Production of matrix metalloproteinase 1 (interstitial collagenase) and matrix metalloproteinase 2 (gelatinase A : 72 kDa gelatinase) by ovine endometrial cells *in vitro*: different regulation and preferential expression by stromal fibroblasts

L. A. Salamonsen¹, H. Nagase², R. Suzuki² and D. E. Woolley³

¹Prince Henry's Institute of Medical Research, PO Box 152, Clayton, Victoria 3168, Australia;

²Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas

City, KS 66103, USA; ³University Department of Medicine, University Hospital of South

Manchester, West Didsbury, Manchester M20 8LR, UK

Ovine endometrial cells (epithelial plus stromal), prepared from ovariectomized ewes treated with oestrogen and progesterone to mimic the luteal phase of the oestrous cycle were maintained in serum-free medium for 48 h in the presence or absence of phorbol myristate acetate (PMA, 100 nmol l⁻¹), a known stimulus for production of matrix metalloproteinases (MMP) in other cells. Matrix metalloproteinase-1 (MMP-1, interstitial collagenase) and matrix metalloproteinase-2 (MMP-2, gelatinase A) activities were expressed by the cells in the absence of PMA; most were in the latent form and required activation by (4-aminophenyl) mercuric acetate (APMA). Exposure to PMA over 48 h resulted in a significant increase in MMP-1 activity but only a modest and nonsignificant increase in MMP-2 activity. Gelatin zymography demonstrated that proMMP-2 (72 kDa) was produced by both PMA-treated and untreated cells and an active form of 67 kDa was also present. Immunolocalization of MMP-1 and MMP-2 was seen within the cells following treatment with monensin. Highly purified epithelial and stromal cells were similarly cultured and analysis of the conditioned medium showed that MMP-1 and MMP-2 were produced predominantly by stromal rather than epithelial cells. Thus, both MMP-1, which degrades interstitial collagens, and MMP-2, an important enzyme for degradation of type IV and V collagens, are synthesized and released by ovine endometrial stromal cells in culture, but MMP-1 is produced primarily upon stimulation, whereas MMP-2 production is constitutive. It is postulated that these enzymes have important roles in endometrial remodelling and implantation.

Introduction

Remodelling of endometrial tissue is an integral part of the normal reproductive cycle, particularly evident in women but also occurring in other species. Implantation of the conceptus in all mammals is accompanied by degradation and remodelling of uterine tissue, the extent of which varies among species depending on the type of implantation (Renfree, 1982). At one extreme is the very invasive situation seen in humans in which the blastocyst penetrates the epithelium and passes into the subepithelial connective tissue where it implants. By contrast, in sheep and other domestic ungulates, implantation is much less invasive and the blastocyst attaches at clearly defined sites, the caruncles, by fusion of binucleate cells from the trophoblast with caruncular epithelial cells to form a syncytial layer. Slender processes from this layer pass through the basement membrane of the luminal epithelium (Wooding, 1984; Wooding and Morgan, 1989). Moreover, degenerative changes in the intercaruncular

regions (the glandular areas between the caruncles) accompany attachment and implantation and the columnar surface epithelium is temporarily lost (Casida and McKenzie, 1932; Boshier, 1969).

During the human menstrual cycle and early pregnancy the major components of endometrial extracellular matrix (ECM) are collagens type III, V and VI and fibronectin. Type IV collagen is confined to epithelial and endothelial basement membranes in the proliferative phase. In pregnancy, it occurs around decidual cells (Aplin *et al.*, 1988; Aplin, 1989). No similar information is available for sheep, but simple histological stains demonstrate an abundance of collagen in both caruncular and intercaruncular areas (L. A. Salamonsen, unpublished observations).

Of particular importance among the local factors involved in remodelling of tissues are the matrix metalloproteinases (MMPs) which operate optimally at neutral pH and between them can degrade all components of the matrix. These enzymes, and the tissue inhibitors of MMPs (TIMPs), may be synthesized and secreted locally by the resident cells of a tissue or by recruited inflammatory cells. Interstitial collagens (types I, II and III) are degraded by a specific interstitial collagenase (MMP-1).

