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Leukemia Inhibitory Factor Can Substitute for Nidatory Estrogen and Is Essential to Inducing a Receptive Uterus for Implantation But Is Not Essential for Subsequent Embryogenesis*

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ABSTRACT

A stage critical in mammalian development is embryo implantation. At this point, the blastocyst establishes a close interaction with the uterine tissues, a step necessary for its continued embryonic development. In many mammalian species, including man, uterine expression of the cytokine, leukemia inhibitory factor (LIF) is coincident with the onset of implantation and in mice LIF is essential to this process. The reasons for implantation failure have not been established. Here we show in LIF-deficient mice that up to the onset of implantation, changes in uterine cell proliferation, hormone levels, blastocyst localization, as well as expression of lactoferrin and Muc-1, do not differ from wild-types. However, the uterus fails to respond to the presence of embryos or to artificial stimuli by decidualizing. In mice, implantation and decidualization are induced by nidatory estrogen. We show that uterine expression of LIF is up-regulated by estrogen and LIF can replace nidatory estrogen at inducing both implantation and decidualization in ovariectomized mice. Implantation of LIF-deficient embryos in the LIF-deficient females, with normal development to term is rescued by ip injection of LIF. Transient expression of LIF on D4 of pregnancy is therefore only required to induce a state of receptivity in the uterus permitting embryo implantation and decidualization. LIF is neither required by the embryo for development nor for the maintenance of pregnancy. (*Endocrinology* **141:** 4365–4372, 2000)

IN MAMMALS, embryo implantation is an essential step in their reproduction. At this stage, the autonomously developing preimplantation embryo enters the uterine lumen and as a blastocyst, establishes a physically closer interaction with the uterine tissues. The extent of this interaction varies between species. In the human and in rodents, the trophoblast invades the uterine tissues and even replaces the capillary endothelium in the uterine blood vessels. In others such as the pig, the embryonic trophoblast remains juxtaposed to the uterine epithelium (1). In all species, the establishment of close physical contact between the embryo and uterine tissues is essential for the continuation of embryonic development.

In preparation for implantation, the uterine tissues undergo distinct cycles of cell proliferation and differentiation. These are induced by the ovarian steroid hormones estrogen (E_2) and progesterone (P_4) (2–5). Some of these changes are either directly mediated by the action of the hormones on the cells or are indirectly regulated through the induction of locally produced growth factors and cytokines, such as epidermal growth factor (EGF) (6, 7), and insulin-like growth factor 1 (IGF-1) (8, 9).

In conjunction with these cycles, the uterus undergoes a change in its receptivity. In mice, blastocysts cannot implant during the first 3 days following mating. Late on the fourth day until early on the fifth day, for about 18 h the uterus becomes receptive (10). At the onset of this period, the blastocysts are in close contact (apposition) with the luminal epithelium. With the start of implantation, the luminal epithelium adjacent to the mural trophectoderm undergoes apoptosis and the trophoblast cells migrate into the underlying endometrial stroma (11). The stroma responds by rapidly proliferating and differentiating to form the decidua. If implantation doesn't occur, the uterus becomes nonreceptive, refractory to implantation, and eventually re-enters the reproductive cycle.

In rodents, implantation is stimulated by a transient rise in circulating levels of E_2—the nidatory surge on the morning of the fourth day of pregnancy (12, 13). Whether E_2 stimulates implantation directly or through secondary factor(s) is currently an area of much interest. One factor essential for embryo implantation is the cytokine leukemia inhibitory factor (LIF) (14). LIF is transiently expressed in the glandular epithelium of mice at ovulation and again on the fourth day of pregnancy (15, 16). In other mammalian species, including the human, LIF expression in the uterus also is up-regulated

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around the onset of embryo implantation, suggesting that LIF may be of general significance to embryo implantation in mammals (17–19).

Female mice carrying a null mutation in the LIF gene are sterile because blastocysts do not implant. Reciprocal transfer of blastocysts between wild-type and LIF-deficient females showed that implantation failure was due to a defective maternal uterine environment rather than deficiencies in the embryo (14). The basis for the inability of the uterus to respond to blastocysts has not been established. Here, we show that in LIF-deficient female mice, up to the onset of implantation, uterine cell proliferation, hormone levels, gene expression, and embryo development does not differ from those observed in wild-type females. However, LIF-deficient uteri do not respond to some decidualizing signals. Embryo implantation in LIF deficient females can be rescued by ip injection of recombinant LIF with the embryos developing to term and surviving to adulthood. Implantation and decidualization can also be induced in hormone primed ovariectomized mice by substituting LIF for nidatory E_2, revealing that nidatory E_2 is only required to induce LIF. Uterine expression of LIF, under the control of nidatory E_2, is therefore essential for inducing a fully receptive state to the uterus and is not essential for subsequent embryonic development or for the maintenance of pregnancy.

