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Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation

(pregnancy/oncostatin M/ciliary neurotrophic factor/infertility/menstrual cycle)

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ABSTRACT **The uterine expression of leukemia inhibitory factor (LIF) is essential for embryo implantation in the mouse. Here, we describe the expression of LIF, related members of this group of cytokines, oncostatin M and ciliary neurotrophic factor, and the LIF receptor β and glycoprotein gp130 in normal human tissues and in the endometrium of fertile women. Our results show that LIF is the only one of these factors expressed at detectable levels in the endometrium of women of proven fertility. LIF expression is restricted to the endometrial glands during the secretory/postovulatory phase but is not present in the endometrium during the proliferative/preovulatory phase. The LIF receptor β is expressed during the proliferative and secretory phases of the cycle and is restricted to the luminal epithelium. The associated signal-transducing component of the LIF receptor, gp130, is also expressed in both the luminal and glandular epithelium throughout the cycle. These results suggest that uterine expression of LIF in humans, like mice, may have a role in regulating embryo implantation, possibly through an autocrine/paracrine interaction between LIF and its receptor at the luminal epithelium.**

A critical stage in early mammalian embryonic development is implantation, when the blastocyst becomes closely associated with the maternal endometrial tissues. Although the regulation of implantation is not clearly understood, it is evident that the steroid hormones, including estrogen and progesterone, induce essential proliferative and differentiative changes in the endometrium that lead to a temporary state of uterine receptivity and permit embryo implantation (1). Recent studies have revealed that many cytokines and growth factors play an essential role in preimplantation development (2–4). These cytokines are expressed in the female reproductive tract and may be the effectors of the steroid hormones. Among these factors, epidermal growth factor (EGF) or an EGF-like molecule has been implicated in regulating endometrial cell proliferation (5) and in some strains of mice embryonic expression of the EGF receptor is essential for development of the peri-implantation embryo (6). Colony-stimulating factor-1 (CSF-1) has an important, although undefined role in a successful pregnancy (7). It has also been suggested that interleukin-1 (IL-1) and its receptor may be required in mediating blastocyst implantation (8). Finally, the expression of leukemia inhibitory factor (LIF) in mice is absolutely essential for implantation, although its precise role is not fully understood (9).

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In the mouse, the principal sites of LIF expression are the endometrial glands of the uterus. Expression in the uterus is temporally restricted, with peak levels occurring at ovulation and just prior to the onset of blastocyst implantation. LIF expression is under maternal control and is not dependent upon the presence of embryos in the uterus (10, 11). Direct evidence that LIF is an absolute requirement for implantation was obtained by the derivation of mice deficient in a functional LIF gene. LIF-deficient male mice are fertile, and while the females ovulate and their embryos develop to the blastocyst stage, the embryos fail to implant. Implantation and normal development of these embryos can occur when they are transferred from the LIF-deficient mothers to wild-type pseudo-pregnant recipients or when an exogenous supply of LIF is provided to the LIF-deficient females (9).

LIF is a member of a family of ligands that include interleukin 6 (IL-6), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), and the recently identified cardiotrophin-1 (12, 13). These factors exhibit functional redundancy, since they have similar effects in a variety of biological systems, including the inhibition of embryonic stem cell differentiation *in vitro* and the induction of acute-phase protein synthesis in hepatocytes (14, 15). They also have structural similarities and all interact with the shared signal-transducing receptor component, glycoprotein gp130. With the exception of IL-6, they can interact with the LIF receptor β (LIFrβ), the principal transmembrane protein that binds LIF (16). The functional redundancies and shared activities of the LIF family of cytokines suggest that LIF, as well as CNTF, OSM, or IL-6 might also be expressed in human endometrium and that any one of these factors could serve to regulate preimplantation development and embryo implantation.

Here we describe the expression of the LIF, CNTF, OSM and the LIF receptor (LIFrβ and gp130) in normal human tissues, with particular emphasis on the expression in the uterus during different phases of the menstrual cycle. We demonstrate that LIF expression is restricted predominantly to the glandular and luminal epithelium, with maximal levels of synthesis occurring during the secretory/postovulatory phase of the menstrual cycle (starting around days 18–21), the time at which implantation is thought to commence. LIF continues to be expressed in the endometrium of first and second trimester pregnancy. Both LIFrβ and gp130 are expressed throughout the cycle, with the LIFrβ being restricted to the

Abbreviations: LIF, leukemia inhibitory factor; OSM, oncostatin M; CNTF, ciliary neurotrophic factor; IL-1 and IL-6, interleukins 1 and 6; PBL, peripheral blood leukocytes; LIFrβ, LIF receptor β; CNTFr, CNTF receptor; EGF, epidermal growth factor.

