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Expression of nuclear factor κ B in human endometrium; role in the control of interleukin 6 and leukaemia inhibitory factor production

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Expression of the rel-A component of nuclear factor κ B (NFκB) by human endometrial cells was investigated by immunocytochemical analysis of cryostat sections cut from endometrial biopsy material and of cultured endometrial epithelial cells. In-vivo expression of rel-A was low in epithelial cells in endometrium obtained during the proliferative phase of the cycle, but increased in these cells during the secretory phase and was maximal at the time of implantation. In-vivo expression of rel-A by stromal cells did not vary greatly throughout the cycle, but showed a slight peak at the time of ovulation. In contrast similar expression of rel-A was seen in short-term cultures of epithelial cells prepared from both proliferative and secretory endometrium. Addition of the NFκB inhibitor SN50 (5 μg/ml) to confluent cultures of endometrial epithelial cells inhibited interleukin (IL)-1α (10 ng/ml) and tumour necrosis factor α (TNFα) (10 ng/ml) stimulated IL-6 (*P* < 0.001 and *P* < 0.01 respectively) and LIF (*P* < 0.01 and *P* < 0.05 respectively) production. The proteasome inhibitor MG132 (0.3 and 3 μmol/l) also caused a dose-dependent decrease in IL-1α and TNFα-stimulated IL-6 (*P* < 0.001 and *P* < 0.001 respectively) and leukaemia inhibitory factor (LIF) (*P* < 0.001 and *P* < 0.001 respectively) production by endometrial epithelial cells. The results support the hypothesis that NFκB mediates signalling between IL-1 and TNFα receptors and the expression of LIF and IL-6 in endometrial epithelial cells.

Key words: cytokines/endometrium/human/NFκB

Introduction

Nuclear factor κ B (NFκB) is a transcription factor which is known to be involved in cellular signalling from interleukin-1 (IL-1) and tumour necrosis factor α (TNFα) receptors in many different cell types (Schutze *et al*., 1995; Wulczyn *et al*., 1996). It consists of a family of proteins (NFκB1, NFκB2, rel-A, rel-b and c-rel) and is present in the cytoplasm of cells where it is attached to an inhibitory protein (IκB) (Baldwin, 1996). Activation is brought about by phosphorylation and ubiquitination of the IκB subunit followed by its degradation by the proteasome, thus allowing NFκB translocation to the nucleus (Wulczyn *et al*., 1996), where it binds to the promoter regions of various genes particularly those of cytokines and adhesion molecules, thus increasing their expression. The signalling pathways between the IL-1 and TNFα receptors and NFκB activation are not entirely understood but may involve activation of acidic sphingomyelinase and the production of ceramide, or the use of TRAF (TNF receptor activating factor) and TRADD (TNFR1-associated death domain) molecules (Baldwin, 1996). It has also been shown that the activated NFκB component can bind to activated steroid receptors including those for oestradiol and progesterone causing mutual inhibition (Van der Burg and Van der Saag, 1996).

The cytokines IL-6 and leukaemia inhibitory factor (LIF) are known to be important in endometrial function and embryo implantation (Smith *et al*., 1998). The importance of LIF in endometrial function was first suggested by experiments with LIF knock-out mice (Stewart *et al*., 1992) in whom implantation cannot occur. Transfer of homozygous LIF-negative blastocysts to pseudopregnant, wild-type mice results in normal implantation and pregnancy outcome, showing that the defect is in the endometrium. Concentrations of LIF in the human endometrium peak at the time of implantation and are decreased in women with unexplained infertility (Laird *et al*., 1997), further suggesting its importance in human reproduction. Endometrial IL-6 concentrations also vary during the menstrual cycle and reach a peak at the time of implantation (Tabibzadeh *et al*., 1995b). Initial studies have shown that IL-6 concentrations are decreased in women who suffer recurrent miscarriage (Lim *et al*., 1997), suggesting that it may also be important in implantation.

Both IL-1 and TNFα have been shown to increase LIF production in endometrial cells (Arici *et al*., 1995) and IL-1 is known to increase IL-6 production in endometrial epithelial cells (Laird *et al*., 1994). Various studies have also suggested that steroids, particularly progesterone may be important in the control of endometrial IL-6 and LIF production (Laird *et al*., 1994,1997; Hambartsoumian *et al*., 1998). The promoter regions of the *LIF* and *IL-6* genes contain NFκB binding sites and expression of these cytokines in other cells has been shown to be controlled by NFκB (Van der Burg and Van der Saag 1996; Bamberger *et al*., 1997). The aim of this study was, therefore, to investigate expression of the rel-A component of NFκB in the cells of the human endometrium. This component of NFκB was chosen as it is a constituent of the

active transcription factor. NFκB inhibitors (SN50 and MG 132) were also used to show that it plays an important role in the signalling pathways between the IL-1 and TNFα receptor and *IL-6* and *LIF* gene expression in human endometrial epithelial cells. SN50 is a peptide, which binds to activated NFκB and prevents its translocation to the nucleus (Lin *et al*., 1995). MG132 inhibits enzymic activity of the proteasome, thus preventing IκB breakdown (Fenterary *et al*., 1995). In addition, as the effect of TNFα on IL-6 production has not been reported, this was investigated first.

