Downregulation of cilia-localized Il-6Rα by 17β-estradiol in mouse and human fallopian tubes

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Am J Physiol Cell Physiol 297: C140–C151, 2009. First published May 6, 2009; doi:10.1152/ajpcell.00047.2009.—The action of interleukin-6 (IL-6) impacts female reproduction. Although IL-6 was recently shown to inhibit cilia activity in human fallopian tubes in vitro, the molecular mechanisms underlying IL-6 signaling to tubal function remain elusive. Here, we investigate the cellular localization, regulation, and possible function of two IL-6 receptors (IL-6Rα and gp130) in mouse and human fallopian tubes in vivo. We show that IL-6Rα is restricted to the cilia of epithelial cells in both mouse and human fallopian tubes. Exogenous 17β-estradiol (E2), but not progesterone (P4), causes a time-dependent decrease in IL-6Rα expression, which is blocked by the estrogen receptor (ER) antagonist ICI-182,780. Exposure of different ER-selective agonists propyl-(1H)-pyrazole-1,3,5-triyl-trisphenol or 2,3-bis-(4-hydroxyphenyl)-propionic acid demonstrated an ER subtype-specific regulation of IL-6Rα in mouse fallopian tubes. In contrast to IL-6Rα, gp130 was detected in tubal epithelial cells in mice but not in humans. In humans, gp130 was found in the muscle cells and was decreased in the perivascular and luteal phases during the reproductive cycles, indicating a species-specific expression and regulation of gp130 in the fallopian tube. Expression of tubal IL-6Rα and gp130 in IL-6 knockout mice was found to be normal; however, E2 treatment increased IL-6Rα, but not gp130, in IL-6 knockout mice when compared with wild-type mice. Furthermore, expression levels of IL-6Rα, but not gp130, decreased in parallel with estrogen-activated oocyte-cumulus complex (OCC) transport in mouse fallopian tubes. Our findings open the possibility that cilia-specific IL-6Rα may play a role in the regulation of OCC transport and suggest an estrogen-regulatory pathway of IL-6Rα in the fallopian tube.

estrogen; oocyte-cumulus complex transport

IN THE MAMMALIAN FALLOPIAN TUBE, luminal epithelial cells that consist of ciliated and secretory cells play an important role in the regulation of multiple tubal functions, including the transport of gametes and embryo and the regulation of tubal fluid flow (6, 26). Moreover, fallopian tube abnormalities result in pregnancy failures and irreversible infertility. Interleukin-6 (IL-6), a pro-inflammatory and immunoregulatory cytokine, exerts potent lesion effects (44, 52) in addition to its physiologic roles in reproduction (8, 29, 35, 41). IL-6 treatment in vitro inhibits cilia activity in human fallopian tube explants (39), and clinical studies show that circulating levels of IL-6 are increased in women with tubal ectopic pregnancies (10) compared with women with normal intrauterine pregnancies (53). These studies suggest that IL-6 signaling may be important for gamete and embryo transport and that abnormal level of IL-6 may have adverse effects on this process. As a member of the class I cytokine receptor family, IL-6 receptors mainly function through activation of intracellular signaling cascades such as STAT 3 and SOCS-1 (44, 52). The receptor comprises two distinct transmembrane proteins: the ligand-binding α-subunit (80-kDa glycoprotein, from here on termed IL-6Rα) and the signal-transducing β-subunit (130-kDa glycoprotein, referred to as gp130) (22, 27). Although binding to both subunits is necessary for signaling cascades, biochemical studies indicate that IL-6Rα can interact with IL-6 directly, whereas gp130 is the signaling coreceptor that mediates intracellular effects of IL-6 following binding to the IL-6Rα (19). Both IL-6Rα and gp130 have been found in human fallopian tubes (30, 59). However, it is not known whether the two receptors are uniformly distributed within the tubal cells, whether they can be regulated in vivo, or what might be the underlying mechanisms for this regulation.