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luminal epithelia and gp130 being expressed in both the glandular and luminal epithelium. These results, together with the known requirement of LIF in the mouse, suggest that LIF may have a similar effect in regulating implantation in human reproduction, possibly through an autocrine/paracrine interaction with the LIFrβ, that acts on the luminal epithelium.

MATERIALS AND METHODS

Isolation of Tissue Samples. Endometrial samples from normal women of proven fertility were obtained by biopsy at tubal ligation. All samples were taken following the informed consent of the patients. All patients were cycling normally, and none had received hormone therapy. Endometrium was collected according to the last menstrual period and confirmed according to the methods of Noyes *et al.* (17). Days in the menstrual cycle were expressed in all figures where the first day of menses is day 1, ovulation is day 14, and the cycle length is 28 days. Samples of endometrium were obtained during pregnancy from the three trimesters and dated as described (17). All samples were transported on ice to the laboratory where they were snap-frozen in liquid nitrogen and stored at −80°C or were immediately fixed in phosphate-buffered 3.5% paraformaldehyde. Specimens of pregnant endometrium from four separate patients were examined by immunohistochemistry, and 20 highly purified mRNA preparations of pregnant endometrium were examined by RNA blot hybridization (Northern) analysis. Utilization of endometrial tissue had been approved by the institutional review board.

Immunoblotting (Western Blotting). The anti-human LIF antibody was raised against a peptide composed of amino acids 162–179 of the mature human LIF protein. The peptide was conjugated to keyhole limpet hemocyanin, emulsified in Freund's complete adjuvant, and injected subcutaneously into a rabbit. After 3 monthly injections, serum was tested by Western analysis of human recombinant LIF protein and endometrial protein extracts. Frozen endometrial tissues were pulverized and then homogenized in buffer containing 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 0.15 M NaCl, 0.01 M Tris·HCl (pH 7.4), 1 mM EDTA, 0.25 mM phenylmethylsulfonyl fluoride, 2 μg of aprotinin per ml, and 1 μg of pepstatin per ml. Lysates (20 μg per lane) were resolved by using Bio-Rad Mini-Gel and transfer apparatus. Membranes were blocked in 2.5% horse sera and 0.5% bovine serum albumin. Anti-human LIF antisera and preimmune sera from the same rabbit were used at 1:1000 dilution, and horseradish peroxidase-linked donkey anti-rabbit IgG antibody (Amersham) was used at 1:5000 dilution. Visualization of signal was by enhanced chemiluminescence (Amersham).

Probes and Labeling. Plasmids containing either human gp130 or human OSM were provided by David Gearing (SyStemix, Palo Alto, CA). The human LIF receptor cDNA fragment was obtained from Nancy Ip (Regeneron Pharmaceuticals, Tarrytown, NY). The human CNTF probe was obtained from David Hirsch (Columbia University). The probe for RPL19 was generated from primers previously described (18). The human actin probe was obtained from Clontech. The human LIF cDNA was purchased from British Biotechnology (Oxford). The probe for CNTF receptor (CNTFr) was generated from the previously published sequence. Probes were purified and labeled to high specific activity by random priming with 6000 Ci/mmol of dCTP (Amersham; 1 Ci = 37 GBq). Labeled fragments were purified through Pharmacia NICK columns.

RNA Isolation and Northern Analysis. Frozen samples of staged human endometrium were homogenized and extracted in 15 ml of RNAzol B (Biotecx Laboratories, Houston). Twenty micrograms of total RNA was electrophoresed, transferred to Genescreen Plus-charged nylon membranes (DuPont/NEN) and hybridized according to the manufacturer's instructions. Uterine mRNA and multiple-tissue Northern blots were purchased from Clontech and hybridized according to the manufacturer's instructions. Data concerning the stage in the cycle of the uterine mRNA on the Clontech filters was not available.

***In Situ* Hybridization and Immunohistochemistry.** Endometrial tissue was removed from patients and placed directly in OCT (embedding compound) tissue tek (Baxter Scientific Products, McGaw Park, IL) or snap frozen prior to OCT, and sectioned at 10-μm thickness. Synthesis of digoxigenin-labeled RNA probes and *in situ* hybridization was performed as described (19). Immunohistochemical localization of LIF antigen was performed on cryostat sections of endometrium taken from patients staged throughout the menstrual cycle and performed as described (20, 21) by using the Vectastain Elite ABC kit (Vector Laboratories) (19). Endometria from pregnancies were fixed in 3.5% paraformaldehyde in phosphate-buffered saline, sectioned at 5 μm, and stained as described (22).

RESULTS

Tissue-Specific Expression of Human LIF, LIF-Related Cytokines, and Receptors. Since the expression of the LIF-related cytokines in humans has not yet been documented, expression of LIF and LIF-related cytokines was examined in 16 normal adult human tissues by Northern analysis (Fig. 1*A*). LIF transcripts (4.4 kb) were detected in all tissues examined except the brain, testis, skeletal muscle, and peripheral blood leukocytes (PBL). A second larger 5.0-kb LIF transcript was also detected in the small intestine and colon. In contrast, a 2.2-kb OSM transcript was strongly expressed only in PBL and a 2.4-kb CNTF transcript was detectable at low levels in the testis.