Materials and methods

Human subjects

Endometrial biopsy samples were obtained throughout the menstrual cycle from normal fertile women attending the Jessop Hospital for Women, in Sheffield, UK for sterilization or hysterectomy for non-endometrial pathology. All women were aged 20–40 years and had regular cycles of 25–35 days. None of the women had taken any steroid hormones for 2 months prior to the study. Informed consent was obtained from all women participating in the study. Samples were either snap-frozen in liquid nitrogen for immunocytochemistry or taken to the laboratory for cell culture. Menstrual dating of the sample was determined by the date of the last menstrual period.

Cell culture

Human endometrial epithelial cells were prepared and cultured as previously described (Laird *et al*., 1994). The endometrial biopsy samples were collected in Hank's balanced salt solution containing streptomycin and penicillin (100 μg/ml). The tissue was chopped finely with scissors and incubated at 37°C for 45 min in 5 ml of Dulbecco's modified Eagle's medium (DMEM) containing 0.2% collagenase (type 1a) (DMEMC). During the incubation and again at the end of the incubation the tissue was pipetted gently to disperse the cells. The epithelial cells were separated from stromal cells by centrifugation at 100 *g*. The supernatant containing stromal cells was removed and the pellet which contained mainly epithelial cells present as glands was incubated at 37°C for a further 45 min in 5 ml DMEMC. The cells were again dispersed by gentle pipetting and the epithelial cells pelleted by centrifugation at 100 *g*.

The epithelial cells were further purified by unit density sedimentation. The cells were resuspended in 2 ml DMEM containing 10% fetal calf serum (FCS), glutamine (4 mmol/l) and penicillin and streptomycin (100 μg/ml) (CDMEM), and gently pipetted onto 8 ml of CDMEM in a test tube and left for 30 min at room temperature. The top 8 ml was discarded and the cells in the lower 2 ml were used for cell culture. Cells were plated into 96-well plates at ~0.8×10^5 cells per well. The cells were grown in CDMEM medium to confluency (usually 48–72 h) at 37°C in an atmosphere of 5% CO_2 and 95% air. At confluency the medium was changed and replaced with CDMEM containing either no supplements or TNFα (0.1–10 ng/ml). In further experiments cells were incubated with TNFα (10 ng/ml) or IL-1α (10 ng/ml) in the presence and absence of the NFκB inhibitor SN50 (Biomol, Plymouth Meeting, PA, USA) (5 μg/ml) (Lin *et al*., 1995) or the proteasome inhibitor MG132 (Biomol) (3 and 0.3 μmol/l) (Fenterary *et al*., 1995). The cells were grown for a further 24 or 48 h, after which the supernatants were removed and stored at –20°C for analysis of IL-6 and LIF. The purity of the cell cultures at the end of the culture period was verified using immunocytochemistry to show the expression of vimentin (stromal cell marker), cytokeratin (epithelial cell marker) and anti-CD45 (leukocyte marker) as previously described (Laird *et al*., 1994).

Immunocytochemistry for rel-A

Cells for rel-A immunocytochemistry were grown in chamber slides in CDMEM at a similar density as in 96 well plates. After 2 days the media was removed and the cells washed and fixed in 3.7% w/v formaldehyde in phosphate-buffered saline (PBS) for 15 min. Sections (5 μm thick) were cut from frozen endometrial biopsy material and immediately fixed in 3.7% w/v formaldehyde in PBS for 15 min. Both sets of slides were then washed twice for 5 min in PBS and then further fixed in methanol (–20°C) for 4 min and acetone (–20°C) for 2 min. After two further 5 min washes in PBS slides were stored at –20°C in sugar storage solution (50:50 0.5 mol/l sucrose: $MgCl_2$ in PBS/glycerol) until used for immunocytochemistry.