Materials and Methods

Mice

LIF-deficient mice were maintained from a previously established colony (14) except that LIF deficient females were maintained on a mixed (BALB/cXC57BL6) background. All wild-type mice were (C57BL6XC3H) F1s. Animal care was provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86–23, 1985). Surgical procedures were performed under tribromoethanol (Avertin) anesthesia according to institutional guidelines. Hormonal priming of ovariectomized mice was performed according to previously established protocols (12) and were as follows; starting 14–18 days after ovariectomy for the first 3 days the mice were injected each day with 100 ng of estradiol-17β (E_2) in arachis oil. Three days later, the mice received 1 injection of 5 mg of 6α-methyl-17-hydroxy-progesterone acetate (Depo-Provera-P_4, Pharmacia, Inc., Peapack, NJ), followed 3 days later by a single injection of 40 ng of E_2. In some instances the last injection of E_2 was omitted or was substituted by ip injection of LIF or the microinjection of 3 μl of LIF or PBS into the right uterine horn. To induce decidualization, the right uterus of the hormone primed or control mice was injected with 50 μl of paraffin oil. The contralateral horn was used as a control. When embryos were used for implantation studies, the embryos were isolated from superovulated mice at the 8-cell stage and cultured overnight in KSOM (20) medium before their surgical transfer into the recipient uteri as blastocysts. For cell proliferation analysis, mice were injected with a single dose of BrdU of 100 μg/g body weight 15 h after the last injection of E_2. Two hours later, the mice were killed and the uteri processed for histological and quantitative analysis. Statistical comparisons were performed using Student's two tailed *t* test or oneway ANOVA to determine whether the treatments were significant ($P < 0.05$).

LIF production

Recombinant LIF was produced using pGeX-mLIF and was expressed as a glutathione S-transferase fusion protein in *Escherichia coli* JM109. The expression, purification, and cleavage of fusion mLIF protein was essentially performed as previously described (21). Purity was determined by inspection of silver-stained SDS-PAGE gels run in a Amersham Pharmacia Biotech. Phast gel system and the biological activity of LIF was determined by the Coomassie Plus Protein Assay and Ba/F3 cell proliferation assay (22).

Hormone measurement

P_4 levels were quantified using by RIA and performed according to the manufactures instructions (Diagostics Systems Laboratories, Inc., Webster, TX).

Histology

Tissues for routine histological analysis were fixed in 4% paraformaldehyde, embedded, sectioned at 6 μm and stained using H and E. Fixation for the BrdU labeled uteri was in 70% ethanol. The tissues were then processed and stained using an antibody to BrdU according to the manufacturer's instructions (DAKO Corp.). Uterine cells undergoing DNA synthesis were counted within a fixed area and expressed as the percentage of the total number of cells within the area. For alkaline phosphatase staining the tissues were fixed and processed according the established procedures although the tissues were embedded and sectioned in 55 C melting point wax (23).

Molecular analysis

Northern analysis and the measurement of LIF mRNA levels by RNase protection was performed as previously described (15) and quantified using NIH image quant software. Probes to Muc-1 and lactoferrin were generated by RT-PCR, cloned into pGEMTeasy and confirmed by sequencing. The primers for the murine Mucin-1 (Muc-1) cDNA were: forward, 5′-TCATCTCAGGACACCAGCAGTTC-3′; reverse, 5′-ACTGTGGACTACTGGAGAGCTGTTG-3′ and corresponded to the region in the Muc-1 cDNA between 1358 and 1657 bp. The primers for the murine Lactoferrin cDNA were: forward, 5′-TTGTGTGAACAGACCAGTGGGAG-3′; reverse, 5′-TTCTGCAAGACAGTGGAGTCCTTC-3′ and corresponded to the region between 1360 to 1740 bp in the cDNA.