The tissue-specific expression of receptor subunit transcripts for the LIF family, LIFrβ, CNTFr, and the signal transducer, gp130, was also examined (Fig. 1*A*). The LIFrβ message is expressed in most tissues as 11.0, 8.0 and 6.5 kb mRNAs except that testis produced an additional 4.4-kb transcript and expression of LIFrβ mRNA in PBL was very low. The CNTFr was detected only in the brain and skeletal muscle as a 2.2-kb transcript. The receptor component gp130 was expressed in all tissues as an 8.5 kb mRNA species (Fig. 1*A*).

Expression of Human LIF in Normal Endometrium. Expression of LIF, CNTF, and OSM transcripts was examined in endometria from women of proven fertility at different days throughout the menstrual cycle and in early pregnancy. Only LIF transcripts were detected, whereas CNTF and OSM transcripts were absent in the same endometrial samples (Fig. 1*B*). LIF transcripts were apparent by the end of the early-secretory phase on day 18 and persisted throughout mid- and late-secretory phases. LIF expression in the endometrium continued into pregnancy with low levels detected during the first trimester (Fig. 1*B*). In the mid-to-late second and third trimester, LIF transcripts were also detected at lower levels (total of eight samples analyzed, and data not shown). Expression of IL-6 in human endometrial epithelium has been previously described and was not reexamined (3).

Localization of LIF mRNA. Specific cell types expressing LIF transcripts were identified by *in situ* hybridization of proliferative and secretory phase endometrium (Fig. 2). Postovulatory endometrial samples ($n = 6$) obtained from women of proven fertility express LIF in the glandular and luminal epithelium but not in the stroma (Fig. 2 *C* and *D*). Proliferative samples, also from women of proven fertility ($n = 2$) show an absence of LIF expression in the glandular and luminal epithelia as well as the stroma (Fig. 2 *A* and *B*).

LIF Protein Expression in Endometrium. Western blot analysis of endometrial protein extracts were used to test the specificity of the anti-human LIF antisera (Fig. 1*D*). Two immunoreactive species of 45 and 40 kDa were resolved from endometrial lysates of secretory-phase samples (Fig. 1*D*, lane