The fallopian tube is a primary reproductive tissue target for estrogens and other steroid hormones (20). The modulation of estrogen receptors (ER), acting as ligand-dependent transcriptional regulators (18), contributes to a diverse array of fallopian tube functions in vivo (1, 20). ERα and ERβ, transcribed from different genes, are the two major ER subtypes identified to date (12, 25). The differential expression, localization, and ligand specificity of ER subtypes (37) and the diverse defects in mouse ovary and uterus following ablation of either ERα/β, ERα, or ERβ (17) suggest that tissue-specific interplay between the two ER subtypes occurs and that there are possible alternate biological functions of these receptors in vivo (4). Whereas ERα is expressed in all cell types in the human and rodent fallopian tubes, research in our laboratory first observed that ERβ is expressed predominantly in the cilia of tubal epithelial cells in rodents (45, 50). Moreover, estrogens have been reported to regulate IL-6 both positively and negatively (57). Therefore, understanding how estrogens regulate the IL-6/IL-6R signaling pathway locally is critically important for...
the molecular interpretation of IL-6 receptor-mediated tubal physiology, and this knowledge may contribute to the development of therapies to treat tubal disease.

To explore the function of the IL-6/IL-6 receptor signaling pathway in the fallopian tube, we investigated the cell specificity of IL-6 receptor expression in both mouse and human fallopian tubal tissues. To establish a physiological role for IL-6 receptors in the appropriate context, we also investigated the molecular and functional processes that mediate the hormonal regulation of IL-6Rα and gp130 expression in mouse fallopian tubes in vivo.

MATERIALS AND METHODS

Antibodies, hormones, and reagents. The primary antibodies used for either Western blot (WB) or immunohistochemistry (IHC) assay were the following: rabbit anti-IL-6Rα (M-20/C-20, 1:250 for WB, 1:50 for IHC, Santa Cruz Biotechnology), biotinylated mouse anti-IL-6Rα (MAB1830, 1:20 for IHC, R&D Systems), rabbit anti-gp130 (M-20, 1:500 for WB, 1:100 for IHC, Santa Cruz Biotechnology), mouse anti-β-tubulin IV (ONSL1A6, 1:500 for WB, Sigma, St. Louis, MO), mouse anti-pan-cytokeratin (C-11, 1:1,000 for WB, Sigma), mouse anti-α-smooth muscle (SM) actin (1A4, 1:1,000 for WB, Sigma), and rabbit anti-Era (MC-20, 1:200 for WB, Santa Cruz Biotechnology). The optimal working dilution was based on different preliminary experiments at different dilutions reported herein. Specificity of the IL-6Rα antibodies was verified by incubating the primary antibody with the corresponding synthetic peptide before use in WB and IHC analyses. Alkaline phosphatase-conjugated goat-anti-mouse IgG (A-1682, Sigma) and alkaline phosphatase-conjugated goat-anti-rabbit IgG (AC31RL, Tropix, MA) were used as secondary antibodies for WB analysis. Tyramide Signal Amplification Biotin system (NEL 700, PerkinElmer Life and Analytical Sciences), Alexa Fluor 488 streptavidin conjugate (emitting green light), and Alexa 488/594 (emitting green/red light) donkey anti-rabbit IgG (Molecular Probes) were used for immunofluorescence assays. 17β-Estradiol (E2) and progesterone (P4) were purchased from Sigma. ERα-selective agonist (55) propyl-(1H)-pyrazole-1,3,5-triyl-trisphenol (PPT), ERβ-selective agonist (32) 2,3-bis-(4-hydroxyphenyl)-propionitrile (DPN), and ER antagonist ICI-182,780 (Faslodex) were purchased from Tocris Cookson (Bristol, UK). Other reagents were purchased from Sigma or Merck AG (Darmstadt, Germany) and were of the highest purity grade available.