The major components of basement membranes are type IV collagen and laminin which are potential substrates for the 72 kDa gelatinase (MMP-2). This enzyme also degrades pericellular type V collagen and has some activity against proteoglycans and fibronectin (Okada *et al.*, 1990b; Murphy *et al.*, 1991; Nagase *et al.*, 1991). Catabolism of basement membranes is thought to be primarily the result of the action of MMP-3 (stromelysin 1; Bejarano *et al.*, 1988) and we have recently demonstrated that ovine endometrial cells in primary culture produce MMP-3, and that production is increased fourfold following stimulation with phorbol myristate acetate (PMA) (Salamonsen *et al.*, 1991a).

The present study was designed to establish whether sheep endometrial cells have the potential to secrete interstitial collagenase and 72 kDa gelatinase, to determine which cell type was responsible and to examine the regulation of these enzymes *in vitro*.

Materials and Methods

Materials

CIDR G Silastic implants were EAZI-breed from Riverina Artificial Breeders Pty Ltd (Albury, Australia). Bacterial collagenase was type 111 from Worthington Biochemical Corp. (Freehold, NJ). Ficoll 400 was from Pharmacia Fine Chemicals (Uppsala). Medium 199 (M199), fetal calf serum (FCS), penicillin–streptomycin–fungizone (PSF), and phosphate-buffered saline (PBS) were from Flow Laboratories (Sydney); monensin, (4-aminophenyl) mercuric acetate (APMA) and phorbol myristate acetate (PMA) were from Sigma Chemical Co. (St Louis, MO). Rabbit antisera against human MMP-1 and MMP-2 were raised as described by Okada *et al.* (1989) and monospecificity was assessed by Western blots using 100-fold concentrated crude culture medium of human rheumatoid synovial fibroblasts. Alkaline phosphatase–antialkaline phosphatase (APAAP) reagents were from Serotec (Oxford, UK). Standard human proMMP-2 was as described by Okada *et al.* (1988, 1990a) and Suzuki *et al.* (1990).

Animals

Six parous Corriedale ewes were ovariectomized and maintained for at least one month before treatment with Silastic implants of oestradiol (days 1–12) plus intravaginal inserts containing progesterone (CIDR-G; days 3–12) to mimic the oestrous cycle (Salamonsen *et al.*, 1985, 1991b). On day 12, the ewes were anaesthetized (initially with 10 ml sodium pentobarbitone (*i.v.*), and then maintained on halothane/oxygen), and hysterectomized. The endometrium was dissected from the myometrium.

Cell isolation and culture

A mixture of endometrial stromal fibroblasts and epithelial cells was prepared from the endometrium using techniques described by Salamonsen *et al.* (1985). In brief, tissue was chopped finely with scissors and digested with bacterial collagenase in a shaking water bath for 75 min. After filtration

through two grades of nylon mesh, the cell mixture was centrifuged (200 g, 5 min) through a Ficoll gradient and the cells lying at the 6–8%, 8–10% and 10–20% interfaces pooled and radioactivity was measured. Cells were resuspended in M199 with 10% FCS and PSF. For enzyme studies, 2×10^6 cells in 2 ml of medium were plated into 24-well dishes and left to attach overnight. For immunocytochemical analyses 6×10^6 cells were plated into 6-well dishes containing glass cover slips that had been soaked overnight in 25% FCS before use. On the following day, cells were washed three times with PBS and media were replaced with M199 containing only antibiotics. Triplicate dishes were either untreated (control) or subjected to added PMA (100 nmol l^{-1}). Cultures were maintained for a further 48 h; media were collected and lyophilized prior to preparation for assay. For time-course studies, medium was removed from triplicate wells (control plus treatment) at each time point. For dose–response studies, triplicate wells contained PMA at 0.1, 1, 10 and 100 nmol l^{-1} . Pure (>95%) endometrial stromal or epithelial cells were obtained from large-scale mixed cell cultures by differential trypsinization (Cherny and Findlay, 1990) and cultured as for the mixed cell preparations.

Enzyme assay

MMP-1 (collagenolytic) activity was measured by the diffuse fibril method of Cawston and Barrett (1979) using [^{14}C]acetylated type I collagen (guinea-pig), incubated at 37°C for 20 h. Gelatinolytic activity (MMP-2 plus MMP-9) was measured by using heat-denatured [^{14}C]acetylated type I collagen (gelatin, guinea-pig), incubated at 37°C for 4 h according to Harris and Krane (1972). One unit of enzyme activity was defined as that amount of enzyme that could degrade $1 \mu\text{g}$ of substrate per minute at 37°C . All samples were assayed for enzyme activity in the pro-(latent) form and in the activated form after preincubation of the samples with APMA (1.5 mmol l^{-1}) at 37°C for 2 h.