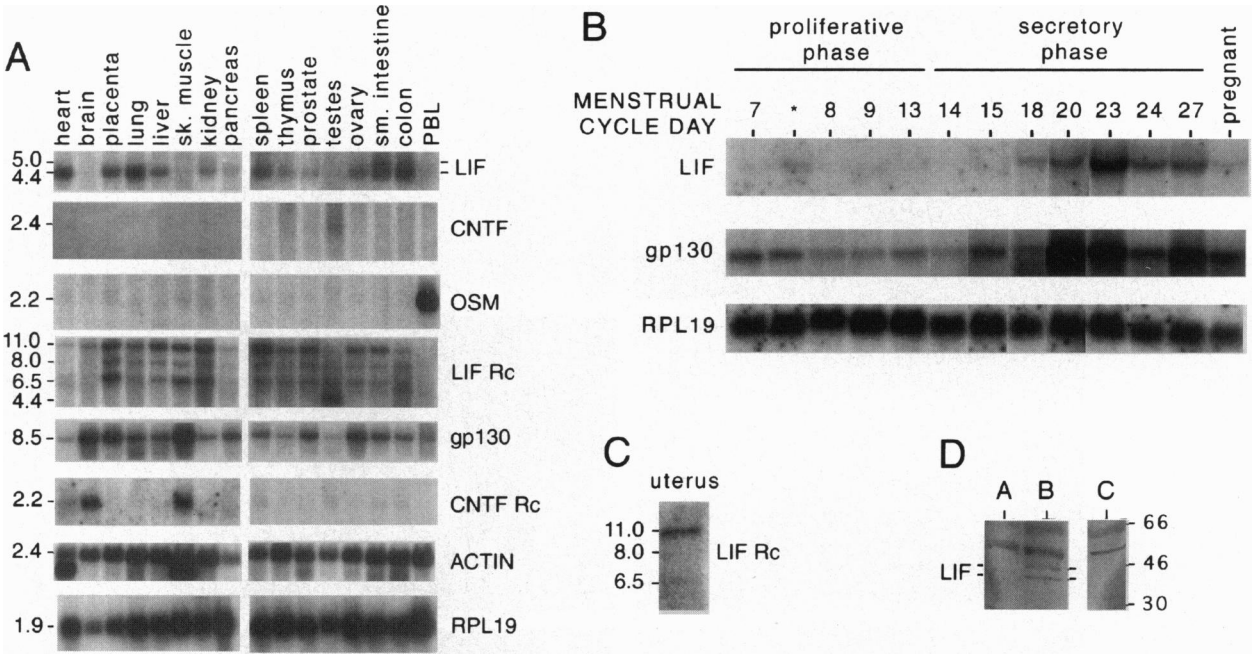

FIG. 1. Cytokine expression in human tissues and endometrium. (*A*) Northern analysis of human multiple tissue blots hybridized to LIF, CNTF, OSM, LIFrβ, gp130, and CNTFr. Actin and RPL19 probes were used to control for loading. (*B*) Northern analysis of RNA expression in human endometrium for LIF and gp130. Endometrial biopsy samples are presented as either in the proliferative or secretory phase and are designated by a cycle day, where the first day of menses is day 1, ovulation is day 14, and the menstrual cycle is 28 days. Endometrial RNA made from first trimester pregnancy is also shown. An RNA sample from a patient diagnosed as anovulatory is designated by an asterisk. The actin message increases during mid- and late-secretory phases and is not an adequate quantitative control for loading, as reported (23). However, the RPL19 transcript, which is nonresponsive to estrogen, can be used as an internal control for endometrial RNA loading (18). (*C*) Northern blot analysis of LIFrβ expression in 2 μg of purified mRNA from pooled human uteri. (*D*) Western blot analysis of human endometrial samples using anti-LIF antibody. Endometrial protein extracts from proliferative-phase day 6 (lane A) and secretory-phase day 18 (lane B). The anti-human LIF antiserum recognizes two protein bands at 45 and 40 kDa in secretory-phase samples but does not recognize these proteins in proliferative samples. Preimmune serum does not recognize these bands (lane C). In competition studies, antibody binding to the 45- and 40-kDa protein bands is competitively blocked with 50 μg of LIF peptide per ml. A second commercially available LIF antibody (R & D Systems) also recognizes these two bands (data not shown).

B). These species were absent from endometrium of the proliferative phase (Fig. 1*D*, lane A). Similar results were also obtained with a commercially available antiserum (R & D Systems; data not shown). Preimmune sera did not detect the 40- and 45-kDa protein species (Fig. 1*D*, lane C). The specificity of the anti-human LIF antibody was tested by competition assay using the LIF peptide, which resulted in disappearance of these two bands (data not shown).

Immunohistochemical staining with the LIF antibody detected LIF protein in the glandular and luminal epithelium, consistent with expression of LIF mRNA in the endometrium during the secretory phase of the menstrual cycle (Fig. 3). Immunoreactive LIF was observed in sections of endometrium from the mid- and late-secretory phases (Fig. 3*B*) but not in sections from the proliferative phase (Fig. 3*A*).

We also determined by Northern analysis that LIF was expressed in postimplantation endometrium and that the level of expression appeared higher during earlier stages of pregnancy (data not shown). An immunohistochemical analysis of the postimplantation endometrium showed LIF staining in the luminal epithelium is more intense in the first trimester (Fig. 3*C*) than in the second trimester (data not shown). In addition, at 5 weeks of gestation, strongly positive cells are detected in the decidualizing stroma at locations immediately adjacent to the luminal epithelium but more distal to the glands (Fig. 3 *C* and *D*). By 16 weeks of gestation, the staining in the decidua is largely absent (data not shown). Immunoreactivity could be competively blocked with LIF peptide (Fig. 3*E*) and was not detected with preimmune serum (Fig. 3*F*).

LIF Receptor Expression During the Normal Menstrual Cycle. Expression of LIFrβ and gp130 was examined in normal endometrial RNA samples (Fig. 1 *B* and *C*). gp130 transcripts were detected in all samples with expression increasing in mid- and late-secretory phases (Fig. 1*B*). LIFrβ transcripts could not be detected by Northern analysis of total RNA, possibly because of low levels of expression or partial degradation of high molecular weight RNA. However, an 11.0-kb LIFrβ transcript was the predominant message detected in purified mRNA from pooled samples of human uteri (Fig. 1*C*). CNTFr transcripts were not detected in human endometrium during any day of the cycle (data not shown), and the OSM receptor has not yet been identified.

Localization of LIF Receptor mRNA. Since the LIFrβ mRNA could not be detected by Northern analysis of total RNA, the presence of the mRNA was examined in the luminal epithelium by *in situ* analysis with an antisense LIFrβ mRNA probe (Fig. 2 *G* and *H*) from both secretory ($n = 3$)- and proliferative ($n = 2$)-phase endometria. LIFrβ transcripts were detected in the luminal epithelium but not in the stroma or glandular epithelium. gp130 was expressed throughout the cycle and was restricted to both the glandular and luminal epithelia, with expression peaking during the secretory phase of the cycle (Fig. 2 *E* and *F*). No gp130 was detected in the stromal tissue in any of the samples analyzed.