Sections were firstly incubated with rabbit serum (10%) in PBS (PBSNRS) for 30 min at room temperature to block non-specific binding. Antibody against the NFκB protein rel-A (Santa-Cruz Biotechnology Inc, CA, USA) (1:100 dilution in PBSNRS) was then placed on the tissue and incubated overnight at 4°C. Sections were then washed twice in PBS for 5 and 30 min and incubated with rabbit anti-goat immunoglobulin G (IgG; Dako, Ely, Cambridgeshire, UK) (1:200 dilution in PBSNRS) for 30 min at room temperature. After a further two washes for 5 min in PBS the slides were incubated with goat peroxidase anti-peroxidase (PAP; Dako) (1:100 dilution in PBS) for 40 min at room temperature. The slides were washed twice in PBS for 5 and 30 min before addition of the peroxidase substrate, 3,3′-diamidinobenzidine (DAB), for 8 min at room temperature. The slides were then washed in distilled water for 5 min and counterstained with haematoxylin (10%) for 10–15 min. Finally the tissue was dehydrated through a series of alcohols (5 min for 50–90% alcohols and 10 min for 95% and 30 min absolute alcohol), cleared in xylene overnight and mounted in DPX. Negative reagent controls were stained in parallel with the primary rel-A antibody, which had been blocked by incubation overnight at 4°C with a specific blocking peptide (Santa Cruz Biotechnology Inc). MCF7 cells (breast cancer cell line) incubated with and without primary antibody were used as a positive tissue control and were included in all staining runs.

Staining was assessed semi-quantitatively using a scoring system of – for no immunoreactivity, + faint immunoreactivity, ++ strong immunoreactivity, +++ intense immunoreactivity (Critchley *et al*., 1998).

IL-6 and LIF assays

The amounts of LIF and IL-6 in cell culture supernatants were assayed using MEDGENIX IL-6 EASIA and MEDGENIX LIF/HILDA kits obtained from Biosource Europe (Fleurus, Belgium). Assays were carried out exactly as per the manufacturer's instructions. For the LIF assay the detection limit was 20 pg/ml and the inter- and intra-assay coefficients of variation were 5 and 8% respectively. For the IL-6 assay, the detection limit was 2 pg/ml and the inter- and intra-assay coefficients of variation were 5 and 7.5% respectively.

Statistical analysis

The Mann–Whitney (non-parametric) *U*-test or the Students *t*-test as appropriate was used to show differences in amounts of IL-6 and LIF produced by control cells and those treated with IL-1α or TNFα in the presence or absence of SN50 and MG132.

Results

Immunocytochemical staining

Figure 1 shows examples of immunocytochemical staining for rel-A in sections from endometrial biopsy samples obtained in the proliferative, early secretory and late secretory phases

