Identification of genes regulated by interleukin-1 β in human endometrial stromal cells

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Abstract

Interleukin-1 β (IL-1 β) is an important immune regulatory factor that in human endometrium plays a role in both menstruation and implantation in the event of pregnancy. It promotes inflammatory-like processes and also stimulates tissue remodelling. We present a cDNA microarray study documenting the major effects of IL-1 β on gene expression in stromal cells from human endometrium. Endometrial stromal cells from five normal healthy women at the mid secretory phase were cultured with or without IL-1 β at 50 and 500 pg/ml for 48 h. cDNA microarrays were used to compare the levels of gene expression in total RNA isolated from cells stimulated with IL-1 β . These cDNA arrays were produced containing 15 164 sequence-verified clones, which included genes known to be important in angiogenesis, immune modulators, apoptosis, cell signalling, extra-cellular matrix (ECM) remodelling and cell cycle regulation. Genes which were regulated by IL-1 β were identified by analysis of the microarray data using the Significance Analysis of Microarrays software package. Upregulated ($n = 23$) and downregulated ($n = 6$) different genes were observed, which changed at least 3-fold, at a false discovery rate of less than 2% ($P < 0.02$). Our results have identified genes regulated by IL-1 β , which are involved in leukocyte recruitment, ECM remodelling and other cellular functions. Changes in three genes, IL-8, colony-stimulating factor 2 and aldo-keto reductase family 1 member 1, which were upregulated by IL-1 β , were verified using real-time PCR. Novel functions regulated by IL-1 β in endometrium, including genes involved in free radical protection, and fatty acid metabolism were also identified. These results also provide new insights into the role of IL-1 β in disorders of the endometrium, especially in implantation-related infertility and endometriosis, in which this cytokine plays a major role.

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Introduction

During each menstrual cycle, the human endometrium undergoes to a complex process of growth and remodelling, in preparation for embryo implantation. Although this cycle is under the control of ovarian steroid hormones, many of their actions are mediated by local factors such as cytokines and growth factors. These regulate functions such as endometrial growth, menstruation, infection prevention, implantation and decidualisation (Arici *et al.* 1993, Sharkey 1998).

Among these factors, interleukin-1 β (IL-1 β) is believed to play a role in tissue remodelling and in inflammatory-like response that occur in the human endometrium at menstruation. It is also implicated in critical reproductive functions such as embryo attachment and decidualisation

(Frank *et al.* 1995, Simon *et al.* 1995, 1997). IL-1 β is a 30 kDa polypeptide produced mainly by blood monocytes as a mediator of the inflammatory response, but it also controls cell proliferation, differentiation and apoptosis (Dinarello 1996). In the human endometrium, IL-1 β immunoreactivity has been detected in the stroma and epithelium as well as on macrophages and endothelial cells, with maximal mRNA expression in the mid to late secretory phase (Kauma *et al.* 1990, Tabibzadeh & Sun 1992). Other members of the IL-1 family include IL-1 α and the antagonist IL-1ra, both of which are expressed in endometrium (Tabibzadeh & Sun 1992, Simon *et al.* 1995). These bind to two receptors: the IL-1 receptor type I (IL-1R1), which is required for signal transduction, and the decoy receptor IL-1R type II. IL-1R1 expression has

