Effect of early luteal phase administration of mifepristone (RU486) on leukaemia inhibitory factor, transforming growth factor β and vascular endothelial growth factor in the implantation stage endometrium of the rhesus monkey

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Abstract

A single, low-dose administration of a potent anti-progesterone such as mifepristone (RU486) in the early luteal phase results in inhibition of blastocyst implantation in primates. The aim of the present study was to examine the status of leukaemia inhibitory factor (LIF), transforming growth factor β (TGFβ) and vascular endothelial growth factor (VEGF) in day 6 gestational endometrium of rhesus monkeys with or without exposure to a single dose (2 mg/kg body weight, s.c.) of mifepristone on day 2 after ovulation. Densitometric analyses of immunoblots of endometrial spent media revealed an increase (P<0·01) in TGFβ pan (TGFβ1, 2, 3 and 5) and a decrease (P<0·01) in VEGF secretion from RU486-exposed endometrial samples compared with control samples. Secretory profiles for LIF, TGFβ1 and TGFβ1 LAP (latency associated peptide) remained unchanged in the two treatment groups. Morphometric analyses of immunohistochemical staining showed altered cell-specific distribution. TGFβ1 (P<0·01) and TGFβ pan (P<0·02) were higher, while VEGF declined (P<0·05) in endometrial glands of RU486-exposed endometria compared with control tissue samples. Stromal cell staining patterns for all experimental cytokines studied remained unchanged. In blood vessels, VEGF was found to be low (P<0·05), while LIF (P<0·05) and TGFβ1 (P<0·01) were higher in mifepristone-exposed endometrial samples compared with control tissue samples. Increased TGFβ secretion together with elevated levels of TGFβ in glandular epithelia and in blood vessels with no apparent change in stromal levels of TGFβ or in levels of TGFβ LAP in any endometrial compartment in the two treatment groups suggest an altered paracrine involvement of this cytokine and an enhanced activation of latent TGFβ in endometrium following mifepristone treatment. Higher levels of TGFβ in gland cells may result in dysregulated growth control and degenerative morphology. Also, higher levels of LIF and TGFβ together with lower levels of VEGF in the vascular compartment in mifepristone-exposed endometrium suggest that endometrial vascular physiology is a target of this anti-progestin during the peri-implantation stage. It is thus plausible that LIF, TGFβ and VEGF in the glandular and vascular compartments of implantation stage endometrium play important roles in rendering the endometrium receptive, and that early luteal phase treatment with an anti-progestin such as mifepristone affects the involvement of these cytokines resulting in endometrial contraception.

Introduction

Mifepristone (RU486) is a potent anti-progesterone. A single dose application of this anti-progestin agent on day 2 after ovulation can inhibit blastocyst implantation, with no notable changes in menstrual cyclicity in women and in monkeys (Gemzell-Danielsson et al. 1993, Ghosh & Sengupta 1993). It has been shown that luteal phase administration of mifepristone to women and to monkeys induced desynchronization of endometrium, repressed glandular secretory differentiation and vascular maturation (Li et al. 1988, Johannisson et al. 1989, Gemzell-Danielsson et al. 1994, Ghosh et al. 1996). We have reported previously that administration of mifepristone on day 2 after ovulation results in differential profiles of secretory proteins (Ghosh et al. 1997a). Thus, progesterone antagonism with mifepristone treatment during the early luteal phase induces changes which render the endometrium non-receptive and the luminal milieu inhospitable for blastocyst implantation (Ghosh et al. 1997b). Besides progesterone, a significant degree of association exists between endometrial maturation and the levels of several cytokines in endometrial cells in relation to blastocyst implantation (see Tabibzadeh & Babaknia 1995).
Leukaemia inhibitory factor (LIF) is a glycoprotein with a remarkable range of biological actions in various tissue systems (Kurzrock et al. 1991, Hilton 1992). LIF is expressed in mouse uterine endometrial glands, especially on the fourth day of pregnancy when implantation occurs; such well-timed expression of LIF in the endometrium in this species is crucial for successful blastocyst implantation (Stewart et al. 1992). LIF expression is also high around the time of implantation in the human (Charnock-Jones et al. 1994, Arici et al. 1995).