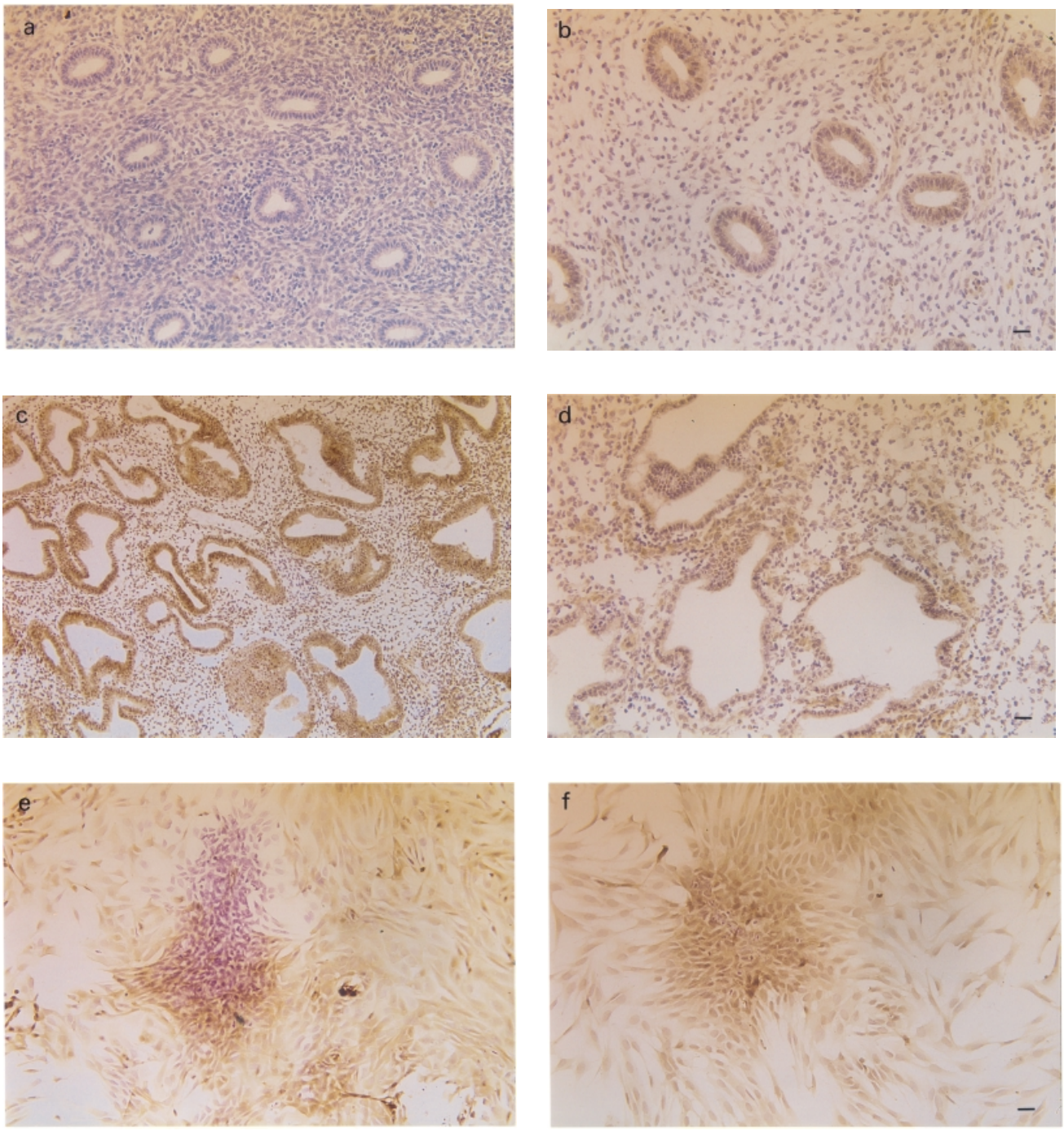

Figure 1. Expression of the nuclear factor κ B (NFκB) protein, rel-A, in human endometrium obtained during the proliferative (**B**), mid-secretory (**C**) and late secretory (**D**) phases of the cycle. (**A**) shows a negative control obtained when the antibody was pre-incubated overnight with a specific blocking peptide (Santa Cruz Biotechnology, California, USA). **E** and **F** show expression of rel-A, by human endometrial epithelial cells prepared from proliferative (**E**) and secretory (**F**) phase endometrium after 2 days in culture. Scale bar = 50 μm.

of the menstrual cycle. Scoring for the intensity of staining seen in sections from 14 biopsies is shown in Table I. Similar staining intensity was seen throughout the cycle in stromal cells with a slight increase in intensity during the late proliferative/early secretory phases. In contrast staining in epithelial cells varied considerably throughout the cycle. Epithelial cell staining was absent or very weak in biopsies obtained during the proliferative phase of the cycle, but staining increased in biopsies obtained during the secretory phase of the cycle and was maximum at the time of implantation, before decreasing towards the end of the cycle. Immunocytochemical staining for rel-A in cultured epithelial cells is shown in Figure 1E and 1F. After 2 days the cultures consisted of small glands surrounded by a monolayer of growing cells. Cells in the glandular fragments of cultures prepared from endometrial tissue obtained during the proliferative phase of the cycle did not stain for rel-A whereas those in glandular fragments of cultures from tissue obtained in the secretory phase stained

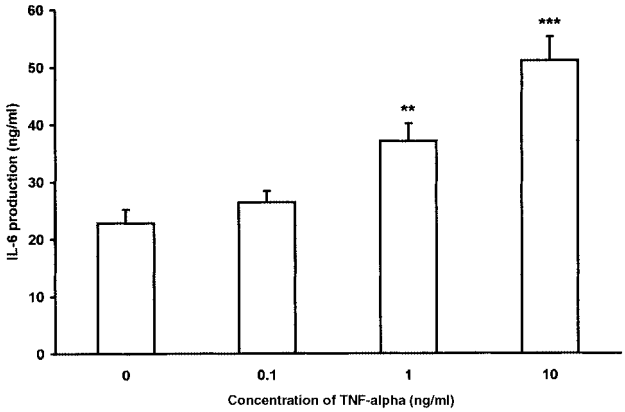

Figure 2. Effect of tumour necrosis factor α (TNFα) on interleukin-6 (IL-6) production by human endometrial epithelial cells. Values are given as mean ± SEM ($n = 4$). The result shown is typical of that obtained in three different experiments. **Significantly different from controls ($P < 0.01$); ***significantly different from controls ($P < 0.001$).

Table I. Staining of rel-A in stromal and epithelial compartments of sections cut from 14 endometrial biopsies obtained from normal fertile women throughout the menstrual cycle

Day	Epithelial staining	Stromal staining
6	–	+
7	–	+
9	–	+/++
10	–	++
12	–/+	++
16	+	+/++
17	++	–/+
18	++	+
20	+++	++
21	++	–
22	++	–
23	+	++
24	+	+/++
26	+/++	+/++

positively for rel-A. In contrast, cells in the monolayer stained positively in cultures prepared from both proliferative and secretory phase endometrium.

Effect of NFκB inhibitors on IL-6 and LIF production

The effect of TNFα on IL-6 production by human endometrial epithelial cells is shown in Figure 2. TNFα caused a dose-dependent increase in IL-6 production. A significant increase was seen at 1 and 10 ng/ml TNFα.