been reported on epithelial and stromal cells, with maximal expression in the mid and late secretory phase (Simon *et al.* 1994, Bigonnesse *et al.* 2001, Boucher *et al.* 2001). Previous reports have demonstrated that IL-1 β stimulates changes in cytokines, growth factors and adhesion molecules in both epithelial and stromal cells of the human endometrium (Simon *et al.* 1994, Nasu *et al.* 2001). Moreover, IL-1 β secreted by the human embryo may act as signal to the endometrium (De los Santos *et al.* 1996, Krussel *et al.* 2003). Indeed, high concentrations of IL-1 β in the culture media of human embryos have been correlated with successful implantation after *in vitro* fertilisation (Sheth *et al.* 1991, Baraño *et al.* 1992, Spandorfer *et al.* 2000). A critical role in early implantation is also suggested by the fact that IL-1ra blocks the response of the endometrium to human chorionic gonadotrophin (hCG) from the pre-implantation in the baboon (Strakova *et al.* 2005). Perhaps the best-documented effect of IL-1 β is on the process of decidualisation, where it has been shown to inhibit stromal cell decidualisation (Frank *et al.* 1995). Finally, this cytokine may be involved in several other pathophysiological functions, such as endometriosis where stromal cells from ectopic endometriotic lesions have been shown to have altered responses to IL-1 β compared with normal eutopic stromal cells (Lebovic *et al.* 2002). It has been argued that the effect of elevated levels of IL-1 β in the peritoneum on refluxed endometrium may contribute to the vascularisation and establishment of ectopic endometriotic lesions.

The present study aimed to determine the effect of IL-1 β on human endometrial stromal cells (HESCs) collected during the secretory phase using a microarray-based approach.

Materials and Methods

Reagents

Human recombinant IL-1 β was purchased from Amersham International. Culture medium consisted of Ham's F-10 (Euroclone, Wetherby, West Yorks, UK) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, 2.5 μ g/ml Fungizone and 10% fetal calf serum (all from Euroclone). Collagenase A was purchased from Roche Molecular Biochemicals (Milan, Italy).

Cells preparation and cultures

Human endometrium was obtained at laparoscopy from five normally cycling fertile healthy volunteers (28–37 years of age). Permission of the Human Investigation Committee of the University of Siena was granted for the experiments, and patients gave their informed consent.

Subjects were free of uterine diseases and did not use any contraceptive steroids for at least 6 months prior to biopsy. All specimens of endometrium were obtained in the mid to late (18–24 day) secretory phase, based on the

date of the last menstrual period, on the ultrasound evaluation and confirmed by histological examination of the samples (Noyes *et al.* 1975). HESCs were separated as described previously (Viganò *et al.* 2002). Briefly, tissue samples were gently minced into small pieces (1–2 mm³) and incubated for 2 h at 37 °C in a shaking water bath in 10 ml Ham's F-10 containing 0.1% collagenase. At the end of the incubation, single stromal cells were separated from large clumps of epithelium by a 10 min period of differential sedimentation at unit gravity. The top 8 ml of medium, containing predominantly stromal cells, were then slowly removed, and the cells were collected by centrifugation, washed twice in culture medium and allowed to adhere selectively to tissue culture dishes for 20 min. Thereafter, any non-attached epithelial cells still present were removed, and a purified stromal preparation was obtained on the surface of the culture dishes. Immunostaining with vimentin was used to verify the purity of the stromal cell cultures, which showed less than 1% epithelial contamination. Cells were counted, and flow cytometric analysis was performed to determine the possible contamination of CD45/CD14-positive cells. Cultures in which there were more than 2% CD45-positive cells were not included in the study. Cells were cultured for 6 days in 60 mm dishes at a density of 5×10^5 /dish to achieve confluence. For the next 48 h the cells were grown in serum-free medium, and then stimulated with IL-1 β at 50 or 500 pg/ml for a further 48 h. In total from five patients we have treated three different sample cultures with or without IL-1 β at 50 pg/ml and three with IL-1 β at 500 pg/ml. All incubations were performed in a humidified atmosphere at 37 °C in 5% CO₂.

RNA extraction from HESCs

Total RNA was separately extracted from HESCs using Trizol Reagent (Invitrogen) and then treated with DNase (Ambion, Inc., Austin, TX, USA) according to the manufacturer's instructions. RNA quality was assessed by loading 200 ng total RNA onto an RNA Labchip (Agilent Technologies, Waldbronn, Germany) and analysed on an A2100 Bioanalyser (Agilent Technologies).