The β type transforming growth factors (TGFβ) are a family of structurally homologous dimeric proteins and they play important roles in the regulation of cell surface molecules, cell proliferation, tissue remodelling, extracellular matrix formation and immunomodulation (Ellingsworth 1990, Clemens 1991). Each of the five forms of TGFβ is initially synthesized as part of a larger precursor molecule with the mature form of TGFβ at the C-terminus. Following proteolytic cleavage, the two portions of the precursor remain together and are secreted as a biologically inactive, noncovalently-bound complex (latent TGFβ) consisting of dimers of both the precursor remainder and mature TGFβ. The precursor remainder is designated the latency associated peptide (LAP). Through a complex and largely unclear mechanism activation of latent TGFβ occurs (Miyazono et al. 1993). Expression of TGFβ has been shown to be high in the preimplantation stage endometrium of mouse (Das et al. 1992) and it has been implicated in embryo development (Paria et al. 1992). TGFβ isoforms display a differential expression in ovine endometrium during the peri-implantation period (Dore et al. 1996). TGFβ1 expression by human endometrial stromal cells is increased by oestradiol and progesterone (Arici et al. 1996) and this cytokine has been shown to be present in maternal endometrium in first-trimester samples (Kauma et al. 1990).

Vascular endothelial growth factor (VEGF) is a dimeric growth factor which is a specific mitogen for endothelial cells and thereby influences angiogenesis (Ferrara et al. 1992). Variant 189 of VEGF also influences vascular permeability (Connolly et al. 1989, Keck et al. 1989). This growth factor has been shown to be expressed in epithelial and stromal cells of human endometrium (Charnock-Jones et al. 1993). Furthermore, it has been suggested that VEGF participates in the increased permeability and angiogenesis occurring in the uterine vascular bed during implantation in the rodent (Chakraborty et al. 1995).

In the present study, our aim was to examine the effect of early luteal phase administration of mifepristone, at a dose which inhibits blastocyst implantation, on endometrial levels and secretion of LIF, TGFβ and VEGF during implantation using the rhesus monkey as a primate model. To this effect, implantation stage endometrial samples were collected on day 6 after ovulation from proven fecund cycles with or without mifepristone treatment on day 2 after ovulation and were semi-quantitatively analysed for the above cytokines using immunohistochemistry and Western blot methods.

Materials and Methods

Animals and treatment

Healthy, mature and proven fertile male and female rhesus monkeys (Macaca mulatta) kept in a semi-natural condition at the Primate Research Facility of the All India Institute of Medical Sciences (AIIMS, New Delhi, India) were used in this study. The details of animal selection, housing and management have been described elsewhere (Ghosh & Sengupta 1992, Ghosh et al. 1996). The present study was approved by the Ethics Committee for Use of Non-Human Primates in Biomedical Research, AIIMS. A vaginal swab from each female monkey was assessed daily. Females (n=19) showing ovulatory menstrual cycles of normal length (26–32 days) were allowed to cohabit with males during days 8–16 of their menstrual cycles. Vaginal smears were checked daily for the presence of spermatozoa. Female monkeys were randomly assigned to two treatment groups: control (group 1, n=8) and mifepristone treatment (group 2, n=11). Female animals from group 1 were injected s.c. with 2 ml vehicle (1:4, benzyl benzoate:olive oil, v/v) on day 2 after their presumed day of ovulation in successfully mated cycles. Animals assigned to group 2 were injected s.c. with a single dose of RU486 (2 mg/kg body weight) in the same volume of vehicle on day 2 after ovulation in mated cycles. We have shown previously that this treatment schedule is one hundred per cent efficacious in preventing implantation in monkeys (Ghosh et al. 1993). The days of ovulation in mated cycles were assessed from the daily profiles of oestradiol-17β (E2) and progesterone (P4) in peripheral serum samples. Steroid radioimmunoassays were performed according to the method of Sufi et al. (1988) using antisera and chemicals obtained from the WHO Matched Reagents Programme. The methodological details are described elsewhere (Ghosh & Sengupta 1989a,b).

