Original Paper

NeuroImmunoModulation

Neuroimmunomodulation 2005;12:157–163
DOI: 10.1159/000084848

Received: April 16, 2003
Accepted after revision: August 11, 2004

Human Endometrial Leukemia Inhibitory Factor and Interleukin-6: Control of Secretion by Transforming Growth Factor-β-Related Members

Sophie Perrier d'Hauterive[a, b] Chantal Charlet-Renard[a] Michel Dubois[b]
Sarah Berndt[c] Frederic Goffin[c] Jean-Michel Foidart[b, c] Vincent Geenen[a]

[a] University of Liège, Department of Medicine, Center of Immunology, Institute of Pathology CHU-B23, Liège-Sart Tilman, [b] University of Liège, Department of Gynecology and Obstetrics, CHR-Citadelle, Liège, and [c] University of Liège, Institute of Pathology CHU-B23, Laboratory of Tumor and Developmental Biology, Liège-Sart Tilman, Belgium

Key Words
Transforming growth factor-β · Leukemia inhibitory factor · Interleukin-6 · Maternal tolerance · Human endometrium

Abstract
Objective(s): The implantation process is closely linked to the fundamental question of the tolerance of the maternal immune system. The main objective of this study was to investigate whether different members of the transforming growth factor-β (TGF-β) superfamily could intervene in the first steps of embryo implantation by modulating the secretion of proimplantatory leukemia inhibitory factor (LIF) and in the tolerance of the fetal graft by regulating proinflammatory interleukin (IL)-6 secretion by human endometrial epithelium (EEC) in vitro. ***Methods:*** EEC were isolated from biopsies collected from 16 informed and consenting fertile women and were cultured for 72 h. Cytokine measurements (LIF and IL-6) were realized by ELISA. ***Results:*** TGF-β_1 (from 10^{-12} to 10^{-8} *M*), -β_2, -β_3 and activin A (10^{-10} and 10^{-8} *M*) increased LIF secretion by EEC cultures. Inhibin B (10^{-10} and 10^{-8} *M*) did not stimulate LIF production by human EEC. Contrastingly, TGF-β_1 (from 10^{-12} to 10^{-8} *M*), -β_2, -β_3 and activin A (10^{-10} and 10^{-8} *M*) reduced IL-6 release by the same cells. Activin A at 10^{-8} *M* also significantly reduced the stimulating effect of IL-1β (10^{-9} *M*) which is known to stimulate LIF production by EEC. Only the highest concentration of inhibin B (10^{-8} *M*) reduced IL-6 secretion by EEC, but did not modulate IL-1β-induced stimulation of IL-6 secretion. ***Conclusion(s):*** Besides their role in the control of the process of implantation and in the induction of embryonic mesoderm, different members of the TGF-β superfamily may also contribute in the reproductive process by enhancing endometrial proimplantatory LIF secretion and reducing proinflammatory IL-6 release by EEC.

Introduction

Human embryonic implantation is a complex process including apposition and adhesion of the blastocyst to the maternal endometrial epithelium followed by invasion into the endometrium and decidua. An appropriate close contact and dialogue between endometrial epithelial cells

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1021–7401/05/0123–0157$22.00/0

Accessible online at:
www.karger.com/nim

Sophie Perrier d'Hauterive, MD, PhD
University of Liège, Department of Gynecology and Obstetrics
Center of Immunology, Institute of Pathology CHU-B23
BE–4000 Liège-Sart Tilman (Belgium)
Tel. +32 4 3662537, Fax +32 4 3662977, E-Mail sperrier@ulg.ac.be

(EEC) and trophoblasts is a crucial step required for further embryonic development. Many molecules such as cytokines, chemokines, growth factors and hormones, from an endometrial or embryonic origin, play crucial roles in regulating endometrial cell function and actively participate to the cross-talk at the embryonic-maternal interface. The implantation process is also closely linked to the fundamental question of the tolerance of the maternal immune system towards the fetal allograft. Most tolerance mechanisms act at the fetal-endometrial interface and in the placenta. Several mechanisms have been proposed such as the modification of the cytokine environment at the time of the implantation. A 9-month dialogue initiated at the time of implantation characterizes the pregnancy. The immune and endocrine molecular parameters of this dialogue need to be finely tuned to ensure the success of the pregnancy.