Preparation of cDNA glass array (printing of HMN cDNAs on GAPSII slides)

The microarray was printed on two slides using cDNA clones derived from four main sources: (i) 5856 cDNA clones came from the Mammalian Gene Collection (<http://mgc.nci.nih.gov/>); (ii) 3360 clones of the HuGen set were purchased from the UK Human Genome Mapping Project (HGMP) Resource Centre (MRC Geneservice, Babraham Bioincubator, Babraham, Cambridge, UK); (iii) 4992 clones from a normalised human testis set IMAGE (<http://image.llnl.gov/>) were also obtained from HGMP; and (iv) the Angiogenesis/Apoptosis cDNA set (956 clones) were developed in the Department of Pathology, University of Cambridge specifically for the study of endometrial biology

(Evans *et al.* 2003). Human cDNA inserts were PCR amplified from bacterial lysates using vector-specific primers and the purified PCR products were printed onto GAPSII aminosilane slides (Corning, NY, USA) in 150 mM phosphate pH 8.5/0.01% SDS buffer using a BioRobotics 610 MicroGrid II robot and MicroSpot 2500 quill pins (BioRobotics, Cambridge, UK). Spot sizes varied from approximately 120 to 160 μ m diameter. Slides were fixed after printing by baking on a hot plate at 80 °C for 2 h. They were then blocked by immersion in 1% BSA (Molecular Biology grade B2518; Sigma-Aldrich) + 0.1% SDS in 3 $\times$ SSC for 20 min at 65 °C, and then denatured by immersion in double-distilled H₂O at 95 °C for 2 min. Slides were finally immersed in isopropanol before drying by centrifugation. They were stored in a dry, dark environment at room temperature.

Preparation of fluorescence-labelled targets and cDNA microarray hybridisation

cDNA synthesis and labelling for hybridisation was carried out using the procedure of Petalidis *et al.* (2003) with minor modifications. One microgram of total RNA was used to synthesise double-stranded cDNA (ds-cDNA) with a SMART PCR cDNA synthesis Kit (Clontech, Oxford, UK) according to the manufacturer's instructions. The ds-cDNA from untreated and IL-1 β -treated HESCs was labelled by Cy3-deoxyuridine triphosphate and Cy5-deoxyuridine triphosphate (Amersham) respectively, using the Bioprime DNA labelling kit (Invitrogen) with random hexamers. These paired samples were purified using Autoseq G50 columns (Amersham), pooled with 5 μ g/ml human Cot-1 DNA (Life Technologies) and 1 μ g/ml Poly dA (Amersham) and hybridised to the cDNA microarray at 50 °C for 16 h. The arrays were washed twice in 2 $\times$ SSC, 0.5% SDS and twice in 0.1 $\times$ SSC, 0.1% SDS for 5 min and twice with 0.1 $\times$ SSC, each at room temperature. The fluorescence signal on microarrays was acquired by using a Genepix 4100 microarray scanner (Axon Instruments, Foster City, CA, USA). The scanned images were processed by using GenePix Pro 3.0 software (Axon Instruments).

Array analysis

The raw data were normalised per spot and per chip using GeneSpring 6 software with intensity-dependent (Lowess) normalisation (per cent of the data used for smoothing 10%) and per chip normalised to the 50th percentile. Low hybridisation signals were removed to give an average of 5000 different genes expressed above background. Experimental interpretation was based on dose treatment of IL-1 β : three samples treated with IL-1 β at 50 pg/ml and three with 500 pg/ml. To identify genes whose expression was altered by IL-1 β the normalised and filtered Cy5 and Cy3 values for each array were analysed with SAM version 1.21 (Significance Analysis of Microarrays; Stanford University) (Tusher *et al.* 2001). SAM identifies genes with statistically significant changes in expression by assimilating a set of gene-specific *t*-tests. Each gene is

assigned a score on the basis of its change in gene expression relative to the s.d. of repeated measurement for that gene. Genes with scores greater than a threshold are deemed potentially significant. The percentage of such genes identified by chance is the false discovery rate (FDR). The criteria used to obtain genes that were significantly up- or downregulated were: an FDR less than 2% and a fold change greater than 3 in all three samples.