Antibodies

The source, specification and dilution of different antisera used for Western blotting (WB) and immunohistochemistry (IHC) are given in Table 1. All antibodies (IgG) were polyclonal except that for TGFβ1, and were purchased from R&D System (Minneapolis, MN, USA). Sensitivities of antibodies were assessed from direct ELISA as suggested by the supplier. Dilutions of stocks of antibodies for WB and IHC as shown in Table 1, were optimized based on 3–5 points titrations. According to the supplier, antibodies for LIF and VEGF have no cross reactivity with other cytokines, that for TGFβ1 has less than 1% cross reactivity with TGFβ2–5, TGFβ pan (TGFβ1–3, 5) has cross
reactivity with TGFβ1, 2, 3 and 5, while TGFβ1 LAP cross reacts only with TGFβ1 LAP and latent TGFβ1, but not with mature TGFβ1. Specificity of antibody liganding and visualization in WB and IHC were assessed by (i) omitting primary antibodies, (ii) replacing primary antibodies with unrelated immunoglobulins from the same species and from other species, and (iii) omitting secondary antibodies, (iv) replacing labelled secondary antibodies with unrelated labelled immunoglobulins from the same species and from other species, and (v) preincubating the antibodies with recombinant human antigens (R&D System). Labelled and unlabelled immunoglobulins, non-immune sera and visualization kits (horseradish peroxidase) were purchased from Vector Laboratories (Burlingame, CA, USA).

Tissue collection and processing

The procedural details of embryo retrieval, collection of endometrial samples and their dating have been described elsewhere (Sengupta et al. 1988, 1989, Ghosh et al. 1993, 1996). Briefly, endometrial dating and embryo age were determined from the presumed day of ovulation (D₀) based on serum profiles of E₂ and P₄ (Sengupta et al. 1989, Ghosh & Sengupta 1992, Ghosh et al. 1997a). Accordingly, endometrial samples were collected on day 6 after ovulation by performing laparotomy and fundal hysterotomy following ketamin (12 mg/kg; Vetlar, Parke-Davis, Mumbai, India) anaesthesia. The presence of a fresh corpus luteum was also checked. Tissue samples were collected only from those animals which yielded a pre-implantation stage embryo on flushing of the entire reproductive tract with sterile Earle’s balanced salt solution (EBSS; Gibco-BRL, New York, USA), pH 7-4. Using this criterion, tissue samples were obtained from 6 control animals (group 1) and 7 mifepristone-treated animals (group 2).

Tissue samples were processed for chemical fixation in phosphate-buffered neutral formaldehyde followed by paraffin embedding as described elsewhere (Ghosh et al. 1993, 1996). Paraffin-embedded tissue samples were used for IHC. Furthermore, tissue samples were processed for incubation in phenol red-free Eagle’s minimum essential medium for 16 h at 37 °C in a humidified environment of 5% oxygen and 95% air (Basha et al. 1980). Spent media were used for WB analysis.

Western immunoblotting

Profiles of experimental cytokines in the incubation medium were characterized by the SDS-PAGE/Western immunoblotting method as described by Ausubel et al. (1994) using electrophoresis and trans-blot equipment from Bio-Rad Laboratories (Richmond, CA, USA). The materials and chemicals were purchased from Bio-Rad Laboratories and Sigma Chemical Co. (St Louis, MO, USA). Prestained molecular markers (Amersham International, Amersham, Bucks, UK) were run with each gel as standard. Samples of the same protein content (10 µg) were separated on 15% SDS-PAGE gels, the proteins were transferred to nitrocellulose membranes which were incubated with primary antibodies. Final visualization was achieved by using Vectastain ABC immunoperoxidase kits (Vector Laboratories) and diaminobenzidine hydrochloride (Sigma Chemical Co.). Positive control, negative control and other controls, as given above, were run to examine the specificity of the procedure. The molecular weights of individual bands were determined based on profile analysis with a calibrated molecular weight standard curve, and semi-quantitative densitometric analysis based on profile analysis, and peak integrations of individual and pooled immunopositive bands were performed using Molecular Analyst image analysis software and a Gel Scanner GS 670 (Bio-Rad Laboratories). The results are shown as Mᵢ of individual bands and semi-quantitative measures (optical density (OD).mm) of pooled profiles of bands, because the basis of protein cleavage in electrophoretic resolution was not examined in the present study.