Among the numerous and redundant factors that may intervene in the first steps of implantation, we were interested in the potent immunosuppressive transforming growth factor-β (TGF-β)-related peptides. TGF-β is a large superfamily of structurally related polypeptides such as inhibins (inhibin A and B), activins (activin A and B), LEFTY-A, Müllerian inhibitory factor, bone morphogenic proteins and growth differentiating factor [1], involved in many biological processes including cell growth, proliferation and differentiation, tissue and extacellular matrix remodeling, angiogenesis, apoptosis and immunosuppression [2–5]. Given that all these events occur during embryo implantation, TGF-β and related factors could be proposed as important mediators for successful implantation and tolerance of the maternal immune system against the fetal allograft. In mammals, there exist three different isoforms of TGF-β: TGF-β_1, -β_2 and -β_3 [6, 7]. In human endometrium and placenta, TGF-β isoforms exhibit spatio-temporal expression patterns consistent with a role in implantation since the maximal expression is observed during the secretory phase [7–11], and TGF-β receptors act in concert with TGF-β ligands in human blastocyst [12]. TGF-β receptors I and II are expressed on human endometrial epithelium [13]. All three activins and inhibins subunits (α, βA and βB) mRNA and protein are expressed by human endometrium, primarily in glandular epithelium, with elevated expression in both endometrial epithelium and stroma in secretory phase and after the onset of decidualization [14–16]. The precise functions of endometrial-derived activin A are not yet known. Since there is an abundance of activin receptors in maternal endometrium and preimplantation embryonic tissues [17], it is hypothesized that activin A could be an important endometrial-derived mediator of early placental development, both through promoting trophoblast invasion into decidua and stimulating placental hormone production [18].

Different cytokines are present at the embryonic-endometrial interface and represent as many potential key molecules for the implantation process and mediators for the dialogue at the apposition-adhesion steps of the implantation. Stewart et al. [19] evidenced first the importance of LIF in murine embryo implantation. Indeed, females lacking the Lif gene *(Lif*$^{-/-}$*)* are fertile, but their blastocysts fail to implant. The blastocysts of *Lif*$^{-/-}$ mice are viable and, when transferred to wild-type pseudopregnant recipients, can implant and develop to term. The question whether LIF plays such a crucial role in human implantation is still unanswered. Evidence is that the pattern of *LIF* and its receptor expression in endometrium and trophoblast strongly suggests an important role for this cytokine at the time of implantation. In vivo studies are consistent since level of LIF in uterine flushings at day LH+10 of the cycle are lower in infertile women compared to fertile ones [20]. Interleukin (IL)-6 is a proinflammatory cytokine also secreted by human endometrium. IL-6 secretion exhibits a temporal profile in the human endometrium (epithelial and stromal fraction) during the menstrual cycle. Low in proliferatory phase, IL-6 secretion rises during the secretory phase and menstruation [21, 22]. Its receptor is present at the surface of the embryo, the placenta and the endometrium [23]. Although only development of concepti is compromised in IL-6$^{-/-}$ mice and not implantation [24, 25], IL-6 seems to play a role in embryo implantation.

To gain further insight into the role played by the members of the TGF-β superfamily at the time of implantation, we have investigated their impact on the endometrial secretion of cytokines participating in the success of implantation and contributing to endometrial receptivity, namely LIF and IL-6. Particularly, we were interested into their secretion by EEC, which are the first cells encountered by the preimplantatory blastocyst when it enters the uterine cavity.

Materials and Methods

Reagents

Recombinant IL-1β was provided by Preprotech (London, UK) and used at a final concentration of 10^{-12} *M*. TGF-β_1 was prepared according to the methodology of Cone [26] and used at 10^{-9}, 10^{-10} and 10^{-12} *M*. Human TGF-β_2, -β_3 from Sigma-Aldrich (St. Louis, Mo., USA), activin A and inhibin B from R&D Systems (Abingdon, UK) were used at two final concentrations: 10^{-8} and 10^{-10} *M*.

Patients

Endometrial biopsies were collected from fertile (one previous delivery) ovulating women (n = 16) undergoing surgery for voluntary sterilization, or during hysteroscopy before assisted medical procreation because of male infertility. The stage of the menstrual cycle was established from women's menstrual history (based on the first day of the last menstruation) and from the histological dating performed by experienced pathologists, according to the criteria of Noyes et al. [27]. The mean age was 33.4 years (18–45 years). None of the patients received any hormonal treatment for 3 months prior to biopsy. The Ethics Committee of Liege Medical School approved the protocol, and informed consent was obtained from the patients.