Real-time PCR verification

In order to verify the results obtained from the cDNA microarray, real-time PCR (Taqman) verification was performed for three genes: IL-8, colony-stimulating factor 2 (CSF2) and aldo-keto reductase family 1 member B1 (AKR1B1). Relative expression levels of each gene in the control and IL-1 β -treated cells were determined using primers obtained from Applied Biosystems (Assays-on-demand) in accordance to the manufacturer's instructions. Standard curves were generated by serial dilution of a standard preparation of total RNA isolated from an untreated HESC sample. Data are expressed in arbitrary units relative to the level of the same gene in this standard RNA. cDNA was produced from each HESC sample by reverse transcription with random hexamers using 5 μ g total RNA with 200 IU Superscript RT (Invitrogen). The expression values obtained were normalised against those from the control ribosomal 18S to account for differing amounts of starting material. Expression levels in the IL-1 β -treated and control cells were compared using the Wilcoxon matched-pairs test. Differences were considered statistically significant at $P < 0.05$.

Results

Microarray analysis

Total RNA was isolated from primary HESCs from five patients and treated *in vitro* with either 50 or 500 pg/ml IL-1 β . RNA from IL-1 β -treated cells was compared by microarray with the corresponding untreated control cells from the same patient. Genes whose expression was altered by either dose of IL-1 β were identified by SAM analysis (Tables 1–3). Only those whose expression was significantly altered as defined by SAM software by more than 3-fold in all patients and with an FDR less than 2% are shown (q value = 0.0195; q value is similar to the well-known P value, but adapted to multiple-testing situations). We identified 13 upregulated genes with IL-1 β 50 pg/ml (Table 1), 23 upregulated genes with IL-1 β 500 pg/ml (Table 2), one downregulated gene with IL-1 β 50 pg/ml and six downregulated with IL-1 β 500 pg/ml (Table 3). We classified the genes into known functional groups based on information retrieved from the National Center for Biotechnology Information/Entrez/Online Mendelian Inheritance in Man database search. In each Table, the genes are ranked in descending order of the average of the fold change. For genes upregulated by IL-1 β , the average

Table 1 IL-1 β 50 pg/ml upregulated genes.

GenBank	Name	Fold change	Signal intensity	Description
<i>Immune modulators/cytokines</i>				
X12510	CXCL1	11.7	25 000	Chemokine (C-X-C motif) ligand 1
M11220	CSF2	9.4	15 000	Granulocyte-macrophage colony-stimulating factor
W47101	IL-1 β	8.1	12 000	Interleukin 1, beta
M26383	IL-8	7.8	9000	Interleukin 8
M36820	CXCL2	3.7	7000	Chemokine (C-X-C motif) ligand 2
K02765	C3	4.5	5000	Complement component 3
AA403048	CCL13	4.5	3000	Chemokine (C-C motif) ligand 13
W39456	CCL7	3.7	4500	Chemokine (C-C motif) ligand 7
R95077	CXCL5	3.6	3300	Chemokine (C-X-C motif) ligand 5
<i>Detoxification and other functions</i>				
R10693	AMPD3	5.2	4700	Adenosine monophosphate deaminase E
R92994	MMP12	4.2	16 000	Matrix metalloproteinase 12
BC007034	MT2A	3.7	14 000	Metallothionein 2A
W56188	AKR1B1	3.3	55 000	Aldo-keto reductase family 1, member B1

Signal intensity is the average signal from all the samples after treatment with IL-1 β .

Table 2 IL-1 β 500 pg/ml upregulated genes.