DISCUSSION

These studies show that the tissue specificity of LIF expression in most human tissues is essentially identical to those in the mouse (10, 24). Both OSM and CNTF expression is more restricted than LIF, with OSM only being detected in PBL. CNTF was expressed at low levels in the testis, similar to results seen in rats (25). Although the cytokines can display functional redundancy *in vitro*, it appears that their expression is strictly

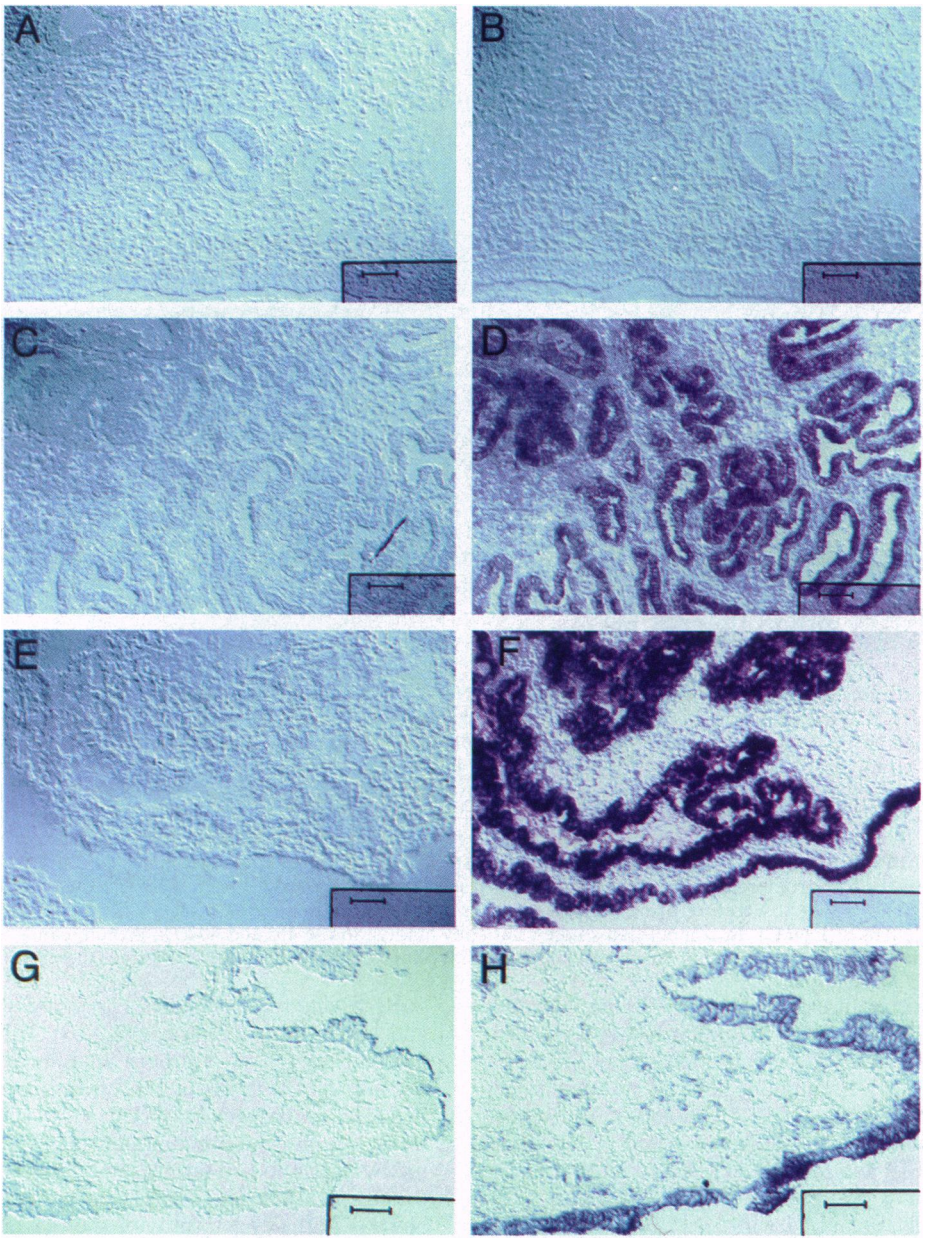

FIG. 2. *In situ* analysis of LIF and LIFrβ RNA expression in endometrium. (*A*) Proliferative-stage biopsy on day 12 of the cycle, digoxigenin (dig)-LIF sense probe. (*B*) Serial section of same biopsy as *A*, with dig-LIF antisense probe revealing no detectable expression of LIF. (*C*) Secretory stage biopsy on day 22 with dig-LIF sense probe. (*D*) Serial section of same biopsy as *C* with dig-LIF antisense probe. The LIF antisense probe binds specifically to luminal and glandular epithelia in secretory-phase samples but not to proliferative-phase samples. (*E*) Signal-transducing portion, gp130 sense probe, hybridized to late-secretory phase/menses endometrium. (*F*) Serial section of *E* hybridized to dig-gp130 antisense probe. (*G*) dig-LIFrβ sense probe hybridized to day 22 secretory-phase biopsy. (*H*) Serial section of same biopsy as *E*. dig-LIFrβ antisense probe hybridizes to the luminal epithelium.

regulated *in vivo* and that in any particular tissue the expression of more than one LIF-related cytokine normally does not occur.