Separation of Endometrial Epithelial from Stromal Cells

Endometrial biopsy samples were collected in Hanks' balanced salt solution containing penicillin (100 IU/ml) and streptomycin (100 μg/ml). EEC were isolated from stromal cells and cultured following the method described by Laird et al. [28]. The tissues were finely chopped with scissors and digested for 45 min at 37°C in 5 ml of Dulbecco's modified Eagle's medium (DMEM) containing 0.2% collagenase type 1a (Sigma-Aldrich). During incubation and at the end of incubation, the tissue was gently pipetted every 15 min to disperse the cells. EEC were separated from stromal cells by centrifugation for 20 min at 100 *g*. Pellets contained mainly epithelial cells present both as small clumps and as separated cells. EEC were further purified by unit density sedimentation. Pellets were re-suspended in 2 ml DMEM containing 10% fetal calf serum (FCS), 1% glutamine and antibiotics, and gently pipetted onto 8 ml of DMEM + 10% FCS in a test tube and left for 30 min at room temperature. Afterwards, EEC were in the lower 2 ml of supernatants. EEC were used for immunocytochemistry and cytokine production. Before cultured in serum-free medium, EEC were centrifuged at 100 *g*, and the pellets were washed twice with DMEM containing 1% glutamine, antibiotics, 0.3% bovine serum albumin and 25 mIU/ml human insulin. The epithelial nature of the cells pelleted was demonstrated by their intense staining with an mAb against human cytokeratin. These cells were not stained either by an mAb against vimentin (specific for stromal cells), or by an mAb staining all white blood cells (anti-CD45RB). Cytokeratin-positive EEC represented more than 95% of the total cell population.

Cell Cultures

EEC were plated into 24-well plates at 2×10^5 cells per ml and per well and cultured for 72 h in DMEM with 1% glutamine, 0.3% bovine serum albumin and 25 mIU/ml human insulin. Each treatment was applied in a micro volume (50 μl) at the beginning of incubation. Each condition was tested in triplicate. Too few epithelial cells after separation resulted in the exclusion of the explant from some treatments. The cells used for immunocytochemistry were cultured on multitest slides (10 wells) (ICN Biomedicals, Aurora, Ohio, USA) in media containing DMEM with 1% glutamine, 25 mIU/ml human insulin and 10% FCS.

Cytokine Immunoassays

Supernatants of cultured cells were collected for assay after 72 h. Collected media were stored for a short period (maximal delay of 15 days) at –20°C until cytokine measurements. All cytokine concentrations (IL-6 and LIF) were measured with specific ELISAs (Biosource-Europe, Nivelles, Belgium) according to appropriate instructions [29]. Sensitivities were 150 pg/ml for LIF and 3 pg/ml for IL-6.

Statistical Analyses

Because of individual variability, all data have been normalized and expressed as the percentage of the mean of control ± SEM. For each experiment (except when specified), statistical analysis of the response to any kind of treatment was performed for the difference with control (untreated cells). The significance of differences was evaluated using unpaired nonparametric Mann-Whitney test with confidence intervals of 95%. Statistical analyses were performed using GraphPad Prism software.

Results

Cytokine Production

In basal conditions, cultured human endometrial epithelium in proliferative and secretory phase spontaneously secreted LIF and IL-6. Treatment with IL-1β (10^{-9} *M*) strongly stimulated LIF and IL-6 production and was used as positive control in all cultures. Results obtained for the different members of the TGF-β superfamily tested (TGF-β_1, -β_2, -β_3, activin A and inhibin B) were first analyzed according to the stage of the menstrual cycle, but no significant difference was observed between the two phases. Results were then pooled without distinction for the cycle phase.

Effect of TGF-β1. An opposite effect on LIF and IL-6 production was observed when EEC were treated with TGF-β_1 (n = 16 patients). Indeed, TGF-β_1 (from 10^{-12} to 10^{-9} *M*) induced a concentration-dependent increase of LIF production and a concentration-dependent reduction of IL-6 production by EEC in culture. The addition of TGF-β_1 did not modify either the stimulating effect of IL-1β on LIF or IL-6 production (fig. 1a).

Effect of TGF-β2 and TGF-β3. TGF-β_2 and -β_3 both stimulated LIF production and reduced IL-6 secretion by EEC in culture (n = 6 patients) (fig. 1b). We could not observe any significant difference between the effect of TGF-β_2 compared to TGF-β_3 in terms of LIF and IL-6 secretion. The addition of TGF-β_2 and -β_3 did not modify the stimulating effect of IL-1β on LIF and IL-6 production by the same cells (data not shown).

Effect of Activin A. Activin A induced an increase of LIF secretion by EEC in vitro and a significant inhibition of IL-6 production (n = 10 patients). Activin A had no impact on the stimulating effect of IL-1β on LIF production but induces a reducing of the stimulating effect of IL-1β on IL-6 production (fig. 2).