GenBank	Name	Fold change	Signal intensity	Description
<i>Immune modulators/cytokines</i>				
X12510	CXCL1	31.2	30 000	Chemokine (C-X-C motif) ligand 1
M17706	CSF3	23.3	15 000	Granulocyte colony-stimulating factor
W47101	IL-1 β	13.3	20 000	Interleukin 1, beta
M11220	CSF2	12.3	22 000	Granulocyte-macrophage colony-stimulating factor
M26383	IL-8	11	14 000	Interleukin 8
M28226	CCL2	10.4	18 000	Chemokine (C-C motif) ligand 2
W39456	CCL7	9.4	13 000	Chemokine (C-C motif) ligand 7
K02765	C3	7.8	8000	Complement component 3
M18403	IL6	7.7	8000	Interleukin 6 (interferon, beta 2)
R95077	CXCL5	7.3	5000	Chemokine (C-X-C motif) ligand 5
AA448801	TNFAIP6	5.2	12 000	Tumour necrosis factor, α -induced protein 6
M36820	CXCL2	5	10 000	Chemokine (C-X-C motif) ligand 2
AA403048	CCL13	3.7	3000	Chemokine (C-C motif) ligand 13
AI015062	LCP1	3	4500	Lymphocyte cytosolic protein (L-plastin)
<i>Multiple cellular functions</i>				
W56188	AKR1B1	3.7	56 000	Aldo-keto reductase family 1, member B1
R10693	AMPD3	3.7	7000	Adenosine monophosphate deaminase E
BC007460	ACPP	3.4	10 000	Acid phosphatase, prostate
J03223	PRG1	3.1	2000	Proteoglycan 1, secretory granule
<i>ECM remodelling</i>				
L23808	MMP12	8.5	8000	Matrix metalloproteinase 12
R94935	PAI2	3.4	3000	Plasminogen activator inhibitor 2
M13509	MMP1	3	9000	Matrix metalloproteinase 1
<i>Detoxifications</i>				
BC007034	MT2A	3.2	10 000	Metallothionein 2A
X14322	SOD2	3	4500	Superoxide dismutase 2, mitochondrial

Signal intensity is the average of the samples after treatment with IL-1 β .

signal intensity of each cDNA for all the samples following IL-1 β treatment is shown in Tables 1 and 2. For downregulated genes, the signal intensity listed in Table 3 is the average of the control (untreated) samples. The signal intensity of the hybridisation for each cDNA on the microarray that was found to change is listed because although this is not always clearly related to the amount of the corresponding transcript, it usually gives some idea of the abundance of that transcript.

From the functional classification, it is clear that several groups of genes with related functions were

upregulated. As expected, the largest group were the immune modulators, with many chemokines and cytokines strongly upregulated. Expression of several metalloproteinases and protease inhibitors was also altered, in agreement with known effects of IL-1 β on endometrial remodelling.

Real-time verification

Real-time PCR analysis of specific gene transcripts in HESC mRNA from untreated cells and cells stimulated with IL-1 β

Table 3 IL-1 β down regulated genes.

GenBank	Name	Fold change	Signal intensity	Description
<i>IL-1β 50 pg/ml</i>				
H19520	STMN2	3.3	6000	Stathmin-like 2
<i>IL-1β 500 pg/ml</i>				
X57766	MMP11	4.5	2900	Matrix metalloproteinase 11
H19520	STMN2	4	5300	Stathmin-like 2
T84395	KLK6	3.5	2000	Kallikrein 6 (neurosin, zyme)
BC012885	TEM7R	3.2	2500	Tumour endothelial marker 7-related precursor
BC019385	FABP5	3	9500	Fatty acid binding protein 5
H81304	ANXA9	3	1500	Annexin A9

Signal intensity is the average of the control (untreated) samples.