Experimental animals. All studies were performed with the approval of and in accordance with guidelines established by the local ethics committee at the University of Gothenburg, Sweden. Intact prepubertal female C57BL/6 mice (25 days old) were obtained from Taconic M&B (Copenhagen, Denmark). The environment of the animal rooms was controlled with a 12-h/12-h light-dark cycle, a relative humidity of 45–55%, and a temperature of 21°C. The animal rooms were controlled with a 12-h/12-h light-dark cycle, a relative humidity of 45–55%, and a temperature of 21°C. The mice had free access to tap water and standard pellet chow. (Teklad global 16% protein rodent diet, 2016, Harland). 2016 does not contain alfalfa or soybean meals, thus minimizing the occurrence of natural phytoestrogens. Mice deficient in IL-6 were originally generated by Merck AG (Darmstadt, Germany) and were of the highest purity grade available.

Hormone treatment and mouse tissue preparation. To examine whether exogenous estrogen and progesterone regulated tubal IL-6 receptor expression, 21-day-old female mice were injected intraperitoneally with either E2 (0.3 mg/kg), or P4 (4 mg/kg), or vehicle (100 μl sesame oil, Sigma). The E2 and P4 treatment paradigm is based upon previous studies of the regulation of estrogen receptors and progesterone receptors in the mouse fallopian tube as it relates to tubal function (45, 51). To determine the effect of steroidal ER antagonist ICI-182,780 on the regulation of IL-6 receptor expression, 21-day-old female mice received (ip) either E2 (0.3 mg/kg), ICI-182,780 (8.3 mg/kg), E2 (0.3 mg/kg) and ICI 182,780 (8.3 mg/kg), or vehicle (100 μl sesame oil) (45). Ideally, the effects of exogenous estrogen on target tissues are studied in ovariectomized animals deficient in endogenous estrogen secretion. However, this approach was intentionally avoided in our study since the mouse and rat ovaries are surrounded by a bursa allied with the infundibulum and the ampulla of the fallopian tube (3). It is therefore possible that ovariectomy-induced trauma to the ampulla might complicate the interpretation of the results more than endogenous estrogen would be likely to do. Intact prepubertal (21–26 days old) rodents used in our previous studies have shown extremely low levels of endogenous E2 concentration (45, 51). In the present study, differences between uterine weights of mice given estrogen and ICI-182,780 indicate that the substances and dosages administrated were sufficient to affect estrogen-responsive tissues. To further determine the specific ER subtype-mediated regulation of IL-6 receptor expression, 21-day-old female mice were injected (ip) either E2 (0.3 mg/kg), PPT (0.1 mg/kg), DPN (0.1 mg/kg), or vehicle (100 μl sesame oil) (45, 50). It is worth noting that the use of ER subtype-selective ligands (PPT and DPN) provides indispensable tools for probing the biological roles of the two ERs in vivo. Mice were euthanized at various time points (indicated in text or legends), and the fallopian tubes were quickly collected, cleaned, and weighed. One fallopian tube from each animal was immediately frozen in liquid nitrogen and stored at −70°C for WB analysis. The other was fixed in 4% formaldehyde neutral buffered solution for 24 h at 4°C and was embedded in paraffin for histochemical analysis. Protein was harvested from a matched portion of the organ in all instances to assure that the results did not differ because of intra-organ heterogeneity.

Superovulation and tissue collection in mice. Intact prepubertal female mice (25 days old, 13–15 g) were superovulated using a hormonal regimen in which 5 IU of pregnant mare serum gonadotrophin (PMSG, Sigma) was injected intraperitoneally followed 48 h later with 5 IU of human chorionic gonadotrophin (hCG, Organon, Oss, Holland) injected intraperitoneally (47). Ovulation was confirmed in hormone-injected animals based on the presence of the corpus luteum. In preliminary studies these doses of PMSG and hCG were found to result in a physiological number of ovulations (between 9 and 12). Superovulated mice were injected intraperitoneally with either E2 (0.3 mg/kg), E2 (0.3 mg/kg) and ICI-182,780 (8.3 mg/kg), or vehicle (100 μl sesame oil) at 12 h after hCG treatment. Mice were euthanized at various time points (indicated in text or legends), and the fallopian tube was quickly collected. The time required for oocyte transport after ovulation was established by direct observation of the cumulus-oocyte complex using a microscope to see through the tubal wall. After the fallopian tube released the cumulus-oocyte complex into PBS, the fallopian tubes from each animal were immediately frozen in liquid nitrogen and stored at −70°C for subsequent WB analysis.