Zymography

Gelatinase activity in the culture media was analysed by zymography. Media samples were subjected to SDS–PAGE on 7.5% acrylamide slab gels containing $1 \text{ mg gelatin ml}^{-1}$ under nonreducing conditions. After washing in buffer containing $50 \text{ mmol Tris-HCl l}^{-1}$, $5 \text{ mmol CaCl}_2 \text{ l}^{-1}$, $1 \mu\text{mol ZnCl}_2 \text{ l}^{-1}$, NaN_3 0.02% (w/v) (pH 7.5) and 1% Triton X-100, the gels were incubated in the same buffer without Triton X-100 at 37°C for 2 h and stained with Coomassie brilliant blue. Gelatinolytic activity was visualized by negative staining.

Immunocytochemistry

After media were harvested from cells on glass cover slips, cells were reincubated in a further 2 ml of medium containing monensin ($3 \mu\text{mol l}^{-1}$) for 4 h. Media were discarded, cells washed twice with PBS, fixed with 70% ethanol for 10 min, dried and stored over silica gel. Cells were subsequently rehydrated with PBS and examined for MMP-1 and MMP-2 production using the standard APAAP method. The primary antibodies were followed by an unconjugated IgG bridge which

Table 1. Production of matrix metalloproteinase (MMP)-1 and MMP-2 by ovine endometrial cells in culture

Treatment	Control	PMA*
MMP-1 without APMA activation	0.14 ± 0.07	0.75 ± 0.14
MMP-1 with APMA activation	0.79 ± 0.43	1.92 ± 0.21†
MMP-2 without APMA activation	0.12 ± 0.04	0.25 ± 0.09
MMP-2 with APMA activation	6.86 ± 3.98	8.17 ± 4.01

MMP activity in culture medium expressed as mean ± SEM units per 10⁶ cells per 48 h.

Combined data from four ewes. Culture dishes in triplicate. APMA: (4-aminophenyl) mercuric acetate.

*PMA: phorbol myristate acetate (100 nmol l⁻¹) added to cells in culture.

†Significantly (*P* < 0.05) different from control.

was subsequently recognized by an alkaline phosphatase-conjugated complex of rabbit APAAP. The bridging step and the alkaline phosphatase-linked step were repeated to enhance staining. Control incubations included similar dilutions of nonimmune sera and specific antigen-absorbed primary antibodies. Staining was developed using new Fuchsin and cells were counterstained using Harris's haematoxylin.

Statistical analyses

Data from enzyme assays was analysed by analysis of variance and Duncan's multiple range *F* test. Differences were considered significant at the 0.05 level.

Results

The production of interstitial collagenase and gelatinase activities by the mixed endometrial cells following 48 h incubation in serum-free medium in the absence and presence of PMA (100 nmol l⁻¹), both before and after activation with APMA, is shown (Table 1). Data shown are combined from parallel experiments with cells from four animals. The production of MMP-1 was significantly enhanced by PMA (*P* < 0.05), although a small amount was always produced by the control cells. Most MMP-1 was in the latent form and required activation. Gelatinase was expressed by the cells in the absence of PMA and as with MMP-1, most was in the latent form requiring activation by APMA. Although there was a modest increase in expression following 48 h stimulation with PMA, this was not significant and was small in comparison with the stimulation of MMP-1 by PMA.

ProMMP-1 was significantly (*P* < 0.05) stimulated by PMA as early as 6 h after addition of PMA to the cultures and this stimulation resulted in continuing MMP-1 accumulation in culture medium for up to 64 h (Fig. 1). Release from control cells did not increase after 24 h (Fig. 1a). Gelatinase activity in the culture medium increased with time up until 64 h, but at no time was stimulation by PMA significant. The dose of PMA chosen (100 nmol l⁻¹) is 100-fold higher than the dose required to elicit a maximum response in MMP-3 release from similar cell

Fig. 1. Time course for release of (a) matrix metalloproteinase (MMP)-1 and (b) MMP-2 into the culture medium. Data are expressed as units of enzymic activity ml⁻¹ culture medium (U ml⁻¹, mean ± SEM, *n* = 3; representative data from one of three experiments). C: control cells (no treatment). P: phorbol myristate acetate-treated cells (100 nmol l⁻¹). □: activity without activation. ▨: activity with (4-aminophenyl) mercuric acetate activation.

preparations (1 nmol l⁻¹; Salamonsen *et al.*, 1991). Lower concentrations (0.1, 1, 10 nmol l⁻¹) were also without effect on MMP-2 production (Fig. 2b). Stimulation of MMP-1 production by PMA was dose dependent and there was significant stimulation (*P* < 0.05) by 100 nmol PMA l⁻¹ and substantial stimulation by 10 nmol PMA l⁻¹, whereas concentrations of 0.1 nmol l⁻¹ and 1 nmol l⁻¹ were without effect (Fig. 2a).