Results

Uterine cell proliferation, gene expression, and P_4 levels in LIF-deficient mice

In the murine reproductive cycle, uterine levels of LIF rise significantly on two occasions. The first is at ovulation and the second, following mating, is on the morning of the fourth day of pregnancy (day plug ≡ day 1). Throughout the remainder of the cycle and during pregnancy LIF continues to be expressed in the uterus at basal levels (15, 16). As LIF is highly expressed at ovulation, it may be required to mediate E_2- and P_4-induced changes in uterine cell proliferation and gene expression before implantation. We compared the distribution and numbers of uterine cells undergoing proliferation in ovariectomized wild-type and LIF deficient females following injection with E_2 alone or in combination with P_4. In normal mice, E_2 alone induces proliferation in the glandular and luminal epithelium. When P_4 is given 3 days after E_2, it suppresses epithelial proliferation, induces morphological changes in the epithelium with the cells assuming a columnar and secretory phenotype and primes the stroma for proliferation. A second injection of E_2, 3 days later, induces stromal cell proliferation with DNA replication peaking 15 h after E_2 injection. A histological and quantitative comparison of the uteri following BrdU labeling revealed no statistically significant differences in the distribution and percentage of cells undergoing DNA synthesis between wild-type and LIF-deficient uteri following both hormonal regimens. This revealed that LIF expression was not essential to mediating the effects of E_2 and P_4 on uterine cell proliferation (Fig. 1A). Similarly, we compared the expression of

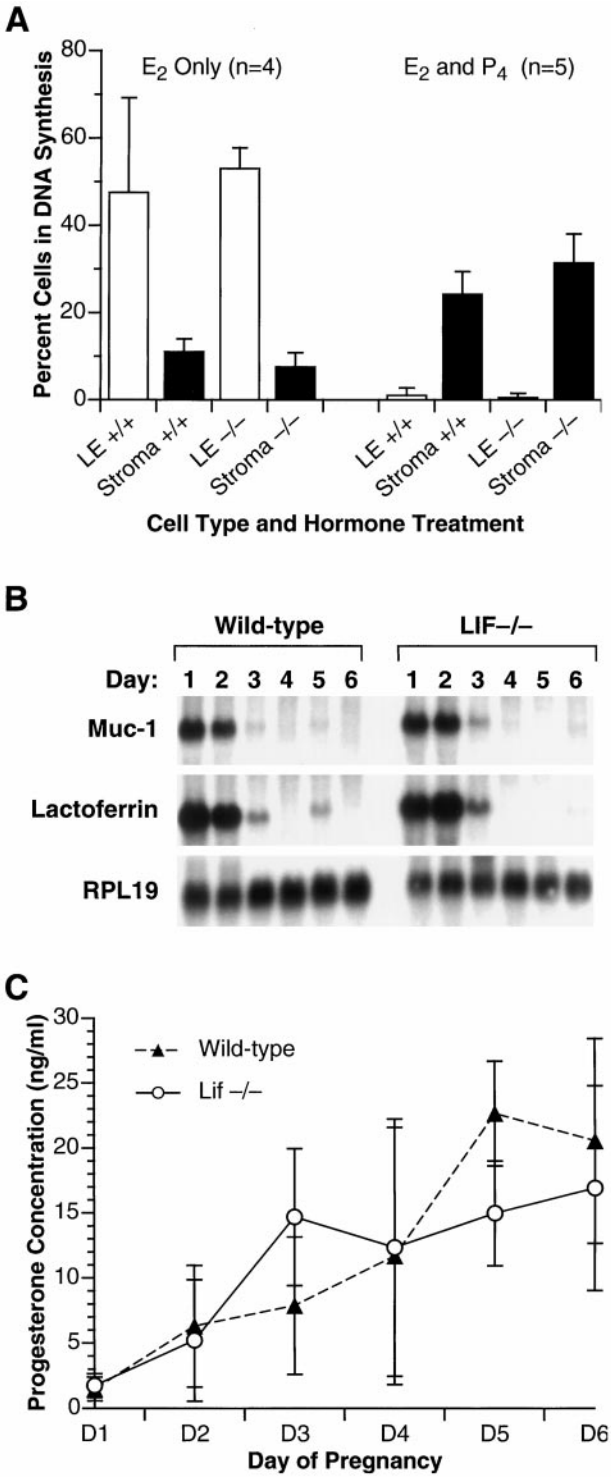

FIG. 1. A, Wild-type (+/+) and LIF-deficient (−/−) labeling indexes in the luminal epithelium (LE) and stroma following E_2 injection are identical. Similarly, P_4 injection following E_2 results in a shift of proliferation to the stroma in both genotypes. A comparison of the mean numbers of labeled nuclei in the epithelium and stroma of wild-type and LIF-deficient mice were compared and were found not to be statistically significant at the 95% confidence level. B, Uterine expression of lactoferrin and Muc-1 are the same in both genotypes except for low level expression of lactoferrin transcripts on D5 of pregnancy in +/+ mice. C, P_4 levels in both +/+ and −/− mice increase at similar rates during the first week of pregnancy.