Human studies. An isthmus portion of the fallopian tubes was obtained from 11 fertile women, aged 28–42 yr, undergoing laparoscopic tubal ligations for sterilization at Sahlgrenska University Hospital (49). Informed consent from the patients and approval by the institutional committee for the use of human subjects in research at the University of Gothenburg were obtained before collection of tissue samples for this study. All women had regular menstrual cycles (25–32 days) and did not receive any form of hormonal treatment in the 3 mo before the procedure. Biopsies were classified according to the criteria of Groothuis and colleagues (13).

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR). Total cellular RNA was isolated from the fallopian tubes of individual animals using the RNeasy Micro Kit (Qiagen, Germany) according to the manufacturer’s instructions and treated with RNase inhibitor (Applied Biosystems, Foster City, CA). A single batch of 0.5
µg of total RNA from each sample was subjected to synthesis of single-stranded cDNA using high-capacity cDNA reverse transcription kits (Applied Biosystems). Primers were synthesized to encompass a specific segment of the cDNA sequence of mouse IL-6Rα (38) (sense, 5′-AAGTGAATCTCCAGGGTGCC-3′ and antisense, 5′-GCTTCCTGGCTGCTG-3′), mouse gp130 (IL-6Rβ) (38) (sense, 5′-CCATCAAGGAACAGGCGCTC-3′ and antisense, 5′-GCATGAA TGAATCGGACTG-3′), and mouse gp130 (IL-6Rβ) (38) (sense, 5′-GGACGCAGTTGGGATCTC-3′). The corresponding cDNA fragments were denatured at 94°C for 2 min, annealed at 57°C for 40 s (except for 3′ and antisense, 5′-GGACGCAGTTGGGATCTC-3′), and of β-actin (sense, 5′-GGTCTGCGGTCTT TAGCCCAT-3′ and antisense 5′-GCAACCGATCCACACAGGT-3′). The PCR products were analyzed on a 1.5% agarose gel, and the bands were visualized using ethidium bromide during exposure to a UV transilluminator.

**Protein extracts and WB analysis.** Whole tissue extracts for protein preparations and WB analysis were essentially carried out as described previously (45). Fifty micrograms of protein were directly electrophoresed on 4–12% one-dimensional bis-Tris gels (Novex, San Diego, CA). In experiments in which both IL-6Rα, gp130, pan-cytokeratin, and α-SM actin were assessed, the gel was cut into two pieces along a line corresponding to ~64 kDa. The section containing higher molecular weights was exposed to the anti-IL-6Rα or anti-gp130, and the lower molecular weight section was exposed to anti-pan-cytokeratin or anti-α-actin. The immunosignal-CDP-Star substrate for alkaline phosphatase system (Tropix, Bedford, MA) was used to visualize protein bands. Immunobblotted signals were visualized using a LAS 1000-cooled charge-coupled device camera (Fujifilm) and ECL-film (Amersham). The amount of protein loaded was quantified by densitometry using the Image Gauge software (Fujifilm). In no case did secondary antibodies alone result in visualization of any immunopositive bands. The correct loading was evaluated by staining the gels with Coomassie blue. Signal intensities of the individual protein were normalized to the gels stained with Coomassie blue and presented as ratios that represent arbitrary densitometric units of relative abundance.