Immunohistochemistry

Immunohistochemical staining for experimental cytokines was performed with formaldehyde-fixed, deparaffinized

Table 1 Characteristics of primary antibodies used

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Specification of antigen</th>
<th>Specification of antisera</th>
<th>Sensitivity* (ng/well)</th>
<th>Dilution**</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIF</td>
<td>rhLIF</td>
<td>Goat IgG</td>
<td>2</td>
<td>5 x 10²</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>rhTGFβ1</td>
<td>Mouse IgG*</td>
<td>10</td>
<td>5 x 10²</td>
</tr>
<tr>
<td>TGFβ1 LAP</td>
<td>rhLAP</td>
<td>Goat IgG</td>
<td>2</td>
<td>10⁻³</td>
</tr>
<tr>
<td>TGFβ pan</td>
<td>rhTGF-βs</td>
<td>Rabbit IgG</td>
<td>—</td>
<td>10⁻¹</td>
</tr>
<tr>
<td>VEGF</td>
<td>rhVEGF</td>
<td>Goat IgG</td>
<td>1</td>
<td>10⁻¹</td>
</tr>
</tbody>
</table>

*Sensitivity was assessed by direct ELISA using WB dilution. **Dilutions of stock (1 mg antibody/ml) were precalibrated based on 3–5 titration points and the information provided by the manufacturer.

*aMonoclonal; rh, recombinant human.
tissue sections using the method described previously (Ghosh et al. 1993, 1996). The source, specification and dilution of different antisera used are given in Table 1. Final visualization was achieved by using Vectastain ABC peroxidase kits (Vector Laboratories), freshly made dianaminobenzidine hydrochloride (Sigma Chemical Co.) and hydrogen peroxide. Positive controls (human osteoblasts for TGFβ, human endometrium for VEGF and LIF) and other controls, as stated above, were run to assess the specificity of immunostaining. The immunohistochemically stained sections were analysed microscopically to estimate morphometrically the areas of immunoprecipitation in different endometrial compartments, namely glandular, stromal and vascular compartments in the functionalis zone using a Leica microscope and a precalibrated computer-assisted video image analysis system (QWIN-Quantimet 500 C+, Leica, Cambridge, UK). In endometrial functionalis, glands of multiple dimensions and different types of blood vessels are seen. Only glands and vessels which were in true cross section were measured using model systems described by Schipper et al. (1989) and Lowe (1984), and collectively termed glandular compartment and vascular compartment respectively. The area of lamina propria minus these two segments was taken as stroma. The details of histometric measurements are given elsewhere (Ghosh et al. 1993, 1996) and the methodological details are given by Baak & Oort (1983), Russ (1990), and Hamilton & Allen (1995). Briefly, glandular (at × 25) and vascular (at × 40) compartments were detected using an interactive planimeter analyser only in cases where discernibility of these structures was distinct, and the immunopositive areas were measured in a particular compartment (segment) by detecting positive profiles in digitized images based on an optimized grey level threshold after shading correction and pixel calibration against the standard provided by the manufacturer.

**Statistical analysis**

Statistical analyses of densitometric and morphometric data were performed using χ²-test and a modified t-test, as applicable (Samuels 1991). Data are shown as means ± s.e.m.

**Results**

Seventy-five per cent of control animals and about sixty-four per cent of mifepristone-treated animals yielded preimplantation embryos. The observed difference in the yield of preimplantation embryos between groups was not statistically significant.