50 and 500 pg/ml is shown in Figs 1–3. We quantified the expression of three genes: IL-8, CSF-2 and AKR1B1. The real-time analysis showed that IL-8 mRNA increased in all six samples treated with IL-1 β relative to the corresponding control cells from the same patients (Fig. 1, mean change 31-fold, range 2- to 100-fold, $P < 0.03$). Similarly, CSF2 mRNA increased in all six samples treated with IL-1 β (Fig. 2, mean change 21-fold, range 1.8- to 50-fold, $P = 0.03$). AKR1B1 mRNA increased in all six samples treated with IL-1 β (Fig. 3, mean change 3.1-fold, range 1.2- to 5.9-fold, $P = 0.03$). The data are consistent with and validate those obtained through the microarray expression profiling analysis, although the levels of fold change are not the same as in the microarray analysis (see Tables). Note that the hybridisation signal for the AKR1B1 cDNA is higher than for IL-8 or CSF2 in both untreated cells and after IL-1 β

treatment, but its increment is much less than IL-8 and CSF2 after IL-1 β treatment.

Discussion

By using a cDNA microarray the changes in the transcriptome of HESCs in response to the cytokine IL-1 β were determined. Twenty-nine genes which are differentially expressed were identified. These fall into several functional categories, reflecting the multiple overlapping actions of this pleiotropic cytokine. By far the largest group of upregulated genes were immune modulators and cytokines. Several genes in this group have previously been reported to be upregulated by IL-1 β , including IL-8, IL-6, CXCL1 (GRO α), CXCL2 (GRO β), CCL2 (MCP-1),

Figure 1 Real-time RT-PCR for IL-8 on HESCs treated with either 50 (samples 1–3) or 500 pg/ml IL-1 β (samples 4–6), compared with untreated control cells from the same patients. Data from six different paired (control and IL-1 β -treated) samples are shown separately. All values are normalised to ribosomal 18S. The results for each sample are shown relative to the level of IL-8 in a reference endometrial RNA sample, used to produce a standard curve for the assay. IL-1 β treatment increased IL-8 mRNA expression by an average of 31-fold (range 2- to 100-fold, both doses considered together) and this increase was statistically significant ($P < 0.03$).

Figure 2 Real time RT-PCR for CSF2 on HESCs treated with either 50 (samples 1–3) or 500 pg/ml IL-1 β (samples 4–6), compared with untreated control cells from the same patients. Data from six different paired samples are shown separately. All values are normalised to ribosomal 18S. The results for each sample are shown relative to the level of CSF2 in a reference endometrial RNA sample, used to produce a standard curve for the assay. IL-1 β treatment increased CSF2 mRNA expression by an average of 2-fold (range 1.8- to 50-fold, both doses considered together) and this increase was statistically significant ($P = 0.03$).

Figure 3 Real time RT-PCR for AKR1B1 on HESCs treated with either 50 (samples 1–3) or 500 pg/ml IL-1 β (samples 4–6), compared with untreated control cells from the same patient. Data from six different paired samples are shown separately. All values are normalised to ribosomal 18S. The results for each sample are shown relative to the level of AKR1B1 in a reference endometrial RNA sample, used to produce a standard curve for the assay. IL-1 β treatment increased AKR1B1 mRNA expression by an average of 3.1-fold (range 1.2- to 5.9-fold, both doses considered together) and this increase was statistically significant ($P = 0.03$).

CSF2 and CSF3 (Arici *et al.* 1993, Chegini *et al.* 1999, Nasu *et al.* 2001), confirming that the arrays were performed correctly. In addition to chemokines and cytokines previously known to be upregulated by IL-1 β in endometrium, several new immune regulators were up increased in HESCs, including CCL-7 (MCP-3), CCL13 (MCP-4), C3, CXCL5 (ENA-78), TNFAIP6 and IL-1 β itself, indicating autocrine upregulation. There is a clear upregulation of both types of chemokines: the CC motif chemokines, which mainly attract and activate mononuclear cells, and the CXC motif chemokines, including IL-8 and CXCL1, which are responsible for recruitment and activation of human neutrophils. Just prior to the onset of menstruation there is a large influx of neutrophils into the endometrium, and it has been proposed that this may be due to IL-8 upregulation, since IL-8 strongly induces the attachment of neutrophils to endothelial cells (Schleimer & Rutledge 1986, Milne *et al.* 1999). IL-8 expression is normally repressed in endometrium by the action of progesterone, and following progesterone withdrawal IL-8 is upregulated around blood vessels. Since we have shown, also with real-time RT-PCR, that IL-8 is strongly upregulated in stromal cells by the action of IL-1 β in the absence of progesterone, the upregulation of IL-8 in endometrium prior to menstruation may be in part due to the action of IL-1 β once progesterone levels begin to decline.