Effect of Inhibin B. Inhibin B did exert any significant effect neither on LIF production, nor on the stimulating

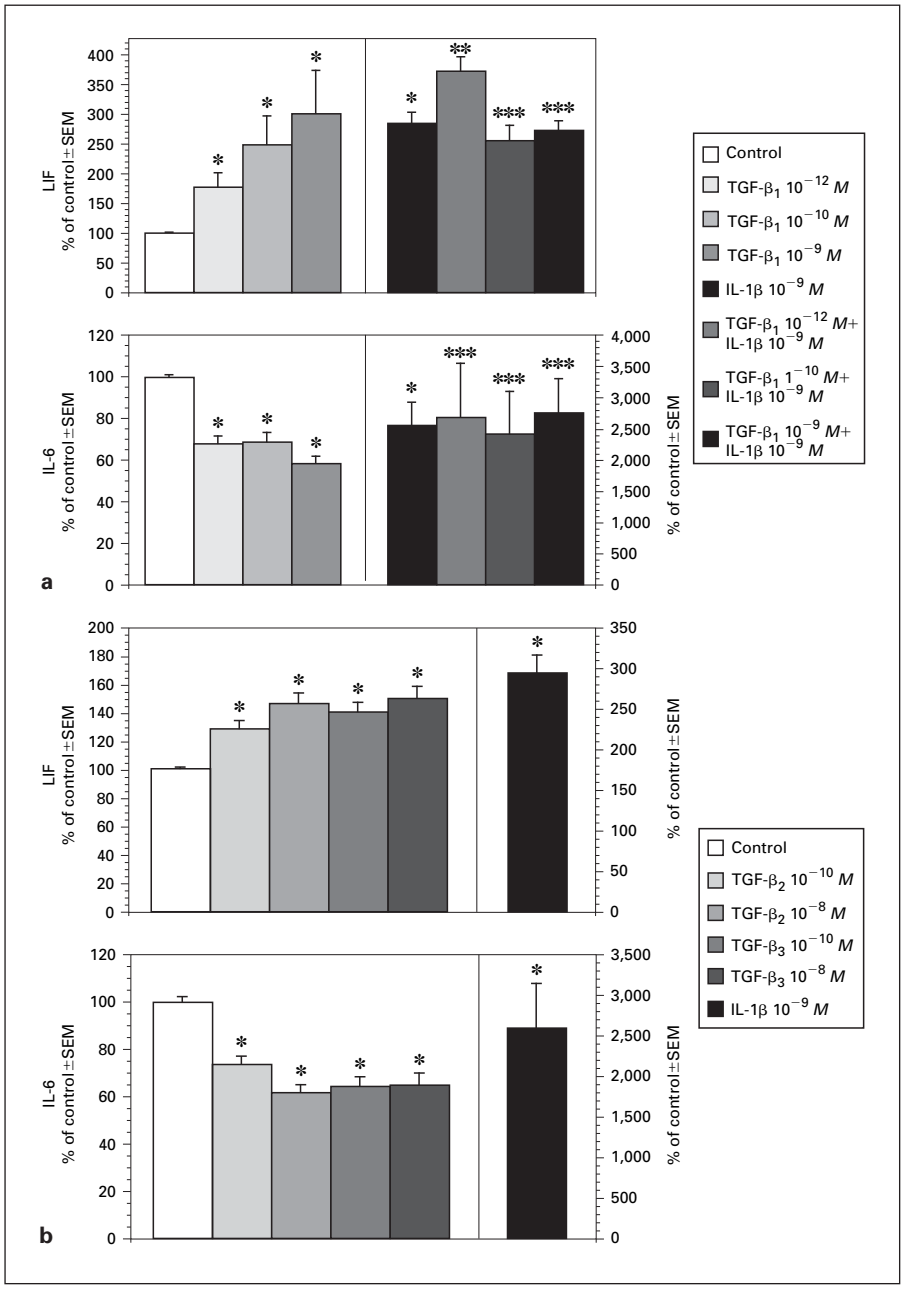

Fig. 1. a Production of LIF and IL-6 by 72-hour cultures of human EEC in basal condition (control), and after treatment with TGF-β_1 (10^{-12} to 10^{-9} *M*). Impact of TGF-β_1 on the stimulating effect of IL-1β on LIF and IL-6 secretion by EEC. Results are expressed as percentage of control (mean ± SEM). n = 16. **b** Production of LIF and IL-6 by 72-hour cultures of human EEC in basal condition (control), and after treatment with TGF-β_2 and TGF-β_3 (10^{-10} and 10^{-8} *M*). Results are expressed as percentage of control (mean ± SEM). n = 6. * $p < 0.0001$ vs. control; ** $p < 0.014$ vs. IL-1β (10^{-9} *M*); *** $p < 0.0001$ vs. control and not significant vs. IL-1β (10^{-9} *M*) by Mann-Whitney test.

effect of IL-1β on LIF or IL-6 secretion by EEC (n = 8 patients). Only inhibin B at 10^{-8} *M* induced a slight reduction of IL-6 secretion by EEC in culture (fig. 3).