As shown in Fig. 1, all experimental cytokines were detected in 2–3 immunopositive bands, and there was no significant change in the band characteristics between the two treatment groups. Furthermore, the total densitometric scores of pooled band profiles from Western blot analysis failed to detect any change in the in vitro secretory levels of LIF, TGFβ1 and latent TGFβ1 (LAP) between the two treatment groups (Table 2). However, there was a significant (P<0.01) increase in TGFβ pan secretion from endometrial samples from mifepristone-treated animals (group 2) compared with control (group 1) endometrial samples (Table 2). In contrast, there was a significant (P<0.01) decrease in the in vitro VEGF secretion from endometrial samples obtained from group 2 (mifepristone-treated) compared with those from group 1 (Table 2).

Table 3 shows the morphometric analysis of the area of immunopositive precipitate for different experimental cytokines in three endometrial compartments - gland, stroma and blood vessels - in the functionalis zone. Figures 2–4 show the representative microphotographs for LIF, TGFβ1, TGFβ pan and VEGF from both treatment groups. No significant changes were noted for LIF and
latent TGFβ1 (LAP) in glandular epithelial compartments between the two treatment groups (Table 3). As shown in Fig. 2, LIF was observed mainly at the luminal side of the glandular epithelium. Significant increases in the scores of TGFβ1 (P<0·01) and TGFβ pan (P<0·02) were noted in the endometrial glandular compartment of mifepristone-treated animals (group 2) compared with control (group 1) animals (Fig. 3). A high degree of nuclear staining for TGFβ1 was frequently observed in mifepristone-treated endometrium (Fig. 3b). The VEGF score was decreased (P<0·05) in RU486-treated endometrial glandular epithelium (Table 3, Fig. 4).

### Discussion

We have demonstrated previously that administration of a potent anti-progestin, such as RU486, on day 2 after ovulation in a mated cycle inhibits blastocyst implantation in the rhesus monkey (Ghosh & Sengupta 1993) and this was associated with marked retardation in endometrial secretory maturation (Ghosh et al. 1996) and significant decline in the viability of preimplantation embryos (Ghosh et al. 1997a). However, there was no change in serum concentrations of ovarian steroid hormones (Ghosh & Sengupta 1993, Ghosh et al. 1996). It was hypothesized in the present study that an alteration in the levels of cytokines such as LIF, TGFβ and VEGF at the endometrial level may mediate the anti-nidatory action of mifepristone.

There is substantial indirect evidence to suggest an involvement of LIF in the process of implantation. Endometrial LIF expression is essential for blastocyst implantation and embryo growth in a variety of species including primates (Fry et al. 1992, Shen & Leder 1992, Stewart et al. 1992, Robertson et al. 1993, Kauma & Matt
Available studies in the human also indicate that endometrial LIF synthesis and secretion are high during the mid-luteal phase, that is, around the time of blastocyst implantation (Charnock-Jones et al. 1994, Kojima et al. 1994, Arici et al. 1995, Chen et al. 1995, Cullinan et al. 1996, Vogiagis et al. 1996). Several cytokines which are influenced by progesterone have been found to enhance endometrial LIF expression (Arici et al. 1995). Ace & Okulicz (1995) also observed that expression of LIF was up-regulated in progesterone-dominated monkey endometrium.

In the present study, mid-luteal phase endometrial secretion of LIF in vitro resolved into two bands at 56 and 27 kDa. LIF molecules are highly glycosylated, single chain molecules varying in molecular masses from approximately 38–67 kDa resulting from differential glycosylation of a protein of approximately 26 kDa in the human (Hilton et al. 1988, Hilton 1992). In the present study, LIF secretion by endometrial samples collected on day 6 after ovulation of proven fecund cycles was not affected by early luteal phase mifepristone treatment in monkeys. Immunohistological examination also failed to detect any change in the level of LIF in the glandular epithelial compartment. Our observation on glandular LIF level following RU486 treatment in monkeys does not agree with the one reported by Gemzell-Danielsson et al. (1997), in which a significant decrease in LIF was noted in mid-luteal phase human endometrium following early luteal phase mifepristone treatment. However, unlike the human study, we have used endometrial samples only from proven fecund cycles. It is notable that the embryo may stimulate endometrial LIF production (Plachot et al.