The effect of the NFκB inhibitor SN50 on TNFα-stimulated LIF and IL-6 production by human endometrial epithelial cells is shown in Figure 3. As expected, TNFα stimulated both IL-6 and LIF production. SN50 inhibited TNFα-stimulated IL-6 and LIF production ($P < 0.01$ and $P < 0.05$ respectively). LIF and IL-6 concentrations in cell culture supernatants from cells incubated with TNFα and the inhibitor were similar to those of controls. The effect of SN50 on TNFα-stimulated cytokine production was refractory. A greater effect was seen after 24 h incubation than after 48 h and no effect was seen, if after the initial incubation the cells were incubated with TNFα and inhibitor for a further period of 48 h. Because of this all further incubations were carried out for 24 h. IL-1α also stimulated IL-6 and LIF production and the amounts of LIF and IL-6 produced in the presence of IL-1α were greater than those seen in the presence of TNFα. SN50 inhibited the IL-1α-stimulated increase in IL-6 and LIF production ($P < 0.001$ and $P < 0.01$ respectively). A similar effect of SN50 on IL-1α and TNFα stimulated IL-6 and LIF production was seen in cells prepared from proliferative and secretory endometrium.

Figure 4 shows the effect of the proteasome inhibitor MG132 on basal and TNFα-stimulated IL-6 and LIF production by human endometrial epithelial cells. MG132 caused a dose dependent-decrease in both basal and TNFα-stimulated IL-6 and LIF production. In the presence of MG132 no stimulatory effect of TNFα on IL-6 production was seen. MG132 also caused a similar dose-dependent decrease in the IL-1-stimulated IL-6 and LIF production by these cells. In the presence of MG132 no stimulatory effect of IL-1 on IL-6 and LIF production was seen. A similar effect of MG132 on IL-1 and TNFα-stimulated IL-6 and LIF production was seen in cells prepared from proliferative and secretory endometrium.

Discussion

Nuclear factor κ B (NFκB) is a transcription factor involved in signalling from IL-1 and TNFα receptors in a number of different cell types (Schutze *et al*., 1995; Wulczyn *et al*., 1996). It is also known to be important in the control of *IL-6* gene expression in various cells (Ray *et al*., 1994) and the promoter regions of both the *IL-6* and *LIF* genes contain recognized NFκB binding sites (Stein and Yang 1995; Bamberger *et al*., 1997). The aim of this study was to investigate the expression of NFκB in the human endometrium and to show that it is involved in signalling between IL-1 and TNFα receptors and IL-6 and LIF production in this tissue by the use of NFκB inhibitors. Although it has previously been shown that IL-1 and TNFα stimulate LIF and that IL-1 stimulates IL-6 production by endometrial epithelial cells (Laird *et al*., 1994; Arici *et al*., 1995), there are no previous reports showing the stimulation of IL-6 by TNFα by these cells. The results in this study clearly show that TNFα is capable of stimulating IL-6 production by human endometrial epithelial cells.

Immunocytochemical analysis of rel-A showed the expression of this component of NFκB in the human endometrium. Expression of NFκB by the mouse endometrium has previously been reported (Shyamala and Guiot, 1992), but this is the first report of the expression of any of the NFκB components in the human endometrium. Rel-A expression was seen in both endometrial and stromal cells, and increased expression in epithelial cells was seen during the mid-secretory phase of the cycle at the time of implantation. Rel-A was also expressed by cultured endometrial epithelial cells. In the monolayer of cells growing out from the glandular endometrium equal expression was seen in cells prepared from both secretory and proliferative endometrium. This difference in patterns of expression *in vivo* and *in vitro* is not understood, but may be related to differences in the rate of growth of cells. These