Gelatin zymography (Fig. 3) demonstrated that the major gelatinolytic activity released by control and PMA-stimulated cells corresponds to human proMMP-2. Comparison with the standard for human proMMP-9 (92 kDa gelatinase) suggests these cells produce little or no gelatinase activity in the form of MMP-9.

Media harvested from purified stromal and epithelial cells cultured in the absence or presence of PMA were assayed for enzyme activities and subjected to zymography to determine which cell type within the endometrium was responsible for production of proMMP-1 and proMMP-2. After activation with APMA, MMP-1 and MMP-2 activities in stromal cell culture media were substantial, whereas in epithelial cell culture media enzyme activities were barely detectable (Fig. 4). The small amounts of MMP-1 and MMP-2 activity assayed in conditioned culture medium from epithelial cells after APMA

Fig. 2. Release of (a) matrix metalloproteinase (MMP)-1 and (b) MMP-2 by ovine endometrial cells in response to increasing concentrations of phorbol myristate acetate (PMA). Data are expressed as units of enzymic activity per 48 h (mean $\pm$ SEM, $n=3$; representative data from one of two experiments); (●) activity without (4-aminophenyl) mercuric acetate (APMA) activation; (▲) activity following APMA activation; *significant stimulation ($P < 0.05$) at the given dose compared with control value.

activation probably represents synthesis by the small number of contaminating stromal cells (<5%). The data for MMP-2 were confirmed by zymography (Fig. 5); both inactive (proMMP-2) and active (MMP-2) forms of the enzyme were visible on zymograms of medium from stromal cell culture, but only very small amounts of enzyme were detected in medium from epithelial cell culture. No stimulation by PMA was evident.

Sheep antisera raised against MMP-1 and MMP-2 were shown to crossreact with ovine MMPs. Immunocytochemical staining for MMP-1 and MMP-2 was demonstrated in mixed cell cultures after stimulation with PMA and inhibition of enzyme release with monensin. However, the staining was mainly confined to a proportion of the cells with fibroblastic morphology. Subsequent localization studies on purified stromal cell cultures demonstrated that these cells were effective producers of MMP-2 and MMP-1. Whereas immunoreactive MMP-2 was clearly associated with perinuclear, Golgi distribution, MMP-1 was more diffusely distributed in the cytoplasm (Fig. 6).

Fig. 3. Zymography of matrix metalloproteinase (MMP)-2 from mixed endometrial cells on polyacrylamide gels containing gelatin. Lane 1: proMMP-2 (human standard); lane 2: proMMP-9 (human standard); lanes 3 and 4: culture medium from ovine endometrial cells, unstimulated; lanes 5 and 6: culture medium from ovine endometrial cells stimulated with phorbol myristate acetate; lane 7: human synovial cell culture medium.

Fig. 4. Release of (a) matrix metalloproteinase (MMP)-1 and (b) MMP-2 activity from purified ovine endometrial stromal and epithelial cells in culture. Data are expressed as units of enzymic activity ml⁻¹ culture medium (U ml⁻¹) (mean $\pm$ SEM, $n=3$ wells per treatment group; representative data from one of five separate experiments). □ control cells (no treatment); ▨ phorbol myristate acetate-treated cells (100 nmol l⁻¹). Samples activated with (4-aminophenyl) mercuric acetate before assay.

Fig. 5. Zymography of matrix metalloproteinase (MMP)-2 from purified stromal or epithelial endometrial cells on polyacrylamide gels containing gelatin. Equivalent cell numbers were present in the stromal and epithelial cultures. Culture media from individual wells of endometrial cells. Lanes 1–6: stromal cells; lanes 7–12: epithelial cells; cells represented in lanes 4–6 and 10–12 were treated with phorbol myristate acetate (100 nmol l^{-1}); lane 13: human synovial cell culture medium.