**Immunoprecipitation studies.** Immunoprecipitation (IP) was performed as described elsewhere (46). Tubal protein extracts (250 µg) were immunoprecipitated with 5 µg of IL-6Rα, gp130, or β-tubulin IV antibodies at 4°C overnight. The immunocomplexes were precipitated with 50 µl of Pansorbin cells (Calbiochem, EMB Biosciences, La Jolla, CA) for 4 h at room temperature. The bound proteins then underwent three sequential washes with 1 ml of RIPA buffer, including 10 mM iodoacetamide to prevent nonspecific disulfide linkages. They were subsequently washed twice with 1 ml of PBS and then processed for WB analysis to detect β-tubulin IV protein expression as described above.

**IHC analyses.** Two different methods of IHC were performed and confirmed in the same fallopian tube tissues. Single-3,3′-diaminobenzidine (DAB) IHC was based on the methodology described previously (45, 50). For immunofluorescence (IF) assay, fallopian tube sections were deparaffinized and rehydrated. Antigen retrieval was performed by boiling the sections in 0.05 M citrate buffer (pH 5.5) for 10 min. Nonspecific binding was blocked for 30 min with 0.5% blocking reagent (FP1020, NEN Life Science Products) in PBS-B2 (0.05% Tween-20). Primary antibody was applied to the sections in PBS-B2 (0.05% Tween-20). The correct loading was evaluated by staining the gels with Coomassie blue. Signal intensities of the individual protein were normalized to the gels stained with Coomassie blue and presented as ratios that represent arbitrary densitometric units of relative abundance.

**Assessment of circulating hormones.** Blood was taken by cardiac puncture, and serum was collected and stored at ~70°C. E2 and P4 levels were determined using radioimmunoassay kits (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland).

**RESULTS**

**Expression of IL-6Rα and gp130 in mouse fallopian tubes.** We first sought to determine whether IL-6Rα and gp130 were present in mouse fallopian tubes using both RT-PCR and WB analysis. As indicated in Fig. 1A, mRNA for the endogenous IL-6Rα and gp130 genes were detected in the fallopian tubes of immature mice. The protein levels of IL-6Rα and gp130, as analyzed by WB, were consistent with the relatively high mRNA expression levels of these specific IL-6 receptors (Fig. 1B). Preabsorption of the IL-6Rα antibody with an excess amount of corresponding antigen peptide (1:2) blocked detection of the IL-6Rα protein band in the fallopian tubes (Fig. 1B).