In addition to its actions in normal endometrium, it has been proposed that inflammatory cytokines such as IL-1 β play an important role in the establishment and maintenance of ectopic endometriotic explants (Lebovic *et al.*

2001). Levels of IL-1 β are elevated in the peritoneal fluid of women with endometriosis and IL-1 β has been shown to upregulate cytokines and growth factors which may contribute to neovascularisation and monocyte chemotaxis in endometriotic explants. Indeed some of the factors which are upregulated by IL-1 β such as IL-8 have direct angiogenic effects on blood vessels (Lebovic *et al.* 2000). Monocytes within endometriotic explants also secrete other angiogenic growth factors, such as vascular endothelial growth factor, which may play a role in establishment of the disease (McLaren *et al.* 1996).

In the event of pregnancy, the process of decidualisation is accompanied by the invasion and differentiation of fetal trophoblast into the maternal decidua. Several of the cytokines and growth factors upregulated by IL-1 β play an important role in this process. CSF2 is the major growth factor for granulocyte and macrophage proliferation, differentiation and survival, but it also stimulates proliferation of non-haemopoietic cell types (Rasko & Gough 1994). Indeed, CSF2 is a growth factor for trophoblast and other placental cells (Giacomini *et al.* 1995), leading to improved placental functioning and fetal survival. IL-1 β stimulates the trophoblast to release hCG through a mechanism involving IL-6 (Masuhiro *et al.* 1991). In pregnancy therefore, growth factors stimulated in HESCs by IL-1 β may control trophoblast differentiation in the decidua. Indeed, IL-1 β levels are reported to be decreased in endometrium of women with recurrent miscarriage, suggesting that a failure to activate the pathways downstream of IL-1 β may contribute to pregnancy failure (von Wolff *et al.* 2000).

Both menstruation and decidualisation involve extensive remodelling of the extracellular matrix (ECM). The matrix metalloproteinase (MMP) gene family is involved in both physiological and pathological ECM remodelling such as occurs in embryonic development, tissue repair and tumour progression (Matrisian 1990). The expression of endometrial MMPs during the menstrual cycle is regulated by steroid hormones and various cytokines, including IL-1 β and tumour necrosis factor- α (TNF- α) (Salamonsen & Wolley 1999). IL-1 α has previously been reported to upregulate MMPs 1, 2, 3, 7 and 9, and IL-1 β upregulates MMP3 via ERK and p38 MAP kinase phosphorylation (Osteen *et al.* 1997, Singer *et al.* 2002, Strakova *et al.* 2003). These effects are antagonised by progesterone, suggesting that ECM remodelling in non-pregnant endometrium is held in check until progesterone levels decline. The upregulation of MMP12 (metalloelastase) and of MMP1 (collagenase), and the downregulation of MMP11 (stromelysin-3) were found. This singular pattern of expression may suggest that IL-1 β induces the degradation of collagen and elastin whereas the effects of stromelysin-3 (MMP11) are arrested. MMP11 has the capacity to degrade the fibronectin and laminin components of the ECM. Since decidualisation is accompanied by the increased deposition of laminin and fibronectin, this process may be assisted by reduced MMP11 activity. The proteins kallikrein 6 and annexin A9,

which are able to degrade and aggregate ECM proteins, were also downregulated and the protease inhibitor plasminogen activator inhibitor type 2 (PAI2) was upregulated. PAI2 is thought to serve as a primary regulator of plasminogen activation in the extravascular compartment. As well as enabling tissue remodelling, many of these proteases can regulate the actions of cytokines and chemokines, by proteolytic cleavage of precursors, or by inactivation of the mature forms. The precise and unique pattern of proteases and their inhibitors, which is induced by IL-1 β , may serve both purposes.