Discussion

We report here that two of the enzymes responsible for the catabolism of extracellular matrix, MMP-1 (interstitial collagenase) and MMP-2 (72 kDa gelatinase), were released from cells derived from endometrium of ovariectomized ewes treated with oestrogen and progesterone. These results together with our previous report of the production of MMP-3 by ovine endometrial cells (Salamonsen *et al.*, 1991a) show that the endometrium can produce a number of enzymes which together can degrade the components of the interstitial matrix and basement membranes. Endometrial stromal, but not epithelial, cells are the source of both MMP-1 and 2 (this study) and of MMP-3 (authors' unpublished observations) in these cultures. The measured MMP-2 activities are more than fourfold higher than MMP-1 activity following stimulation and are tenfold higher than those of MMP-3 released from similar cell cultures following stimulation by PMA (Salamonsen *et al.*, 1991a).

Whereas production of MMP-1 and MMP-3 can be stimulated by PMA, substantial amounts of proMMP-2 are apparently produced from ovine endometrial cells without PMA stimulation. However, some data from Western analyses and from zymography demonstrate a small increase in proMMP-2 release following PMA treatment, and these are supported by a small though not significant increase in enzyme activity. One explanation for these observations could relate to the status of the animals producing maximal stimulation of proMMP-2 (but not of proMMP-1 or proMMP-3) *in vivo*, which is retained throughout the studies *in vitro*. Previous studies with similar cells have demonstrated a 'memory of responsiveness' (in terms of individual secreted proteins) to steroid hormones for a number of days of culture in steroid-free medium (Salamonsen *et al.*, 1985). The rationale for the treatment of the ewes *in vivo* in the present study was to mimic the luteal phase of the cycle which prepares the uterus for implantation and the endometrium was thus exposed to both oestradiol and progesterone *in vivo*. Furthermore phenol red, which has inherent low oestrogenic activity, was present in all culture media. In long-term cultures

of human cervical fibroblasts, progesterone was reported to modulate synthesis of MMP-1, MMP-3 and TIMP (Sato *et al.*, 1991) and thus it is possible that progesterone or oestrogen stimulates proMMP-2 production by endometrial cells. Constitutive release of proMMP-2 compared with regulated release of proMMP-1 and proMMP-3 as described here and by Salamonsen *et al.* (1991a) has also been described for human rheumatoid synovial fibroblasts in culture (Okada *et al.*, 1990a). To date two gelatinolytic enzymes of molecular masses of 72 kDa and 92 kDa (products of different genes) have been isolated from many cell types including mesenchymal, haemopoietic and tumour cells. The zymographic analyses in this study suggest that the only gelatinase released from ovine endometrial cells is the 72 kDa form and that the 92 kDa gelatinase (gelatinase B; MMP-9) is not produced by these cells.

Physiological activity of all MMPs *in vivo* depends not only upon local regulation of their production but also upon development of conditions necessary for enzyme activation *in vivo*. The natural activators of proMMP-1 include proteinases such as trypsin, plasmin and also MMP-3 (Suzuki *et al.*, 1990) and it is possible that the co-production of proMMP-1 and proMMP-3 from endometrial cells favours activation of proMMP-1 *in vivo*. Proteinases that activate proMMP-2 are not yet known. As the amino acid sequence similarity in the activation regions of proMMP-1 and proMMP-3 (Phe-Val-Arg-Arg-Lys-Asp (Nagase *et al.*, 1992)) is not present in the corresponding region of proMMP-2, it is likely that a specific proteinase yet to be identified may activate proMMP-2 at a quite different site. APMA activates all three proMMPs *in vitro* with an accompanying change in molecular mass of MMP-1 from 52 to 41 kDa (Grant *et al.*, 1987) and of MMP-2 from 72 to 67 kDa (Stetler-Stevenson *et al.*, 1989; Okada *et al.*, 1990b; Nagase *et al.*, 1992). The concentrations of the ubiquitous specific tissue inhibitors of MMPs (TIMPs) which inactivate the enzymes by binding to them with 1:1 stoichiometry are also critical for the activity of MMPs *in vivo*. The potential interference by TIMPs in the present studies was not addressed.