Figure 2 Immunohistochemical localization of LIF in endometrial samples from control (a,b) and mifepristone-treated (c,d) animals. Haematoxylin counterstained. × 500 (a,c) and × 790 (b,d).
Figure 3 Immunohistochemical localization of TGFβ1 (a,b) and TGFβ pan (c,d) in endometrial samples from control (a,c) and mifepristone-treated (b,d) animals. A high degree of nuclear staining for TGFβ1 in the mifepristone-treated endometrial sample (b) is evident. Haematoxylin counterstained. × 500 (a,b) and × 790 (c,d).

Figure 4 Immunohistochemical localization of VEGF in endometrial samples from control (a) and mifepristone-treated (b) animals. Haematoxylin counterstained. × 790.
have shown previously in the rhesus monkey that the endometrium on day 6 of a fecund cycle has distinct morphological and biochemical characteristics which distinguish it from the endometrium on day 6 of non-mated ovulatory cycles (Ghosh & Sengupta 1988, 1989b, Sengupta et al. 1988, Ghosh et al. 1993). The results of the present study support an earlier observation that LIF production in human endometrium in vitro was not affected by a low dose of RU486 (Chauvat et al. 1995). Similar to earlier reports in the human (Charnock-Jones et al. 1994, Arici et al. 1995, Chen et al. 1995, Vogiagis et al. 1996), we also observed only low to moderate levels of LIF in the stromal compartment of mid-luteal phase endometrium in the rhesus monkey. There was, however, a marginal increase in the level of LIF in the vascular compartment. Thus, it remains to be investigated further whether LIF plays the same critical role in the process of implantation in primates as has been demonstrated in mice (Stewart et al. 1992).

TGFβ may also play a significant role in preimplantation stage endometrium and its level may be affected around the time of blastocyst implantation as a result of early luteal phase application of mifepristone. Expression of TGFβ has been shown to be high in mouse and ovine preimplantation stage endometrium (Das et al. 1992, Dore et al. 1996), which has been implicated in embryo development (Paria et al. 1992). Furthermore, TGFβ1 expression by human endometrial stromal cells is regulated by ovarian steroid hormones (Casslen & Ohlsson 1981, Gong et al. 1991, Arici et al. 1996) and this cytokine has been shown to be present in first-trimester maternal endometrial samples (Kauma et al. 1990). In an elegant study, Casey and MacDonald (1996) have suggested that TGFβ may interact in a very complex manner in progesterone-dominated endometrium in defining its functional destiny.

In the present study, TGFβ1, TGFβ1 LAP and TGFβ pan were immunodetected in three bands in the range of 50–45, 30–25 and 20–15 kDa respectively. Similar immunodetectable bands have been observed in other species (Lyons et al. 1988, Miyazono et al. 1988, R&D Systems 1994). The fact that a lower score of TGFβ1 LAP compared with TGFβ1 was detected in Western blots possibly supports the notion that TGFβs are secreted in mature forms to act upon adjacent cells (Lyons et al. 1988). On the other hand, a higher score of TGFβ1 LAP compared with TGFβ1 was observed in immunohistology presumably due to higher sensitivity of antibody against LAP compared with that against TGFβ1 (see Table 1). The immunodetectable levels of TGFβ1 and TGFβ pan were dramatically enhanced in endometrium together with higher secretion of TGFβ pan following RU486 treatment, with no change in TGFβ1 LAP. It appears that endometrial receptivity towards blastocyst implantation is significantly associated with the level of TGFβ1 in the endometrium; it tends to be low at that time in the rhesus monkey. Following early luteal phase mifepristone administration, there was a significant increase in endometrial TGFβ during the peri-implantation period, resulting in desynchronisation of epithelial cell maturation and consequent failure of implantation. TGFβ is inhibitory to epithelial cell mitosis, and it also induces apoptosis (Rotello et al. 1991, Murphy & Ballejo 1994). Apoptosis is significantly high in mifepristone-treated endometrium (Ghosh et al. 1992, Rotello et al. 1992, Ghosh et al. 1996). TGFβ in endometrium is also very high during menstruation (Casey & MacDonald 1996). In fact, it was suggested by Gold et al. (1994) that TGFβ may play a significant paracrine role between different endometrial compartments, and simultaneous overexpression or accumulation of TGFβ, especially in glandular epithelium, is indicative of dysregulated growth control. Interestingly, Gold et al. (1994) also observed a high degree of nuclear TGFβ1 staining in abnormal endometrial glands and stroma. Such a higher degree of nuclear TGFβ1 staining may be associated with its inhibitory activity on DNA synthesis and inductive effect on apoptosis (Ignott & Massague 1987, Rotello et al. 1991).