**Distinct cellular localization of IL-6Rα and gp130 in mouse fallopian tubes.** Because the fallopian tube is a complex tissue composed of functionally and morphologically distinct cell types (45, 50), we investigated the expression patterns of IL-6Rα and gp130 using immunofluorescence microscopy. In all regions examined, cell type-specific expression of IL-6 receptors was apparent. With the use of both polyclonal (rabbit) and monoclonal (mouse) antibodies, specific labeling for IL-6Rα was identified in the tubal epithelium with prominent staining of the apical membrane, placing it close to gametes and/or early embryos during tubal transport (Fig. 2A, and supplemental Fig. A and B). The intensity of this immunostaining was robustly reduced (Fig. 2A, bottom) following preabsorption of the IL-6Rα antibodies with the corresponding antigen peptide, indicating that these labels are IL-6Rα specific. Brightfield images of staining using DAB revealed a consistent cilia-localized immunoreactivity for IL-6Rα in mouse fallopian tubes (Fig. 2B). There was no detectable staining when both IL-6Rα antibodies were omitted, replaced by nonimmune serum. Furthermore, there was a progressive decrease in the proportion of IL-6Rα-positive cells from the infundibulum to the isthmus regions (Fig. 2A), in agreement with the gradient of the ciliated epithelial cell number found in the fallopian tubes (1, 20, 28). Similar to IL-6Rα, gp130 was observed in the tubal epithelia with most pronounced immunoreactivity in the epithelial cell membrane. However, gp130 was found to have a diffuse distribution throughout the cytoplasm (Fig. 2C). Levels of both IL-6Rα and gp130 were undetectable in tubal smooth muscle cells (Fig. 2, A and C). Specificity of IL-6Rα and gp130 staining was based on negative controls that featured either the omission of primary antibodies (Fig. 2C, right), omission of both primary and secondary antibodies, or gradual overloading
Specific downregulation of IL-6Rα by 17β-estradiol via ERα in mouse fallopian tubes. To determine whether ovarian steroid hormones could regulate IL-6 receptor expression in mouse fallopian tubes, WB analysis was performed in mice treated with E2 or P4. The expression of IL-6Rα protein was gradually downregulated from 6 h up to 48 h following E2 treatment but not following P4 treatment (Fig. 3, A and B). In contrast, no alteration of gp130 was noted in mice treated with E2 or P4. Furthermore, concomitantICI-182,780 treatment attenuated E2-induced downregulation of IL-6Rα expression (Fig. 3C). Because both ERα and ERβ have similar binding affinity for E2 and estrogenic substances (24), we examined which ER subtype was involved in an estradiol-mediated inhibition of IL-6Rα in mouse fallopian tubes. Whereas PTT (an ERα-selected agonist) caused a downregulation of IL-6Rα expression comparable to E2 (Fig. 3A), DPN (an ERβ-selected agonist) failed to regulate IL-6Rα expression (Fig. 3D). IL-6Rα expression and ER agonist/antagonist systems vary concurrently and inversely. Whereas the role of ER subtypes in the regulation of cellular responsiveness to their selective ligands may merit consideration in dosing regimens of E2-like pharmaceutical compounds in vivo, a significant decrease in the speed of ovulated oocyte-cumulus complexes (OCC) transport has been recently documented after treatment with DPN in a dose-dependent manner in rat fallopian tube (48). To strengthen the observation that the lack of reduction of IL-6Rα by DPN in mouse fallopian tube, both dose (10, 100, and 250 mg/kg) and time course (6, 12 and 24 h after the 100 mg/kg dose) experiments were performed in two independent groups of animals. Neither dose nor time point (with a selected dose of DPN) was observed to alter IL-6Rα expression in mouse fallopian tube (data not shown). Therefore, it is likely that estrogenic regulation of IL-6Rα expression is not fully dependent on ERβ in the fallopian tube. In addition, no significant changes were observed in circulating level of IL-6 in mice treated with E2, P4, or ICI-182,780 (data not shown).

IL-6 depletion blocks estrogenic suppression of IL-6Rα in mouse fallopian tubes. To gain mechanistic insights into whether E2-induced downregulation of IL-6Rα in mouse fallopian tubes requires IL-6 signaling, WB analysis was performed in IL-6−/− and WT mice treated with E2 or vehicle. Although there was no difference in IL-6Rα expression between IL-6−/− and WT mice treated with vehicle, opposite trends in IL-6Rα expression were observed in IL-6−/− and WT mice following E2 stimulation (Fig. 4, A and B), suggesting that the expression of IL-6Rα is not required for IL-6 expression but is critical for E2 stimulation. However, E2 treatment had no effect on gp130 protein levels in IL-6−/− and WT animals (Fig. 4, A and C). Similar ERα protein levels were detected in IL-6−/− and WT mice, and ERα was downregulated by E2 in both IL-6−/− and WT mice (Fig. 4, A and D). Whereas no apparent differences in serum P4 levels were observed (Fig. 4F), serum E2 levels were increased in mice treated with E2 (Fig. 4E).