Our analysis revealed that human LIF expression occurs predominantly in the uterine endometrium; that the onset of transcription starts around days 18–21 of the cycle, in agreement with previous observations (26, 27); and that uterine expression persists throughout the first trimester of pregnancy. A combined mRNA *in situ* and immunohistochemical analysis indicated that LIF expression is restricted to the glandular and luminal epithelium during the secretory/postovulatory phase of the cycle. Western analysis on the uterine samples revealed that two forms of the LIF protein are expressed. The molecular basis for producing these two forms has not yet been established and may either correspond to alternatively spliced forms or to differences in glycosylation that are found in the mouse (28, 29).

The patterns of LIF expression in the human and mouse endometrium differ slightly. In the mouse, two peaks of expression are detected, with the first occurring at ovulation and the second around the onset of implantation. Both bursts of expression last ≈24 hr (10, 11). The cellular localization of the ovulatory peak has not yet been determined, whereas the peak at implantation is restricted to the epithelium of the endometrial glands. In the human, we found no evidence to indicate a peak of expression occurring around the time of ovulation. Human LIF expression occurs in the luminal epithelium, as well as the glands, whereas, murine LIF expression is restricted to the glands. In both species, if pregnancy occurs, LIF expression in the endometrium persists in the luminal and glandular epithelium. During pregnancy additional and as yet unidentified strongly LIF-expressing cells are found in the