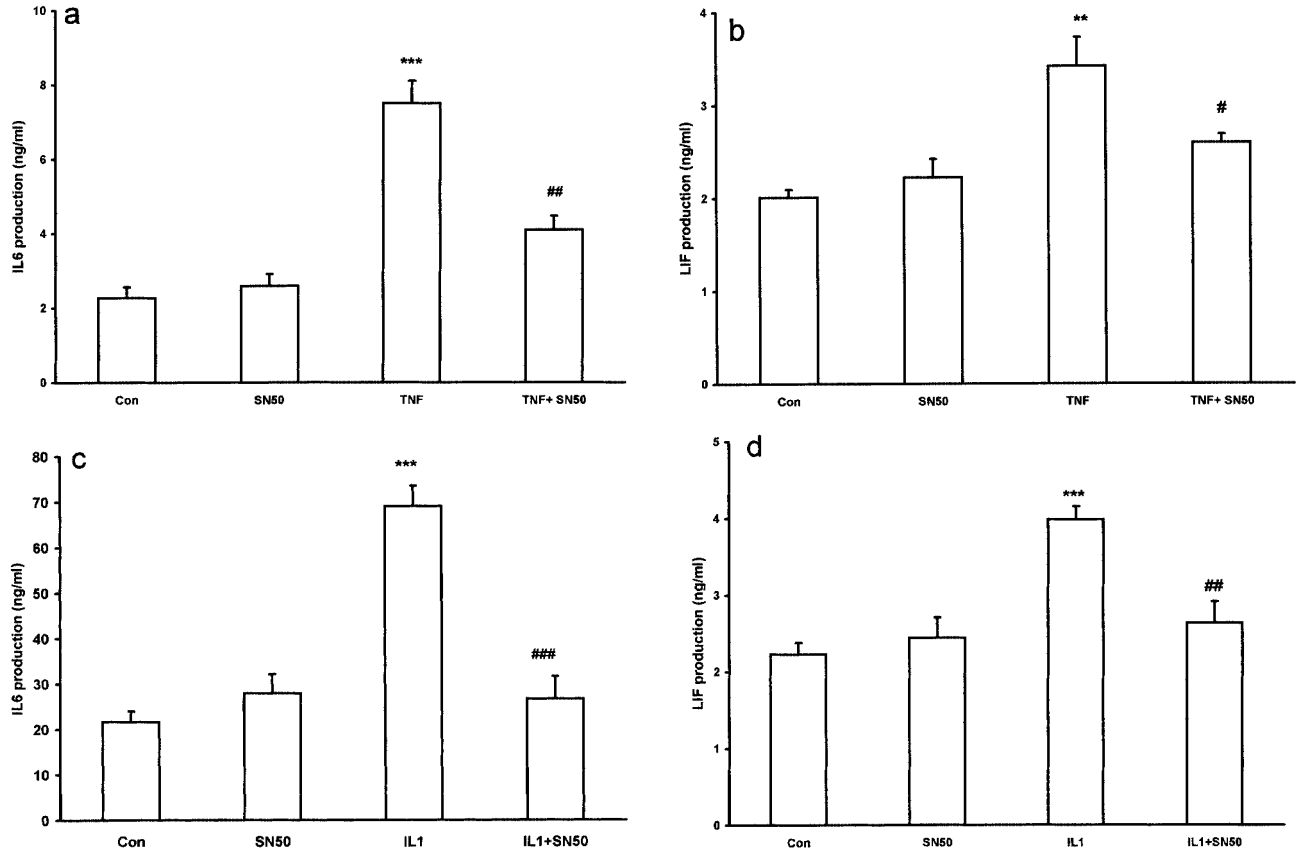

Figure 3. Effect of the nuclear factor κ B (NFκB) inhibitor SN50 (5 μg/ml), on the production of interleukin (IL-6) and leukaemia inhibitory factor (LIF) by human endometrial epithelial cells stimulated with (**a**, **b**) tumour necrosis factor α (TNFα) (10 ng/ml) and (**c**, **d**) IL-1 (10 ng/ml). Values are given as mean ± SEM ($n = 4$). Results shown are typical of those obtained in three different experiments, except for (**c**) which was repeated four times. In two experiments SN50 alone increased IL-6 production, while in the other two no effect was seen, as was the case in (**a**). **Significantly different from controls ($P < 0.01$); ***significantly different to controls ($P < 0.001$). # Significantly different from TNFα-stimulated cytokine production ($P < 0.05$). ## Significantly different from IL-1 or TNFα-stimulated cytokine production ($P < 0.01$). ### Significantly different to IL-1-stimulated cytokine production at ($P < 0.001$).

differences also suggest that care should be taken in extrapolation of the cell culture results to the in-vivo situation. However, the fact that expression of rel-A in culture is similar to that seen *in vivo* at the time of implantation suggests that effects seen *in vitro* may reflect changes which would be possible in the endometrium at this important time. Rel-A is only one component of the NFκB family. The activated form consists of homodimers of rel-A or heterodimers made up of rel-A and the p50 component. These homo and heterodimers are held in the cytoplasm in the inactive form bound by IκB of which there are a number of forms (Wulczyn *et al.*, 1996). The expression of these other components of the NFκB family of proteins by the human endometrium has not yet been investigated, but would be important in understanding the mechanism of NFκB activation in this tissue.