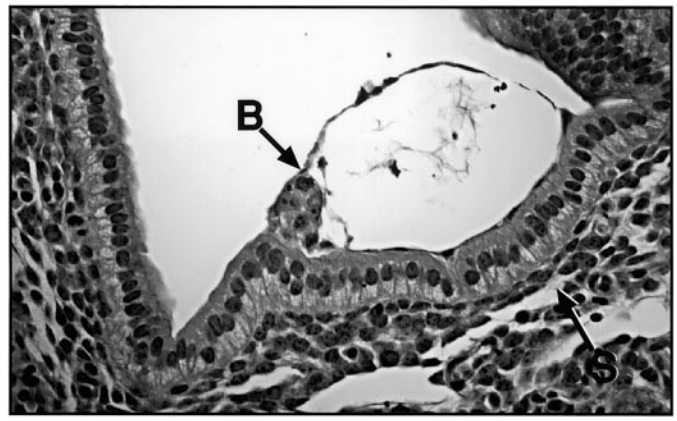

FIG. 2. A 7-day LIF−/− blastocyst (B) in apposition to the luminal epithelium in a LIF−/− uterus. The stroma (S) shows no evidence of decidualization and the luminal epithelium in contact with the blastocyst is intact and not undergoing apoptosis.

two proteins lactoferrin and Muc-1, which are expressed in the glandular and luminal epithelia and are regulated by E_2 and P_4 (24, 25). Both proteins are expressed in the epithelium during preimplantation development with their levels declining during the first 3–4 days of pregnancy. Again, we saw no detectable differences in their levels of expression in the uteri of wild-type and LIF-deficient mice apart from a weak signal on D5 in the wild-type uteri that was not seen in the LIF-deficient uteri (Fig. 1B). We also measured P_4 levels over the first 7 days of pregnancy following mating. Although there was substantial variation between individuals in the systemic levels of P_4, in both wild-type and LIF-deficient mice, P_4 concentrations steadily rose over the first 7 days following mating to levels previously reported, indicating that loss of LIF had no significant role in regulating P_4 levels (Fig. 1C). From these results, we conclude that LIF expression at ovulation is not a significant factor in mediating the changes in uterine cell proliferation, gene expression, and in the increase in P_4 during the first 7 days of pregnancy.

Preimplantation development in the LIF-deficient mice

In mice, the uterine morphology changes on the fourth day of pregnancy with the lumen's shape changing from a circular to slit-like profile. The blastocysts come to lie at the antimesometrial side of the lumen in apposition with the uterine epithelium. In normal pregnancies, blastocysts are in apposition to the luminal epithelium early on D4, with implantation starting late on day 4. By day 7, the embryo has formed an egg cylinder with the trophoblast invading the endometrium, which has also differentiated to form the decidua, making it impossible to flush embryos from the uterus. Previously, we had shown that blastocysts from LIF-deficient females could be readily flushed from the uteri of D7 pregnant mice (14). This indicated that the blastocysts were not tightly attached to the uteri and had not invaded the uterus. Histological analysis of D7 uteri from pregnant LIF-deficient females mated to LIF null males resulted in the identification of 13 blastocysts in four uteri. In all instances, the blastocysts had hatched from their zonae and were located at the antimesometrial side of the lumen. Twelve of the 13 blastocysts