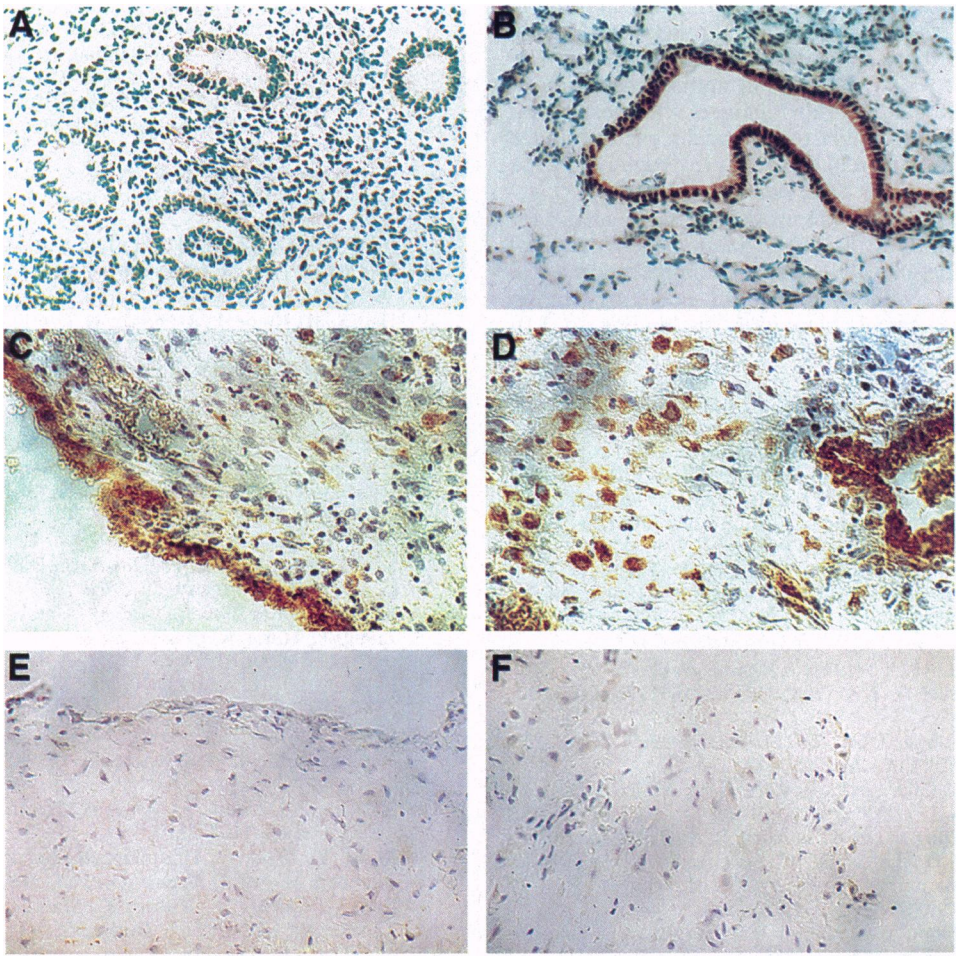

FIG. 3. LIF expression in human endometrium during the proliferative and secretory phase of the menstrual cycle and in pregnant endometrium. Immunohistochemical analysis of endometrial sections using rabbit anti-LIF antibody (*A*). LIF staining of endometrium late-proliferative to early-secretory phase. (*B*) LIF staining of glandular epithelium day 22 secretory phase. (*C* and *D*) LIF staining of pregnant endometrium, 5 weeks gestation. Note staining of both the glandular and luminal epithelium. (*E*) LIF peptide inhibition of LIF staining of decidualized endometrium. (*F*) Preimmune sera staining. Other staining cells, probably macrophages, are found scattered in the stroma (*C* and *D* from a total of six individual samples analyzed). The identity of the epithelial cells was confirmed by staining adjacent sections with rabbit anti-cytokeratin antibody (data not shown).

decidualizing stroma at locations immediately adjacent to the luminal epithelium. Likewise, low levels of LIF expression occur in murine endometrium and decidua throughout pregnancy (10). We do not yet know if the continued expression of LIF is essential for the maintenance of pregnancy.

The significance of any differences in LIF expression between the mouse and human is not yet apparent. In the mouse, it is unlikely that there is an absolute requirement for the ovulatory burst of LIF expression, since mice deficient in LIF can ovulate and form viable blastocysts. Implantation of these embryos can be induced in the LIF-deficient mice if an exogenous source of LIF is provided on day 3 of gestation, indicating that LIF expression at ovulation is not required for preimplantation development or embryo implantation (9).

Although there is an absolute requirement for LIF at implantation in the mouse, neither CNTF nor IL-6 are required, as mice deficient in either of these factors show no impairment in their development or reproduction (30–32). Similarly, humans deficient in CNTF have been identified with no overt phenotypic effect (33). As a preliminary attempt to determine whether defective LIF expression might have a role in human infertility, we analyzed expression in samples taken from 49 patients with some form of infertility. All but two patients expressed detectable LIF transcripts during their secretory phase, indicating that alterations in LIF expression are unlikely to be a major contributory factor to infertility in females. The cause for the apparent lack of LIF expression in the two remaining patients remains unclear, although it is still under investigation.

In LIF-deficient mice, decidualization does not occur after the appropriate hormonal priming followed by oil injection or traumatization of the uterus, revealing that LIF is normally required to place the endometrium in a receptive state where embryos can implant and the stroma decidualize (unpublished observations). Our analysis of the cellular pattern of expression of LIF and the LIFrβ in humans has provided some important indications as to how LIF may regulate implantation. The results reveal that expression of LIFrβ is restricted to the luminal epithelium, with expression of gp130 occurring in both the luminal and glandular epithelium. Neither of these two receptor components appears to be expressed in the stromal cells. A similar restriction in expression of these two factors has also been noted in the mouse (unpublished observations). The patterns of expression of LIF and its receptor strongly suggest that LIF does not directly affect the stroma but acts on the luminal epithelium, possibly through an autocrine/paracrine route. In response to elevated levels of estrogen (10), LIF may act to initially "sensitize" the luminal epithelium and this, together with an additional stimulus such as that associated with either blastocyst attachment or injection of oil into the uterine lumen, induces a luminal epithelium–stromal interaction, resulting in decidualization of the stroma. This is

consistent with the observations that initiating decidualization in rodents requires a stimulus to act through the luminal epithelium (34) and that in rats, decidua formation cannot be induced *in utero* in which the luminal epithelium has been removed (35). Whether LIF also induces changes in the luminal epithelium to permit blastocyst attachment and induce other factors, such as EGF-related factors (6), required by the implanting embryo remains to be determined.

In summary, these observations provide further evidence for the crucial role cytokines play in regulating fetal–maternal interactions during pregnancy. The data strongly support a role for the endometrial synthesis of LIF in mediating uterine receptivity for blastocyst implantation by binding to LIF receptors on the luminal epithelium.