Fig. 6. Immunocytochemical localization of matrix metalloproteinase (MMP)-1 and MMP-2 in purified stromal endometrial cells. The APAAP method of immunolocalization was applied with haematoxylin counterstain and the cells were examined under a Zeiss Photomicroscope 111. (a) Control incubation; (b) MMP-2 distribution; (c) MMP-1 distribution. Arrows highlight positive staining. Bar = 10 µm.

MMP-1 and MMP-2 have quite different substrate specificities. MMP-1 acts specifically on interstitial collagens (I, II, III, VII and X) (Welgus *et al.*, 1981; Schmid *et al.*, 1986; Seltzer *et al.*, 1989). Although MMP-2 is commonly known as a type IV collagenase, Okada *et al.* (1990b) have shown that MMP-3 is far more effective at digesting type IV collagen than is MMP-2 and that a more representative function for MMP-2 is the digestion of type V interstitial collagen. Although synergistic effects of MMP-1 and MMP-2 on collagen fibril degradation have been demonstrated *in vitro* (Sunada and Nagai, 1983; Murphy and Reynolds, 1985), differences in responsiveness of MMP-2 synthesis to inductive stimuli, when compared with that of other MMPs as seen in this

and other studies (Overall *et al.*, 1989; Okada *et al.*, 1990b) suggest that the role of MMP-2 in matrix catabolism may be separate from those of MMP-1 and MMP-3.

Since endometrial remodelling, angiogenesis and embryo implantation all involve the breakdown of connective tissue barriers, such as the stromal matrix and basement membranes, it seems likely that MMPs will make a significant contribution to these processes. Elucidation of the mechanisms of release, activation and actions of MMPs in the uterus therefore offers a substantial challenge for the future.

The authors are grateful to B. Doughton for assistance with the animal surgery, to the Director of the Animal Research Institute at Werribee, Victoria for provision of surgical facilities and to L. Tetlow for expert technical assistance. This work was supported by the NH and MRC of Australia and by NIH grant AR39189. The animal experimental work was approved by Animal Ethics Committees at the Monash Medical Centre and at the Animal Research Institute.

References

- Aplin JD (1989) Cellular biochemistry of the endometrium. In *Biology of the Uterus* (2nd Edn), pp. 89–108 Eds RM Wynn and WP Jollie. Plenum Medical Book Co., New York.
- Aplin JD, Charlton AK and Ayad S (1988) An immunohistochemical study of human endometrial extracellular matrix during the menstrual cycle and first trimester of pregnancy *Cell and Tissue Research* **253** 231–240
- Bejarano PA, Noelken ME, Suzuki K, Hudson BG and Nagase H (1988) Degradation of basement membranes by human matrix metalloproteinase 3 (stromelysin) *Biochemical Journal* **256** 413–419
- Boshier DP (1969) A histological and histochemical examination of implantation and early placental formation in sheep *Journal of Reproduction and Fertility* **19** 51–61
- Casida LE and McKenzie FF (1932) The oestrous cycle of the ewe: histology of the genital tract *University of Missouri, College of Agriculture Research Bulletin* **170** 4–28
- Cawston TE and Barrett AJ (1979) A rapid and reproducible assay for collagenase using (1–14C)acetylated collagen *Analytical Biochemistry* **99** 340–345
- Cherny RC and Findlay JK (1990) Separation and culture of ovine endometrial epithelial and stromal cells: evidence of morphological and functional polarity *Biology of Reproduction* **43** 241–250
- Grant GA, Eisen AZ, Marmer BL, Roswit WT and Goldberg GI (1987) The activation of human skin fibroblast collagenase *Journal of Biological Chemistry* **262** 5886–5889
- Harris ED and Krane SM (1972) An endopeptidase from rheumatoid synovial tissue culture *Biochimica et Biophysica Acta* **258** 566–576
- Murphy G and Reynolds JJ (1985) Progress towards understanding the resorption of connective tissues *Bioessays* **2** 55–56
- Murphy G, Cockett MI, Ward RV and Docherty AJP (1991) Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa, 72 kDa gelatinases, stromelysin-1 and -2 and punctuated metalloproteinase (PUMP) *Biochemical Journal* **277** 277–279
- Nagase H, Ogata Y, Suzuki K, Enghild JJ and Salvesen G (1991) Substrate specificities and activation mechanisms of matrix metalloproteinases *Biochemical Society Transactions* **19** 715–718
- Nagase H, Suzuki K, Morodomi T, Enghild JJ and Salvesen G (1992) Activation mechanisms of the precursors of matrix metalloproteinases 1, 2 and 3 *Matrix (Supplement 1)* 237–244
- Okada Y, Harris ED and Nagase H (1988) The precursor of a metalloendopeptidase from human rheumatoid synovial fibroblasts: purification and mechanisms of activation by endopeptidase and 4-aminophenylmercuric acetate *Biochemical Journal* **254** 731–741
- Okada Y, Takeuchi N, Tomita K, Nakanishi I and Nagase H (1989) Immunolocalization of matrix metalloproteinase 3 (stromelysin) in rheumatoid synovial cells (B cells): correlation with rheumatoid arthritis *Annals of Rheumatic Diseases* **48** 645–653