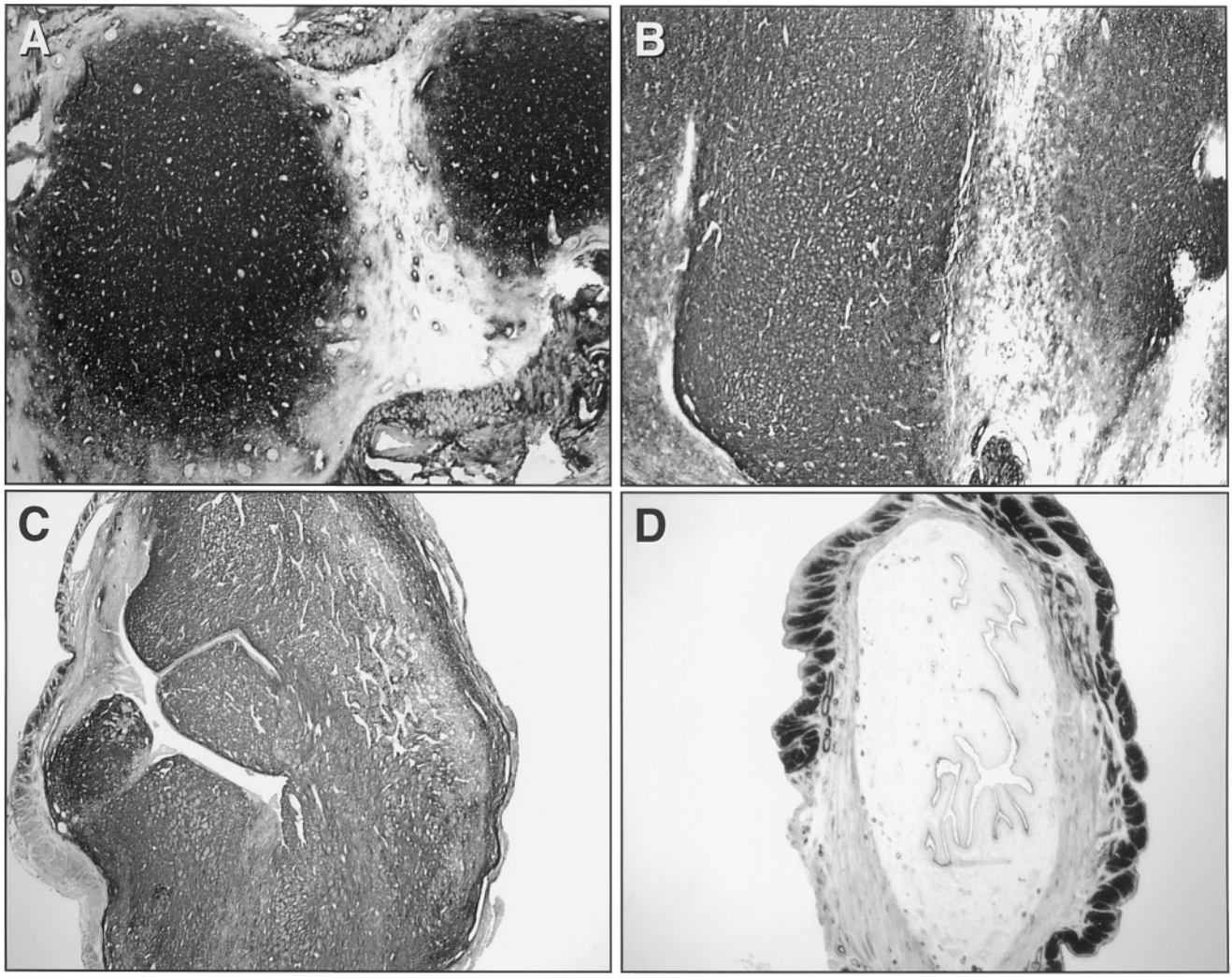

FIG. 3. A, Wild-type uteri decidualize following crushing in the absence of nidatory E_2. The black/gray staining is indicative of alkaline phosphatase activity, a marker for decidual cells (5/8 mice treated). B, Wild-type uteri decidualize following nidatory E_2 and oil injection (15/17 mice treated). C, LIF−/− uteri partially decidualize following crushing. (5/8 mice treated). D, LIF−/− uteri do not decidualize following E_2 and oil injection. (0/20 mice treated).

were in tight contact by their mural trophectoderm with the luminal epithelium, with many of the blastocysts being surrounded by the epithelium. The epithelium juxtaposed to the embryo showed no overt indication of it separating from underlying basement membrane or undergoing apoptosis, two morphological changes associated with the onset of implantation in normal mice. Furthermore, the underlying stroma retained a fibroblastic morphology with no evidence of it decidualizing (Fig 2). Similarly, systemic injection of pregnant LIF-deficient females, on day 5 with Pontamine Blue revealed no evidence of localized uterine edema, which is one of the earliest indications of implantation starting.

LIF-deficient mice fail to respond to some decidualizing stimuli

Our preceding analysis revealed preimplantation changes in the uterus and blastocyst localization did not differ in any significant way between wild-type and LIF-deficient females. The only difference was that in the LIF-deficient mice implantation and decidualization was absent. In receptive rodents, the uterus responds to the presence of an embryo by decidualization of the stroma. What triggers this response has been an area of considerable interest. Although the blastocyst is the normal stimulus, a variety of artificial stimuli can also induce a decidual response following appropriate hormonal priming. These stimuli have usually consisted of the injection of a small volume of oil into the lumen of hormonally primed uteri. However, more traumatic stimuli, such as crushing the P_4-treated uterus, are also effective and do not depend on E_2 (26). Oil injection into twenty ovariectomized, hormonally primed with E_2 and P_4 and nidatory E_2 LIF-deficient females, did not result in any indication that decidualization was induced, as assessed by an increase in uterine weight or the appearance of alkaline phosphatase positive stromal cells, a marker of decidualization. In wild-type uteri, decidualization was readily apparent following oil injection in 15/17 mice. However, when uteri of P_4-treated LIF deficient mice were crushed in several places using a

hemostat, decidualization was apparent in 5 out 7 wild-type uteri and in 5 of 8 LIF deficient uteri (Fig. 3, A–D).