Discussion

The reliability of the technique used for the separation of EEC from endometrial biopsy was confirmed by immunocytochemical analyses, with level of purity of more than 95%. IL-1β stimulates LIF production by human endometrium both by stromal [30] and epithelial cells [31]. Vandermolen and Gu [22] and Laird et al. [32] evidenced that IL-1β stimulates IL-6 endometrial production. These effects are probably mediated by the interaction of IL-1β with its receptor (IL-1R type I) which has been previously evidenced in human EEC [33, 34]. In our model, IL-1β (10^{-9} *M*) also stimulates LIF and IL-6 production by EEC in culture and this was used as positive control in all our experiments. Since the degree of cyto-

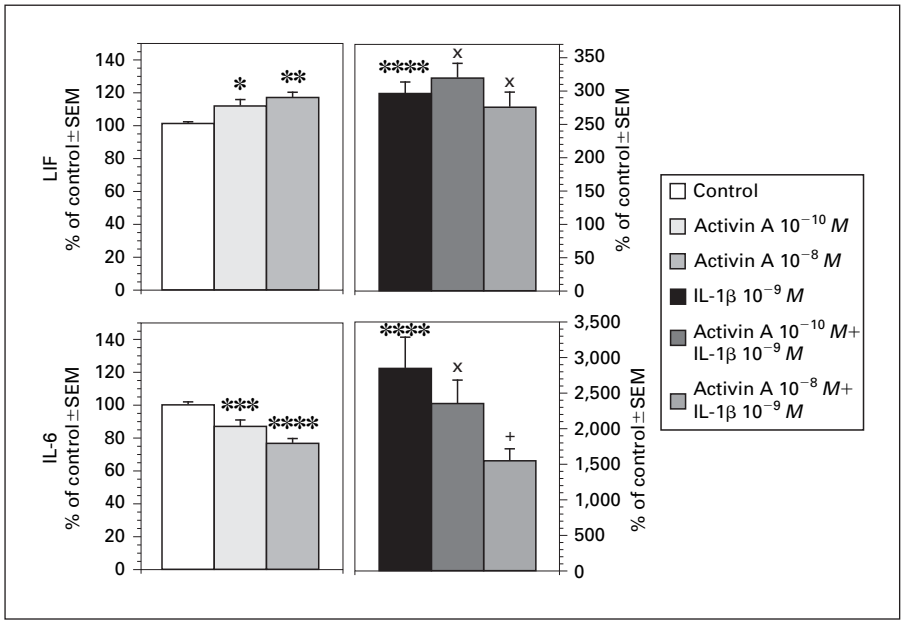

Fig. 2. Production of LIF and IL-6 by 72-hour cultures of human EEC in basal condition (control), and after treatment with activin A (10^{-10} and 10^{-8} *M*). Impact of activin A on stimulating effect of IL-1β (10^{-9} *M*) on LIF and IL-6 secretion. Results are expressed as percentage of control (mean ± SEM). n = 10. * p = 0.03, ** p = 0.0004, *** p = 0.0009, **** p < 0.0001 vs. control; + p = 0.04, × = not significant vs. IL-1β (10^{-9} *M*) by Mann-Whitney test.

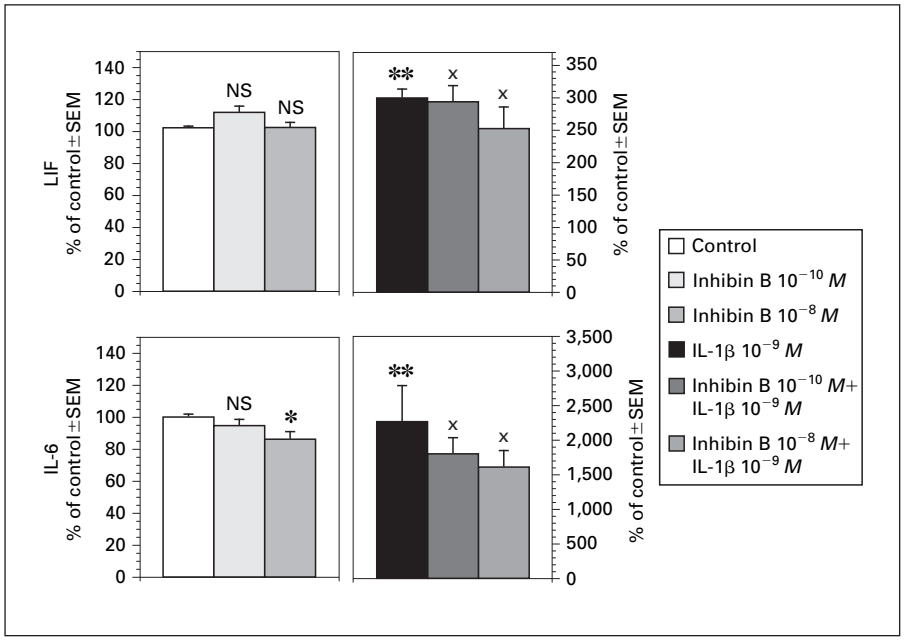

Fig. 3. Production of LIF and IL-6 by 72-hour cultures of human EEC in basal condition (control), and after treatment with inhibin B (10^{-10} and 10^{-8} *M*). Impact of inhibin B on stimulating effect of IL-1β (10^{-9} *M*) on LIF and IL-6 secretion. Results are expressed as percentage of control (mean ± SEM). n = 8. NS = Not significant, * p = 0.003, * p < 0.0001 vs. control; × = not significant vs. IL-1β (10^{-9} *M*) by Mann-Whitney test.