- Okada Y, Gonoji Y, Nakanishi I, Nagase H and Hayakawa T (1990a) Immunohistochemical demonstration of collagenase and tissue inhibitor of metalloproteinases (TIMP) in synovial lining cells of rheumatoid synovium *Virchow's Archives. B. Cell Pathology* **59** 305–312
- Okada Y, Morodomi T, Enghild JJ, Suzuki K, Yasui A, Nakanishi I, Salvesen G and Nagase H (1990b) Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts: purification and activation of the precursor and enzymic properties *European Journal of Biochemistry* **194** 721–730
- Overall CM, Wrana JL and Sodek J (1989) Independent regulation of collagenase, 72 kDa progelatinase and metalloproteinase inhibitor expression in human fibroblasts by transforming growth factor- β *Journal of Biological Chemistry* **264** 1860–1889
- Renfree MB (1982) Implantation and placentation. In *Reproduction in Mammals* (2nd Edn), pp 26–69 Eds CR Austin and RV Short. Cambridge University Press, Cambridge
- Salamonsen LA, O WS, Doughton BW and Findlay JK (1985) The effects of estrogen and progesterone in vivo on protein synthesis and secretion by cultured epithelial cells from sheep endometrium *Endocrinology* **117** 2148–2159
- Salamonsen LA, Nagase H and Woolley DE (1991a) Production of matrix metalloproteinase-3 (stromelysin) by cultured ovine endometrial cells *Journal of Cell Science* **100** 381–385
- Salamonsen LA, Hampton AL, Clements JA and Findlay JK (1991b) Oestrogen and progesterone regulate gene expression and cellular localisation of prostaglandin synthase in the ovine endometrium *Journal of Reproduction and Fertility* **92** 393–406
- Sato T, Ito A, Mori Y, Yamashita K, Hayakawa T and Nagase H (1991) Hormonal regulation of collagenolysis in uterine cervical fibroblasts: modulation of procollagenase, prostomelysin and tissue inhibitor of metalloproteinases synthesis by progesterone and oestradiol-17 β *Biochemical Journal* **275** 645–650
- Schmid TM, Mayne R, Jeffrey JJ and Linsenmayer TF (1986) Type X collagen contains two cleavage sites for a vertebrate collagenase *Journal of Biological Chemistry* **261** 4184–4189
- Seltzer JL, Eisen AZ, Bauer EA, Morris NP, Glanville RW and Burgeson RE (1989) Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin *Journal of Biological Chemistry* **264** 3822–3826
- Stetler-Stevenson WG, Kruttsch HC, Wachter MP, Margulies IMK and Liotta LA (1989) The activation of human type IV collagenase proenzyme: sequence identification of the major conversion product following organomercurial activation *Journal of Biological Chemistry* **264** 1353–1356
- Sunada H and Nagai Y (1983) Mechanism of collagen degradation by collagenase: a transition process of the collagen molecule from collagenase-substrate to gelatinase-substrate *Biomedical Research* **4** 61–70
- Suzuki K, Enghild JJ, Morodomi T, Salvesen G and Nagase H (1990) Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin) *Biochemistry* **29** 10 261–10 270
- Welgus HG, Jeffrey JJ and Eisen AZ (1981) The collagen substrate specificity of human skin fibroblast collagenase *Journal of Biological Chemistry* **256** 9511–9515
- Wooding FB (1984) Role of binucleate cells in feto-maternal cell fusion at implantation in the sheep *American Journal of Anatomy* **170** 233–250
- Wooding FB and Morgan G (1989) Fetomaternal cell fusion at ruminant implantation. In *Blastocyst Implantation*, pp 117–123 Ed. K Yoshinaga. Adams Publishing Group, Boston.