Regulation of LIF expression and substitution of nidatory E_2 by LIF

Up-regulation of uterine LIF expression at ovulation and implantation is coincident with increased E_2 levels, suggesting that LIF expression is regulated by E_2. To directly determine that LIF could be induced by E_2 we injected ovariectomized wild-type mice with 100ng of E_2 and then assayed LIF mRNA expression in the uterus at various times after injection. Within 1 h, LIF levels had increased 3-fold, but were declining by 5 h and had returned to basal levels by 12 h after injection. Injection of P_4 had no effect on LIF expression (Fig 4). We then sought to determine whether LIF could substitute for nidatory E_2.

Female wild-type mice mated to fertile males were ovariectomized on the afternoon of the third day of pregnancy and injected with P_4. Three days later, the mice were injected ip with varying doses of recombinant LIF and the mice examined a further 3 days later for evidence that the embryos had implanted. The results are presented in Table 1a and show that a single injection of 10 μg was sufficient to result in embryo implantation at rates comparable to those achieved with E_2. Similar rates of implantation were also attained by giving 4 injections of LIF of 1 μg over an 8-h period. However, six injections of LIF at 0.5 μg were unsuccessful at inducing implantation. Injection of the mice with PBS did not result in implantation and unimplanted blastocysts showing the typical morphology of those in delay were recovered from the uteri of these females.

We then repeated these experiments by direct injection of LIF into the uterine lumen using ovariectomized wild-type females treated with P_4. Three days after P_4 injection, nidatory $E_2$was substituted by LIF, at doses ranging from 25 ng to 1 μg, which were injected into the right uterine horn in a 3 μl volume followed by the transfer of 5–8 blastocysts derived from 8-cell stage embryos cultured overnight. Three days later, the mice were examined for the presence of decidual swellings, which were then dissected to determine whether they contained embryos. These results are presented in Table 1b and show that LIF at a dose as low as 25 ng when injected directly into the uterine lumen of wild-type mice was

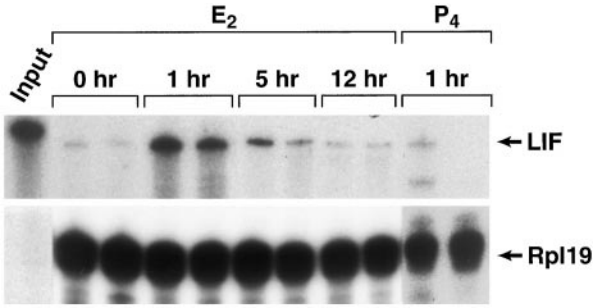

FIG. 4. E_2 induction of LIF. Ovariectomized mice were injected with 100 ng E_2 and total RNA isolated at different times after injection and measured by RNase protection assay. LIF transcripts rise rapidly within 1 h after E_2 and then decline to basal levels by 12 h. P_4 had no effect on LIF expression. Rpl19 was used as a loading control.

TABLE 1a. Dose of LIF by ip injection required to induce embryo implantation in ovariectomized +/+ mice without nidatory E_2

Treatment (μg/mouse × injections)	Nos. Mice ovxd	Nos. Mice with Deciduae	Mean Nos. Implanted Embryos
E_2 (25 ng)	8	6	7.8
LIF 10 μg × 1	8	7	8.6
LIF 1 μg × 4	10	8	6.4
LIF 0.5 μg × 6	6	0	0 (25 blastocysts recovered)
PBS/Saline	5	5	0 (27 blastocysts recovered)

TABLE 1b. Dose of LIF injected intraluminally to replace nidatory E_2 and induce embryo implantation in ovariectomized wild-type and LIF −/− mice

Nos. of Mice	LIF Dose μg	Nos. Embryos Transferred	Nos. Mice pregnant (Embryos Implanted)	Nos. Unimplanted Embryos Recovered
6	1	42	6 (24)	—
6	0.1	36	4 (14)	—
6	0.05	42	5 (6)	—
4	0.025	32	1 (5)	9
4	0	26	0	10