The NFκB inhibitor SN50 inhibited both IL-1α and TNFα stimulated LIF and IL-6 production by endometrial epithelial cells in culture. Experiments with inhibitors were only carried out on cultured epithelial cells as it has previously been shown that cultured stromal cells do not produce significant amounts of LIF and IL-6 (Laird *et al.*, 1994, 1997; Chen *et al.*, 1995). In this study SN50 had effects on human endometrial epithelial cells at a concentration similar to that seen in other cells (Lin *et al.*, 1995). The inhibitory effect of SN50 was transient; a greater effect was seen after 24 h incubation than at 48 h and no effect was seen if, after changing the media, the cells were further incubated in the presence of cytokine and inhibitor for a further 48 h. Studies in other cells have shown that after activation and translocation to the nucleus NFκB dimers are recycled back to the cytoplasm bound to IκB and are thus inhibited (Arenzana-Seisdedou *et al.*, 1997). The refractory nature of the inhibitory effect seen in this study may be related to this process. Overall SN50 had no effect on basal IL-6 and LIF production, suggesting that transcription factors other than NFκB are involved in the constitutive production of these cytokines.

The proteasome inhibitor MG132 also inhibited IL-1 and TNFα-stimulated IL-6 and LIF production at similar concentrations as has been found to be effective in other tissues (Fenterary *et al.*, 1995). The role of the proteasome is to breakdown proteins targeted by ubiquitination. NFκB activation involves the phosphorylation of IκB followed by ubiquitination and subsequent degradation by the proteasome (Baldwin *et al.*, 1996). The inhibitory effect of both MG132 and SN50 on IL-1 and TNFα-stimulated IL-6 and LIF production strongly suggests an active role for NFκB in the signalling pathways between the IL-1 and TNFα receptors and IL-6 and LIF production in human endometrial cells. The effect of these