An exciting feature of this study is the identification of several new genes representing novel functions not previously known to be regulated by IL-1 β in endometrium. For example, AKR1B1 is an aldose reductase enzyme that is secreted into the extracellular space where it has multiple functions: it can reduce benzaldehyde, glyceraldehyde, glucose and several other carbonyl-containing compounds. AKR1B1, with superoxide dismutase-2 and metallothioneins, may act to protect cells from unstable reactive radicals and heavy metals implicated in the pathology of a number of organs including endometrium (Kagi 1991, Ishikawa *et al.* 1993, Sies 1993). These toxic compounds and oxygen free radicals may be detrimental to the attachment, implantation and development of the embryo (Orsi & Leese 2001) and AKR1B1 may play a role around the time of implantation to protect the embryo and to create a safe environment for reception of a fertilised ovum. Similarly at menstruation, these proteins may serve to mop up free radicals generated by highly activated leukocytes during tissue necrosis. The second aspect of this poorly understood enzyme is that AKR1B1 has been associated with prostaglandin (PG) F synthase activity. The corresponding bovine enzyme, AKR1B5, can transform PGH2 into PGF2 α in reproductive tissue (Madore *et al.* 2003). This leads us to hypothesise that AKR1B1, together with cyclooxygenases 1 and 2, may be an important enzyme to synthesis PGs in human endometrium. Finally, AKR1B1 has recently been shown to be an obligatory mediator of TNF- α signalling, leading to an increase in the expression of adhesion molecules and increased binding of monocytes to the endothelium (Ramana *et al.* 2004). Thus AKR1B1 may be involved in mechanism by which IL-1 β recruits monocytes to the endometrium.

Among the downregulated genes, STMN2 expression decreased at both doses of IL-1 β . This poorly defined peptide plays a 'functional' role in neuronal differentiation and in modulating membrane interaction with the cytoskeleton during neurite outgrowth in the adult brain. It also plays a 'development' role during embryonic and perinatal periods, by regulating cell proliferation, differentiation and maturation during tissue development (Koppel *et al.* 1990). In addition, we identified a 3-fold decrease in transcripts encoding FABP5, which binds free fatty acids and regulates lipid metabolism and transport. The high expression of FABP5 in the endometrium in the absence

of IL-1 β suggests an important role for it in cholesterol transport in this tissue, perhaps for local steroid hormone biosynthesis or steroid hormone binding.

In conclusion, the data presented herein offer the opportunity to investigate the multiple roles of IL-1 β in stromal cells of the endometrium. IL-1 β is expressed *in vivo* at the decision point when endometrium may undergo menstruation or decidualisation and implantation. Many of the genes regulated by IL-1 β in stromal cells could play a role in both these outcomes. The study identified new genes involved in functions previously known to be regulated by IL-1 β in endometrium such as leukocyte recruitment and activation as well as matrix remodelling (MMPs). However, we also identified novel functions which appear to be regulated by IL-1 β in endometrium, including several genes involved in detoxification/free radical protection, and fatty acid metabolism. The identification of genes regulated directly in the stroma by IL-1 β in this study will permit investigation of how IL-1 β is involved in situations where menstruation or decidualisation and implantation are disturbed. For example in recurrent miscarriage, where IL-1 β is reported to be decreased, we will seek to determine whether the downstream genes regulated by IL-1 β are also altered. This will permit investigation of the role of IL-1 β in very early events of pregnancy.

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