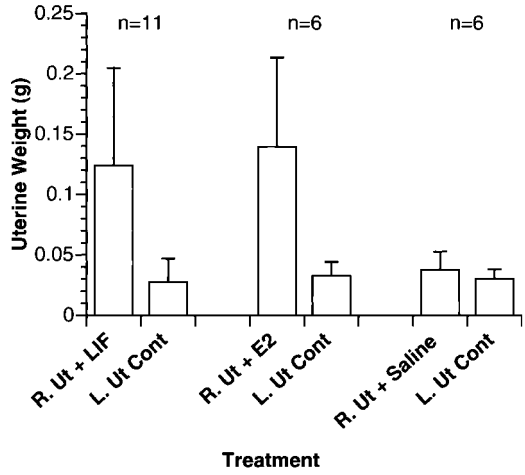

FIG. 5. Comparison between LIF injection and E_2 at the extent of decidualization induced following either treatment. Uterine wet weights were measured 3 days after injection of either factor. There was no statistically significant difference between either treatment ($P \geq 0.7$).

able to induce blastocyst implantation with subsequent normal embryogenesis. Controls, in which only PBS was injected into the lumen, did not result in blastocyst implantation and unimplanted blastocysts were recovered following flushing of the uteri. We also compared the extent to which decidualization could be induced by either LIF or E_2 following oil injection into the uterine lumen. Ovariectomized wild-type mice were hormonally primed with E_2 and P_4 and then injected with either 5 μg of LIF or 40 ng of E_2 3 days after P_4. Twelve hours later 50 μl of paraffin oil was injected intraluminally, and the uteri were removed and weighed 48 h later. These results are presented in Fig. 5 and show that LIF injection was equally effective as E_2 at inducing decidual-

TABLE 2. Rescue of pregnant LIF−/− by ip injection of LIF

Day of LIF injection (μg/mouse × injections)	Nos. mice injected	Nos. pregnant	Nos. offspring born (mean)
D4 (5 × 2)	25	10	28 (3)
D4 (2 × 3)	5	3	6 (2)
D5 (10 × 1)	8	2	7 (3)

ization with no statistically significant difference between LIF and E_2 in the level to which decidualization was induced.

Restoration of implantation and embryo development in LIF-deficient mice

Previously we had attempted to rescue the failure of blastocyst implantation in LIF-deficient mice by the administration of recombinant LIF to pregnant LIF-deficient females using miniosmotic pumps inserted into the peritoneal cavity (14). Although implantation was induced in a few of the females, all the implanted embryos had died by day 9 of pregnancy. Death may have been due to a requirement for LIF to sustain postimplantation development, as LIF is expressed at low levels in the uterus throughout pregnancy and the LIF receptor is highly expressed in the placenta (15, 27). Alternatively, embryonic failure may have been due to traumatic effects on their development due to the pump being placed in the peritoneum. We repeated these experiments, with LIF being administered by ip injection. LIF-deficient females were caged with LIF-deficient males and checked daily for copulation plugs. On the fourth or fifth day of pregnancy, mated females were given a single ip injection of recombinant LIF at various doses. Approximately half of the females became pregnant and delivered viable offspring 15–16 days later that continued their development to adulthood (Table 2). Southern analysis on their genotypes confirmed that all the offspring were homozygous for the mutated LIF allele (data not shown). These results demonstrated that administration of LIF to pregnant LIF deficient females restored embryo implantation, with the implanted embryos developing normally to term.

Discussion

Here we have further characterized the role of LIF in regulating embryo implantation in mice. Despite the relatively high levels of LIF expression in the uterus at ovulation, absence of LIF does not overtly affect preparation of the uterus for embryo implantation, as measured by a variety of parameters. Cell proliferation, gene expression and P_4 levels did not significantly differ from wild-type mice. Embryo development to the blastocyst stage was also normal with the blastocysts coming to lie at the antimesometrial end of the lumen, in tight contact with the luminal epithelium. There was, however, no evidence for the uterus in the LIF-deficient mice, even by day 7 of gestation, responding to the blastocyst by undergoing localized edema, apoptosis of the luminal epithelium or decidualization of the underlying stroma. Furthermore, it was apparent that the uterus in ovariectomized and appropriately hormonally primed, LIF-deficient mice was unresponsive to decidua inducing signals such as oil injection. Decidua formation, however, could be partially induced using traumatic stimuli such as crushing of the P_4 primed uterus.