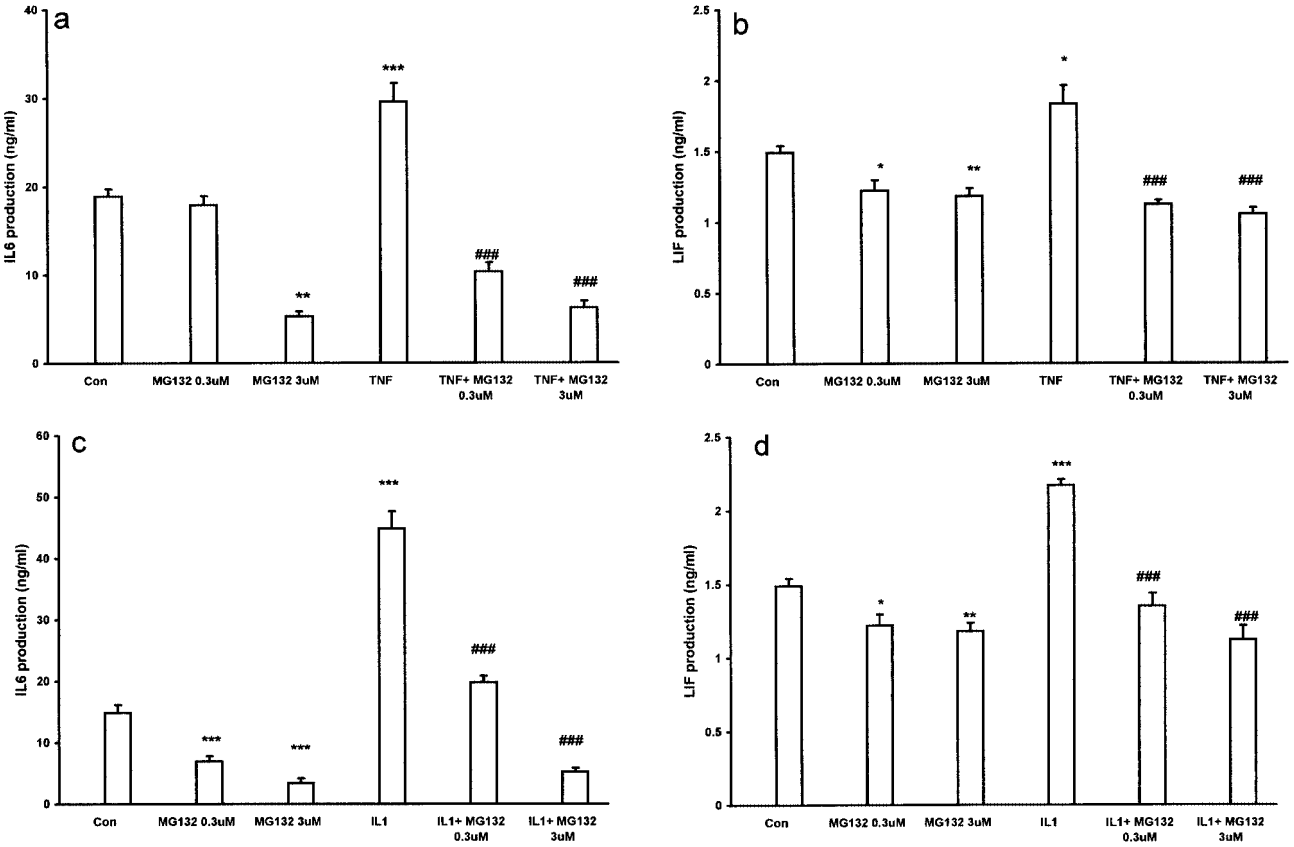

Figure 4. Effect of the proteasome inhibitor MG132 on interleukin (IL-6) and leukaemia inhibitory factor (LIF) production by human endometrial cells stimulated with (**a**, **b**) tumour necrosis factor α (TNFα) (10 ng/ml) and (**c**, **d**) IL-1 (10 ng/ml). Values are given as mean ± SEM ($n = 4$). The results shown are typical of that obtained in three different experiments. *Significantly different from controls ($P < 0.05$); **significantly different from controls ($P < 0.01$); ***significantly different from controls ($P < 0.001$). ### Significantly different from IL-1 or TNFα-stimulated cytokine production at ($P < 0.001$).

inhibitors on cell growth was not investigated in this study. However, the fact that the effects seen were transient and optimal after 24 h incubation suggest that inhibition of IL-6 and LIF secretion is not due to an effect on cell growth.

The results from these experiments suggest that rel-A may be important in increasing LIF and IL-6 production by human endometrial epithelial cells. Therefore, it follows that the increased expression of rel-A in these cells during the secretory phase of the cycle *in vivo* should be associated with increased LIF and IL-6 production. Previous studies have suggested that in-vivo endometrial production of IL-6 and LIF is greatest in the secretory phase of the cycle and particularly at the time of implantation (Tabibzadeh *et al*., 1995b; Laird *et al*., 1997; Smith *et al*., 1998). Production of IL-6 and LIF by cultured epithelial cells prepared from tissue obtained at different times in the cycle shows a slightly different pattern, with maximal amounts produced during the late proliferative and early secretory phases of the cycle (Laird *et al*., 1994, 1997; Chen *et al*., 1995). In this study, expression of rel-A in epithelial cells prepared from proliferative endometrium, as well as from secretory endometrium was shown. Therefore a possible explanation for the increased production of LIF and IL-6 by cells prepared from proliferative endometrium is the increased expression of rel-A which is seen in these cells.

There are two types of IL-1 receptor (IL-1 RtI and IL-1 RtII) but signalling occurs mainly through the IL-1 RtI receptor. *IL-1 RtI* mRNA is found in the human endometrium throughout the menstrual cycle but amounts are increased during the secretory phase at the time of implantation (Simon *et al*., 1993). Expression of IL-1 by the human endometrium has also been reported and again amounts are thought to increase during the secretory phase of the cycle (Kauma *et al*., 1990; Tabibzadeh and Sun, 1992). It has also recently been suggested that the production of IL-1 by the embryo may be important in preparing the endometrium for implantation (Simón *et al*., 1998). There are also two forms of the TNFα receptor (TNFr-I and TNFr-II) and NFκB is thought to be more important in signalling from TNFr-II. Both forms of TNF receptor are expressed by endometrial epithelial cells throughout the cycle (Tabibzadeh *et al*., 1995a), while the expression of TNFα appears to show two peaks of expression in the late proliferative and late secretory phases of the cycle (Hunt *et al*., 1992; Tabibzadeh *et al*., 1995a; Laird *et al*., 1996). Thus both the IL-1 and TNFα cytokine ligand and receptor are present in the endometrium at the time of implantation. The maximum expression of rel-A also at the time of implantation shown in these studies may be a way of maximizing the response of IL-6 and LIF to IL-1 and TNFα at this time. Concentrations of LIF and IL-6 are decreased in women with reproductive failure (Laird *et al*., 1997; Lim *et al*., 1997) and blastocyst implantation does not occur in *LIF* gene-deficient mice (Stewart *et al*., 1992) suggesting the importance of these cytokines in the

implantation process. Therefore, any means of increasing their production will be important in enhancing the chances of successful pregnancy.

In this study no attempt was made to analyse the cellular location of the rel-A component. Incubation with IL-1 or TNFα would be expected to result in rel-A translocation from the cytoplasm to the nucleus of the cell. These types of experiment would provide further evidence for a role of NFκB in signalling pathways from the IL-1 and TNFα receptors and are at present being carried out in our laboratory. In addition, NFκB activity in human endometrial tissue needs to be examined by electrophoretic mobility studies to show any differences in biological activities at different stages in the cycle.

In summary these results show the expression of rel-A in human endometrium for the first time and suggest that epithelial cell expression of rel-A is cyclical and is maximal at the time of implantation. The inhibition of IL-1 and TNFα-stimulated IL-6 and LIF production by the NFκB inhibitors SN50 and MG132 suggests that NFκB components play an active role in the signalling pathways between the receptors for IL-1 and TNFα and the production of IL-6 and LIF in this tissue.

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