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1. Finn, C. A. & Martin, L. (1974) *J. Reprod. Fertil.* **39,** 195–206.
2. Pampfer, S., Arceci, R. J. & Pollard, J. W. (1991) *BioEssays* **13,** 535–540.
3. Tabibzadeh, S. & Sun, X. Z. (1992) *Hum. Reprod.* **7,** 1214–1221.
4. Tabibzadeh, S. (1991) *Endocr. Rev.* **12,** 272–290.
5. Nelson, K. G., Takahashi, T., Bossert, N. L., Walmer, D. K. & McLachlan, J. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 21–25.
6. Threadgill, D., Dlugosz, A. A., Hansen, L. A., Tennenbaum, T., Lichti, U., Lee, D., LaMantia, C., Mourton, T., Herrup, K., Harris, R. C., Barnard, J. A., Yuspa, S. H., Coffey, R. J. & Magnuson, T. (1995) *Science* **269,** 230–234.
7. Pollard, J. W., Hunt, J. S., Wiktor-Jedrzejczak, W. & Stanley, E. R. (1991) *Dev. Biol.* **148,** 273–283.
8. Simon, C., Frances, A., Piquette, G. N., el, D. I., Zurawski, G., Dang, W. & Polan, M. L. (1994) *Endocrinology* **134,** 521–528.
9. Stewart, C. L., Kaspar, P., Brunet, L. J., Bhatt, H., Gadi, I., Kontgen, F. & Abbondanzo, S. J. (1992) *Nature (London)* **359,** 76–79.
10. Bhatt, H., Brunet, L. J. & Stewart, C. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11408–11412.
11. Shen, M. M. & Leder, P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8240–8244.
12. Gearing, D. P. (1993) *Adv. Immunol.* **53,** 31–58.
13. Pennica, D., Shaw, K. J., Swanson, T. A., Moore, M. W., Shelton, D. L., Zioncheck, K. A., Rosenthal, A., Taga, T., Paoni, N. F. & Wood, W. I. (1995) *J. Biol. Chem.* **270,** 10915–10922.
14. Hilton, D. J. (1992) *Trends Biochem. Sci.* **17,** 72–76.
15. Piquet, P. C., Grey, L., Mereau, A. & Heath, J. K. (1994) *Exp. Cell Res.* **213,** 340–347.
16. Kishimoto, T., Taga, T. & Akira, S. (1994) *Cell* **76,** 253–262.
17. Noyes, R. W., Hertig, A. T. & Rock, J. (1950) *Fertil. Steril.* **1,** 3–25.
18. Park, O. K. & Mayo, K. E. (1991) *Mol. Endocrinol.* **5,** 967–978.
19. Wilkinson, D. G. & Nieto, M. A. (1993) *Methods Enzymol.* **225,** 361–373.
20. Hsu, S. M., Raine, L. & Fanger, H. (1981) *Am. J. Clin. Pathol.* **75,** 734–738.
21. Lessey, B. A., Castelbaum, A. J., Buck, C. A., Lei, Y., Yowell, C. W. & Sun, J. (1994) *Fertil. Steril.* **62,** 497–506.
22. Daiter, E., Pampfer, S., Yeung, Y. G., Barad, D., Stanley, E. R. & Pollard, J. W. (1992) *J. Clin. Endocrinol. Metab.* **74,** 850–858.
23. Guo, J. Z. & Gorski, J. (1988) *Mol. Endocrinol.* **2,** 693–700.
24. Robertson, M., Chambers, I., Rathjen, P., Nichols, J. & Smith, A. (1993) *Dev. Genet.* **14,** 165–173.
25. Ip, N. Y., McClain, J., Barrezueta, N. X., Aldrich, T. H., Pan, L., Li, Y., Wiegand, S. J., Friedman, B., Davis, S. & Yancopoulos, G. D. (1993) *Neuron* **10,** 89–102.
26. Kojima, K., Kanzaki, H., Iwai, M., Hatayama, H., Fujimoto, M., Inoue, T., Horie, K., Nakayama, H., Fujita, J. & Mori, T. (1994) *Biol. Reprod.* **50,** 882–887.
27. Charnock, J. D., Sharkey, A. M., Fenwick, P. & Smith, S. K. (1994) *J. Reprod. Fertil.* **101,** 421–426.
28. Smith, A. G., Heath, J. K., Donaldson, D. D., Wong, G. G., Moreau, J., Stahl, M. & Rogers, D. (1988) *Nature (London)* **336,** 688–690.
29. Rathjen, P. D., Toth, S., Willis, A., Heath, J. K. & Smith, A. G. (1990) *Cell* **62,** 1105–1114.
30. Masu, Y., Wolf, E., Holtmann, B., Sendtner, M., Brem, G. & Thoenen, H. (1993) *Nature (London)* **365,** 27–32.
31. Poli, V., Balena, R., Fattori, E., Markato, A., Yamamoto, M., Tanaka, H., Ciliberto, G., Rodan, G. A. & Costantini, F. (1994) *EMBO J.* **13,** 1189–1196.
32. Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H. & Kohler, G. (1994) *Nature (London)* **368,** 339–342.
33. Takahashi, R., Yokoji, H., Misawa, H., Hayashi, M., Hu, J. & Deguchi, T. (1994) *Nat. Genet.* **7,** 79–84.
34. Finn, C. A. & Porter, D. G. (1975) *The Uterus* (Elek Science, London).
35. Lejeune, B., Van Hoeck, R. & Leroy, F. (1981) *J. Reprod. Fertil.* **38,** 235–240.