In rodents, implantation and decidualization are normally initiated by a nidatory surge in E_2 levels (12, 13). The transient rise of E_2 on the fourth day of pregnancy has pleiotropic effects, inducing a variety of transcription factors, growth factors, and changes in cell proliferation in the uterine stromal cells (28). Some of these factors may be essential to the implantation process. Among these is LIF, in which transcription is up-regulated in the glandular epithelium within 1 h of estrogen administration, with expression persisting for 5–6 h before declining to basal levels. By substituting LIF for nidatory E_2 in ovariectomized mice, we showed that it is an essential factor downstream to E_2 that probably functions by initiating changes in the uterine epithelium that result in blastocyst implantation and decidualization. Furthermore, ip injection of LIF into pregnant LIF-deficient females was sufficient to rescue embryo implantation failure, resulting in the LIF-deficient females giving birth to viable offspring. This revealed that during the mouse's life cycle, LIF is essential for initiating implantation but is not required for embryonic development or for the maintenance of pregnancy as has been previously suggested (29). However, it remains unclear what is the role of increased levels of LIF at ovulation. One possibility is that this is a consequence of the high levels of estrogen at ovulation inducing LIF, but the uterus is unresponsive to LIF in the absence of P_4.

The ip doses of LIF required to induce implantation were relatively high. This may have been due to the rapid clearance of recombinant LIF from the circulation (t1/2 = 3–5 min) and that injected LIF is accumulated at high levels, by the liver, pancreas, spleen and lungs, preventing access of sufficient biologically active LIF to the uterus (30). This was supported by the observation that much lower doses of LIF when directly injected into the uterine lumen were effective at inducing embryo implantation.

How LIF acts to induce implantation is still not understood. It is possible that it could act on the hypothalamic-pituitary axis where LIF can influence hormone synthesis and therefore may indirectly affect ovarian and/or uterine function (31). However, at present we favor a paracrine mechanism in that the target for LIF's action in the uterus is the luminal epithelium. The heterodimeric LIF receptor, consisting of the LIFrβ and the transmembrane protein gp130, are localized to the glandular and luminal epithelia (17, 32, 33). Neither component is expressed at detectable levels in stromal cells. Secreted LIF has also been detected in uterine washings (34, 35), and we have evidence that intact luminal epithelium isolated from the uterus responds to LIF by the activation of a variety of signal transduction pathways including the phosphorylation and nuclear translocation of STAT transcription factors (33). Blastocysts also express the heterodimeric LIF receptor (32). However, it is unlikely that blastocyst responsiveness to LIF at implantation is essential, as embryos homozygous for loss of either the LIF receptor β or gp130, both of which are required to form a functional receptor, can implant and undergo postimplantation development (36, 37). Furthermore, direct injection of LIF, at relatively low doses, into the uterine lumen is effective at inducing embryo implantation. Therefore, LIF, secreted from

the glandular epithelium in response to a rise in nidatory E_2, binds to receptors on the luminal epithelium and so activates signal transduction pathways that result in transcriptional changes in the epithelium. In turn, these result in a change in receptivity of the luminal epithelium allowing the blastocyst that is in apposition, to start to invade the epithelium and underlying stroma. The stroma responds to the invading blastocyst and now responsive epithelium by undergoing localized decidualization at the site of implantation. Once these changes have been initiated, LIF is no longer required by either the mother or the embryo for fetal development to term. Because ovulation, fertilization, and development to the blastocyst stage occur in LIF-deficient females, and implantation is rescued by the injection of LIF, this demonstrates that preimplantation development of the embryo and preparation of the uterus up to blastocyst apposition with the luminal epithelium may also be independent of LIF.

Changes in uterine cell proliferation and gene expression are driven by the ovarian steroid hormones E_2 and P_4. These hormones act either directly on cells or through locally produced cytokines/growth factors that act in an autocrine/paracrine manner. The steroidal regulation of many growth factors and cytokines has been well documented (38, 39). However, which factors are essential to mediating the changes in uterine physiology in response to E_2 and P_4 is only being established by the use of gene targeting experiments or the identification of spontaneous (14, 40–44). As an example, the evidence for the epidermal growth factor family (EGF) regulating uterine cell proliferation in response to E_2 has been compelling (7, 45–48). It is nevertheless evident that there is substantial redundancy within this family of growth factors regarding their roles in regulating cell proliferation and other changes in the uterus, as mice simultaneously deficient for three of the factors (EGF, TGF-α and amphiregulin) are fertile (49).

In conclusion, transient expression of LIF in the uterus, induced by the nidatory rise in E_2 levels, at the time of embryo implantation, is essential to inducing a state of receptivity in the uterine epithelium, allowing both blastocyst invasion and stromal decidualization. LIF is neither required for both pre- and postimplantation embryogenesis nor for the maintenance of pregnancy. Current investigations are focused on determining what factors are regulated in the luminal epithelium by the action of LIF on this tissue. Identification of these factors should provide deeper insights into how this complex, but vital process of implantation is regulated.

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