kine response significantly varied from one woman to another, all the cytokine data were normalized (see Materials and Methods section). Our studies indicate that TGF-β_1, -β_2 and -β_3 isoforms significantly enhance LIF secretion by EEC in cultures, but this stimulating effect was not synergic with IL-1β. The three isoforms of TGF-β reduced IL-6 release by EEC in culture. However, this effect was not able to interfere with the stimulating effect of IL-1β (10^{-9} *M*) on IL-6 production. In human, TGF-β_1 is critical for regulating the protease network thereby effectively controlling the extent to which the trophoblast may invade the maternal endometrium [35–37]. In vitro studies evidenced that TGF-β_1 (and other isoforms) inhibits trophoblast proliferation and promotes their differentiation into syncytiotrophoblast, controlling then the process of placentation [38, 39]. Later in the implantation process, TGF-βs intervene in the embryo development and in the induction of mesoderm within the embryo [40]. Earlier in the implantation process, tissue-derived TGF-β isoforms from a receptive secretory endo-

metrium could contribute to the adhesive phase of implantation by modulating embryo and early trophoblast fibronectin-integrin interaction. Our results further suggest such an early role for TGF-β isoforms in the implantation process by enhancing the production of the proimplantatory LIF by EEC. Infertile bonnet monkeys present a derangement in LIF and TGF-β_2 expression profiles during the peri-implantation. This suggests that TGF-β_2 signaling intervenes in the regulation of the progesterone-driven cascade of events leading to endometrial receptivity, and that any aberration in this signaling may adversely affect the subsequent molecular events such as expression of LIF [41].

The elucidation of the molecular mechanisms by which the immunologically distinct embryo is not rejected by the mother's immune system is an important question in reproductive medicine. Our experiments evidence that TGF-β isoforms induce an important reduction of the proinflammatory IL-6 production by EEC in vitro. More experiments are needed to elucidate whether the TGF-β isoforms could contribute to the maternal tolerance of the fetal graft through such an in vivo reduction of IL-6 by EEC during pregnancy. McLennan and Koishi [42] have recently demonstrated that in mice, concepti are exposed to significant levels of both fetal and maternal TGF-β_1. The level of fetal TGF-β_1 is determinant of whether a conceptus is rejected or not, although the maternal TGF-β_1 and other factor are also important. Their data are consistent with maternal and fetal TGF-β_1 interacting to maintain pregnancy, within immune-competent mothers.

Activin A is expressed and produced by both the embryo and the human endometrial epithelium during the menstrual cycle [14]. In early pregnancy, activin A may facilitate implantation, because of its ability to promote cytotrophoblast differentiation towards an invasive phenotype and to stimulate the production of paracrine or endocrine agents involved in invasion such as placental hormones. Our results provide further information regarding the positive influence of activin A present at the maternal-fetal interface during the first steps of human implantation by enhancing proimplantatory LIF production. Interestingly, activin A was also shown to strongly reduce IL-6 production by a direct action on EEC and through the interference with the stimulating effect of IL-1β. Activin A is an immunomodulatory molecule, but its role during the inflammatory response remains unclear. It appears to exert both pro- and anti-inflammatory effects, as defined by its impact on cytokine production [43].

Inhibins were originally isolated based on their ability to specifically suppress follicle-stimulating hormone secretion from pituitary cells in vitro. Though inhibin is secreted by endometrial gland [44], our results suggest that inhibin B is less implicated in the implantation process in term of LIF and IL-6 secretion by EEC.

In conclusion, this study evidences that treatment of human EEC in culture by the different TGF-β-related peptides tested (inhibin B excepted) increases proimplantatory LIF secretion and inhibits proinflammatory IL-6 secretion, suggesting a positive influence of these factors during the first steps of implantation, when embryo encounters the endometrial epithelium.

Acknowledgements

Sophie Perrier d'Hauterive is Research Fellow and Vincent Geenen is Research Director of the Belgian National Fund of Scientific Research (NFSR). This study was supported by Fondation Léon Fredericq (Liège Medical School) and Fédération Belge contre le Cancer.