IL-6Rα correlates with E2-enhanced OCC transport in mouse fallopian tubes. Once superovulation was achieved by gonadotropin (PMSG/hCG) stimulation in mice, the ovulated OCC were recovered in the enlarged and translucent ampulla of the fallopian tube at 24 h after hCG treatment (Fig. 5A). As shown in supplemental Fig. 2, in the ampulla,
the expression of IL-6Rα was clearly visible in the cilia. PMSG-induced increase in endogenous E2 levels coincide with a downregulation of IL-6Rα expression in the fallopian tube (Fig. 5C), consistent with the effects seen following exogenous E2 treatment (Fig. 3, A and C). These data demonstrate that downregulation of IL-6Rα may be induced by estrogens in the tube. Exogenous E2 treatment has been shown to enhance fallopian tubal transport acceleration in vivo (36). To further understand the physiological relevance of IL-6 receptors in the fallopian tube transport, we examined the consequences of manipulation of OCC transport in superovulated mice treated with E2 or E2 in the presence of ICI-182,780. As shown in Fig. 5B, we observed no effects of E2 or E2/ICI-182,780 on oocyte transport after 24 h. When examined at 36 h after hCG treatment, 20% of the mice treated with E2 showed tubal retention of OCC, as opposed to mice treated with the vehicle (80%) or E2/ICI-182,780 (100%). The high rate of tubal OCC retention following E2/ICI-182,780 treatment was notable (80%) compared with a 0% retention rate in mice treated with the vehicle or E2 48 h after hCG treatment (Fig. 5B). Furthermore, there was a time-dependent decrease in IL-6Rα, but not in gp130 expression, in E2-treated mice compared with mice treated with the vehicle (Fig. 5C). Again we saw that estrogentic downregulation of IL-6Rα was blocked by ICI-182,780, consistent with our previous data (Fig. 3C).
Fig. 3. Time-dependent regulation of IL-6Rα and gp130 protein expression in mouse fallopian tubes following steroid hormone treatment. A: IL-6Rα expression, but not gp130, was decreased following E2 injection in a time-dependent manner. B: no regulation of IL-6Rα or gp130 was found following injection of P4. C: ICI-182,780 treatment blocked the E2-induced downregulation of IL-6Rα. D: PPT treatment resulted in a downregulation of IL-6Rα, whereas DPN had no effect on IL-6Rα expression, indicating that ERα is a critical component in mediating estrogenic regulation of ciliated IL-6Rα expression. The expression of pan-cytokeratin protein was included as a positive control for protein content of epithelial cells. Tubal tissues were pooled (five organs per lane at each time point). Values are presented as means ± SE of three independent observations. Data were analyzed by Kurskal-Wallis test followed by Mann-Whitney U-test for individual group comparisons. *P < 0.05 vs. each control sample.
Expression and regulation of IL-6Rα and gp130 in human fallopian tube. To determine whether any diversity in IL-6 receptor expression exists in human fallopian tubes compared with mouse fallopian tubes, immunofluorescence and WB analyses were performed in fallopian tubes from women with normal reproductive cycles. Staining of human fallopian tube tissues (Fig. 6A) with IL-6Rα antibody showed that IL-6Rα protein was expressed in the cilia of epithelial cells (Fig. 6A1), in agreement with results from mouse fallopian tubes (Fig. 2A). However, no immunostaining of gp130 was found in the...
epithelial cell layer, although its immunoreactivity was detected in the tubal muscle cells (Fig. 6B). An adjacent tubal section with no primary antibody applied is presented as a negative control (Fig. 6C). As shown in Fig. 6D, although statistically insignificant, there was a trend toward decreasing IL-6Rα expression in both periovulatory and luteal phases. In addition, a significant reduction of gp130 protein level in both periovulatory and luteal phases was noted.

**DISCUSSION**

The principal finding from our study is that the regulation of cilia-specific IL-6Rα following modulation of estrogenic signaling may be involved in the OCC transport process. We provide the first in vivo demonstration that 1) IL-6Rα and gp130 are differentially expressed and regulated in mouse and human fallopian tubes; 2) E2 regulates IL-6Rα through ERα, but not ERβ, although both ERβ and IL-6Rα are localized in the cilia; 3) estrogenic downregulation of IL-6Rα is dependent on the presence of IL-6 using IL-6−/− mice; and 4) IL-6Rα localized in the cilia is associated with estrogenic accelerated OCC transport in mice in vivo. Taken together, these results provide insights into the molecular mechanisms regulating IL-6Rα expression and function in fallopian tubes and form an important basis for interpretation of future studies of the patho-physiological role of IL-6R in vivo.

