receptor occurs only in highly differentiated mammary epithelial cells in contrast to milk protein gene expression which occurs much earlier during cell differentiation.

This situation in the mammary gland is more complicated than for other organs where pIgR is expressed. Many studies have shown that oestradiol alone increases pIgR gene expression in a variety of tissues (Sullivan *et al.* 1983*b*; Kaushic *et al.* 1995). Treatment with oestradiol and progesterone increased slightly pIgR mRNA levels. We think that oestradiol and progesterone are not sufficient to stimulate pIgR gene expression and that glucocorticoids must be injected after ovarian steroid hormones for induction of pIgR gene expression in the mammary gland. This stimulatory effect of glucocorticoids is in agreement with several studies showing that glucocorticoids increase pIgR expression in rat hepatocytes (Wira & Colby, 1985) and in salivary glands (Wira & Rossoll, 1991). Glucocorticoids were also shown to enhance steady state mRNA levels in developing intestine and in IEC-6 cells (Li *et al.* 1999). The recent demonstration that glucocorticoid response elements (GRE) are present in

human and murine pIgR promoter (Martin *et al*. 1998; Haelens *et al*. 1999) give a mechanistic explanation for the stimulatory effect of glucocorticoids on pIgR gene expression in many biological systems. In the mammary gland, it has been known for a long time that glucocorticoids also amplify the effects of prolactin to induce mammary cell differentiation and milk protein gene expression (Devinoy & Houdebine, 1977). When we used bromocryptine to block prolactin secretion during experimental induction of mammary development, pIgR gene expression was also inhibited indicating that glucocorticoids are ineffective without prolactin. Recent studies performed in marsupial (Adamski & Demmer, 1999) indicate also that pIgR gene expression in the mammary gland is positively correlated with plasma prolactin levels.

In conclusion, pIgR expression in the mammary gland appears to be tightly controlled by complex interactions between steroids and peptide hormones. The most important period appears to be the time of parturition when the rapid decrease of progesterone plasma level induces a prolactin surge concomitantly with an increase of glucocorticoids. During lactation it appears that there is an additional increase of pIgR expression. Prolactin at this period is probably crucial to maintain at high levels pIgR expression but additional factors are probably involved. These factors are presently under investigations in our laboratory.

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