References

1 Kingsley DM: The TGF-β superfamily: New members, new receptors, and new genetic tests of function in different organisms. Genes Dev 1994;8:133–146.
2 Haralson MA: Transforming growth factor-β, other growth factors, and the extracellular matrix. J Lab Clin Med 1997;130:455–458.
3 Tanaka T, Umesaki N: Cytokine regulation of apoptotic susceptibility in a human endometrial epithelial cell line. J Reprod Immunol 2000;47:105–119.
4 Tabibzadeh S: Homeostasis of extracellular matrix by TGF-β and lefty. Front Biosci 2002; 7:1231–1246.
5 Lin HY, Wang HM, Li QL, Liu DL, Zhang X, Liu GY, Qian D, Zhu C: Expression of Smad2 and Smad4, transforming growth factor-β signal transducers in rat endometrium during the estrous cycle, pre-, and peri-implantation. Anim Reprod Sci 2004;80:303–316.
6 Graycar JL, Miller DA, Arrick BA, Lyons RM, Moses HL, Derynck R: Human transforming growth factor-β_3: Recombinant expression, purification, and biological activities in comparison with transforming growth factors-β_1 and -β_2. Mol Endocrinol 1989;3:1977–1986.
7 Tabibzadeh S, Lessey B, Satyaswaroop PG: Temporal and site-specific expression of transforming growth factor-β_4 in human endometrium. Mol Hum Reprod 1998;4:595–602.