Expression of endometrial VEGF is high during implantation and it presumably participates in increased vascular permeability and angiogenesis occurring in maternal endometrium during blastocyst implantation (Sharkey et al. 1993, Chakraborty et al. 1995, Smith 1996). Thus, we hypothesized that following early luteal phase mifepristone treatment, endometrial VEGF could be affected at the time of implantation resulting in implantation failure. In the present study, endometrial VEGF was detected in two bands at 46 and 28 kDa. It has been noted by others that VEGF is a dimeric protein (~46 kDa) consisting of two identical (~23 kDa) subunits (see Ferrara et al. 1992). Similar to the observation in women (Charnock-Jones et al. 1993), immunodetectable VEGF was primarily observed in the epithelium of implantation stage endometrium in the present study. Although band characteristics in Western blot did not change with mifepristone treatment, there was a significant decrease in the levels of VEGF in endometrium and in its secretion in vitro following mifepristone treatment. Although we have no clear idea about the regulation of VEGF in endometrium by oestradiol and progesterone, a cell line (RL-95) has been shown to express significantly higher levels of VEGF in response to progesterone (Smith 1996).

Although it is true that in vitro secretion of a cytokine and its histochemical distribution do not provide direct information about the secretary status of the test cytokine in situ, such information generally provides an effective operative knowledge (Ballock & Roberts 1993). Thus, the observation that the experimental cytokines were affected not only in the glandular compartment, but also in the vascular compartment of preimplantation stage endometrium following early luteal phase mifepristone treatment appears important because changes in the
vascular bed are integral to the process of implantation (Finn & Porter 1975, Ghosh et al. 1993, Abberton & Rogers 1995). It is generally known that VEGF and fibroblast growth factor promote angiogenesis, LIF inhibits angiogenesis and TGFβ can both inhibit and promote angiogenesis depending on its concentration and overall endocrinology and paracrinology of the tissue (Klagsbrun & D’Amore 1991, Pepper et al. 1992, 1993, 1995, Casey & MacDonald 1996). LIF, TGFβ and VEGF are also known to influence leukocytic margination and emigration, vascular integrity and remodelling, as well as organisation of extravascular matrix (Ignotz & Massague 1987, Schäinberg et al. 1988, Hilton 1992, Murphy & Ballejo 1994, Smith 1996). Therefore, the observed changes in the cytokines characterized by high LIF and TGFβ together with decreased VEGF in the vascular compartment are suggestive of a major functional change in this compartment. Previous morphological studies also revealed that small blood vessels were affected together with microhaematoma and enhanced emigration of leukocytic cells into the extracellular matrix in mid-luteal phase endometrium after mifepristone administration in human volunteers (Johannisson et al. 1989) and in monkeys (Ghosh et al. 1996).

To our knowledge, this is the first report documenting a pooled status of LIF, TGFβ and VEGF in glandular and vascular compartments of implantation stage endometrium with or without early luteal phase mifepristone treatment in a primate species. The observed changes in these cytokines in endometrium are indicative of dysregulated glandular maturation and vascular incompetence resulting in endometrial inadequacy and loss of preimplantation embryo viability following early luteal phase mifepristone treatment in the rhesus monkey (Ghosh et al. 1996, 1997a).

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