8 Godkin JD, Doré JJ: Transforming growth factor-β and the endometrium. Rev Reprod 1998; 3:1–6.
9 Chegini N, Zhao Y, Williams RS, Flanders KC: Human uterine tissue throughout the menstrual cycle expresses transforming growth factor-β_1 (TGF-β_1), TGF-β_2, TGF-β_3, and TGF-β type II receptor messenger ribonucleic acid and protein and contains [^{125}I]TGF-β_1-binding sites. Endocrinology 1994;135:439–449.
10 Casslén B, Sandberg T, Gustavsson B, Willén R, Nilbert M: Transforming growth factor-β_1 in the human endometrium. Cyclic variation, increased expression by estradiol and progesterone, and regulation of plasminogen activators and plasminogen activator inhibitor-1. Biol Reprod 1998;58:1343–1350.
11 Kauma S, Matt D, Strom S, Eierman D, Turner T: Interleukin-1β, human leukocyte antigen HLA-DRα, and transforming growth factor-β expression in endometrium, placenta, and placental membranes. Am J Obstet Gynecol 1990;163:1430–1437.
12 Osterlund C, Fried G: TGF-β receptor types I and II and the substrate proteins Smad 2 and 3 are present in human oocytes. Mol Hum Reprod 2000;6:498–503.
13 Piestrzeniewicz-Ulanska D, Brys M, Semczuk A, Jakowicki JA, Krajewska WM: Expression of TGF-β type I and II receptors in normal and cancerous human endometrium. Cancer Lett 2002;186:231–239.
14 Petraglia F, Florio P, Luisi S, Gallo R, Gadducci A, Vigano P, Di Blasio AM, Genazzani AR, Vale W: Expression and secretion of inhibin and activin in normal and neoplastic uterine tissues. High levels of serum activin A in women with endometrial and cervical carcinoma. J Clin Endocrinol Metab 1998;83: 1194–1200.
15 Jones RL, Salamonsen LA, Critchley HO, Rogers PA, Affandi B, Findlay JK: Inhibin and activin subunits are differentially expressed in endometrial cells and leukocytes during the menstrual cycle, in early pregnancy and in women using progestin-only contraception. Mol Hum Reprod 2000;6:1107–1117.
16 Florio P, Severi FM, Luisi S, Ciarmela P, Calonaci G, Cobellis L, Petraglia F: Endometrial expression and secretion of activin A, but not follistatin, increase in the secretory phase of the menstrual cycle. J Soc Gynecol Investig 2003; 10:237–243.
17 Welt CK: The physiology and pathophysiology of inhibin, activin and follistatin in female reproduction. Curr Opin Obstet Gynecol 2002; 14:317–323.
18 Ni X, Luo S, Minegishi T, Peng C: Activin A in JEG-3 cells: Potential role as an autocrine regulator of steroidogenesis in humans. Biol Reprod 2000;62:1224–1230.
19 Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Köntgen F, Abbondanzo SJ: Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992; 359:76–79.
20 Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC, Zhang X: The production of leukaemia inhibitory factor by human endometrium: Presence in uterine flushings and production by cells in culture. Hum Reprod 1997;12:569–574.
21 Tabibzadeh S, Kong QF, Babaknia A, May LT: Progressive rise in the expression of interleukin-6 in human endometrium during menstrual cycle is initiated during the implantation window. Hum Reprod 1995;10:2793–2799.
22 Vandermolen DT, Gu Y: Human endometrial interleukin-6: In vivo messenger ribonucleic acid expression, in vitro protein production, and stimulation thereof by interleukin-β. Fertil Steril 1996;66:741–747.
23 Sharkey AM, Dellow K, Blayney M, Macnamee M, Charnock-Jones S, Smith SK: Stage-specific expression of cytokine and receptor messenger ribonucleic acids in human preimplantation embryos. Biol Reprod 1995;53: 974–981.
24 Robertson SA, Mayrhofer G, Seamark RF: Uterine epithelial cells synthesize granulocyte-macrophage colony-stimulating factor and interleukin-6 in pregnant and nonpregnant mice. Biol Reprod 1992;46:1069–1079.
25 Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, Köhler G: Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 1994;368:339–342.
26 Cone JL, Brown DR, DeLarco JE: An improved method of purification of transforming growth factor, type β from platelets. Anal Biochem 1988;168:71–74.
27 Noyes RW, Hertig AI, Rock I: Dating the endometrial biopsy. Fertil Steril 1950;1:3–25.
28 Laird SM, Li TC, Bolton AE: The production of placental protein 14 and interleukin-6 by human endometrial cells in culture. Hum Reprod 1993;8:793–798.
29 De Groote D, Zangerle PF, Gevaert Y, Fassotte MF, Beguin Y, Noizat-Pirenne F, Pirenne J, Gathy R, Lopez M, Dehart I, et al: Direct stimulation of cytokines (IL-1β, TNF-α, IL-6, IL-2, IFN-γ and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. Cytokine 1992;4:239–248.
30 Arici A, Engin O, Attar E, Olive DL: Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium. J Clin Endocrinol Metab 1995;80: 1908–1915.
31 Delage G, Moreau JF, Taupin JL, Hambartsoumian E, Frydman R, Tartzkovsky B, Chaouat G: In vitro modulation of the production of the cytokine HILDA/LIF, secreted by human endometrial explants: Preliminary results (in French). Contracept Fertil Sex 1995; 23:622–625.
32 Laird SM, Tuckerman E, Li TC, Bolton AE: Stimulation of human endometrial epithelial cell interleukin-6 production by interleukin-1 and placental protein 14. Hum Reprod 1994; 9:1339–1343.
33 Simon C, Piquette GN, Frances A, Polan ML: Localization of interleukin-1 type I receptor and interleukin-1β in human endometrium throughout the menstrual cycle. J Clin Endocrinol Metab 1993;77:549–555.
34 Bigonnesse F, Labelle Y, Akoum A: Triphasic expression of interleukin-1 receptor type I in human endometrium throughout the menstrual cycle of fertile women and women with unexplained infertility. Fertil Steril 2001;75:79–87.
35 Graham CH, Lysiak JJ, McCrae KR, Lala PK: Localization of transforming growth factor-β at the human fetal-maternal interface: Role in trophoblast growth and differentiation. Biol Reprod 1992;46:561–572.
36 Bischof P, Campana A: Molecular mediators of implantation. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:801–814.
37 Karmakar S, Das C: Regulation of trophoblast invasion by IL-1β and TGF-β_1. Am J Reprod Immunol 2002;48:210–219.
38 Graham CH, Lala PK: Mechanisms of placental invasion of the uterus and their control. Biochem Cell Biol 1992;70:867–874.
39 Graham CH, Connelly I, MacDougall JR, Kerbel RS, Stetler Stevenson WG, Lala PK: Resistance of malignant trophoblast cells to both the anti-proliferative and anti-invasive effects of transforming growth factor-β. Exp Cell Res 1994;214:93–99.
40 Dunn NR, Vincent SD, Oxburgh L, Robertson EJ, Bikoff EK: Combinatorial activities of Smad2 and Smad3 regulate mesoderm formation and patterning in the mouse embryo. Development 2004;131:1717–1728.
41 Sachdeva G, Patil V, Katkam RR, Manjramkar DD, Kholkute SD, Puri CP: Expression profiles of endometrial leukemia inhibitory factor, transforming growth factor-β_2 (TGF-β_2), and TGF-β_2 receptor in infertile bonnet monkeys. Biol Reprod 2001;65:1–8.
42 McLennan IS, Koishi K: Fetal and maternal transforming growth factor-β_1 may combine to maintain pregnancy in mice. Biol Reprod 2004;70:1614–1618.
43 Phillips DJ, Jones KL, Scheerlinck JY, Hedger MP, de Kretser DM: Evidence for activin A and follistatin involvement in the systemic inflammatory response. Mol Cell Endocrinol 2001;180:155–162.
44 Mylonas I, Jeschke U, Winkler L, Makovitzky J, Richter DU, Briese V, Friese K: Immunohistochemical expression of inhibin-α in human endometrium and the in vitro secretion of inhibin, estradiol and cortisol in cultured human endometrial glandular cells. Arch Gynecol Obstet 2003;268:142–150.