Ample evidence indicates that a number of genes expressed in the fallopian tube that are critical for dramatic and cyclic changes in the epithelial cells (i.e., ciliation and secretion) and for normal fallopian tubal function (1, 20) are regulated by estrogens (40, 58). Using RT-PCR, immunofluorescence, and WB approaches, we have identified cilia-localized IL-6Rα as a target of E2 regulation in mouse and human fallopian tubes. Mounting evidence indicates that E2 has stimulatory and inhibitory effects on IL-6 production and secretion and that these effects are cell-type specific (57). Although epithelial cells in the human fallopian tube are capable of secreting IL-6 (9, 30), treatment with E2 did not change circulating or tubal IL-6 levels (data not shown). Our studies using IL-6−/− mice show that E2-induced downregulation of IL-6Rα in the fallopian tube is absent in these animals. Therefore, we assume that selective E2 regulation of IL-6Rα might be dependent on the presence of rather than the accumulation of IL-6 in the fallopian tube. Since the promoter regions of both human and mouse IL-6Rα do not contain either the consensus estrogen-response element or multiple copies of the estrogen-response element half-site (41, 52), and since blockade of IL-6 transcription by E2 is mediated by the transcription factor nuclear factor-κB (42, 56), it is likely that IL-6Rα is an indirect target of E2 in the fallopian tube. E2 elicits biological responses principally via the two nuclear ERs (18). We found that estrogenic regulation of tubal IL-6Rα is blocked by the ER antagonist ICI-182,780. Moreover, treatment with 2-methoxyestradiol, a metabolite of...
E2 with little affinity for ERs, fails to regulate IL-6Rα (data not shown). These results provide support that the inhibitory effects of E2 on IL-6Rα expression are ER mediated. The differential regulation of E2 signaling in the fallopian tube can be attributed to the differential expression of ER subtypes (45, 50). Previous studies in vitro have shown that the expression of IL-6Rα is markedly reduced by exogenous E2 in Gg-Ad cells (a decidual cell line) expressing only ERβ (7). Our studies show that both ERβ (45, 50) and IL-6Rα (Fig. 2A) are expressed in ciliated epithelial cells in rodent fallopian tubes and that this expression is specifically located in the cilia. Thus the involvement of which ER subtypes in response to E2 effects was further investigated through the use of specific ER subtype agonists. Surprisingly, IL-6Rα expression was decreased by selective activation of ERα by PTT, but no effects were observed by DPN, an ERβ-selected agonist. Our data suggest that downregulation of cell-type specific IL-6Rα in mouse fallopian tube is regulated by E2 acting via ERα.

Although gp130 is located in the epithelia of the mouse fallopian tube, regulation of gp130 is independent of ovarian steroid hormones and not comparable to that of IL-6Rα. In contrast to the mouse fallopian tube, the expression of gp130 is not found in the epithelium and is altered in the human fallopian tube during the reproductive cycle with low levels in the periovulatory and luteal phases. The pattern of gp130 immunostaining reported here differs from a previous report showing a more widespread immunostaining in both epithelial and muscle cells of human fallopian tubes (59). These differences may be explained either by differences in the specificity of the antibodies employed or perhaps by the use of different methodologies, tissue fixation, and detergents, which often are used to improve access for staining reagents, and they raise an interesting issue for further study. Certainly, demonstration of variable expression and regulation of IL-6Rα and gp130 in adult mice during the estrus cycle may facilitate comparisons between the mice and humans. However, it is worth noting that there are differences in the E2 profile in humans and rodents during the reproductive cycle. At this stage, our data concerning the location and regulation of gp130 suggests species-specific differences between human and mouse fallopian tubes.

Cilia are microtubule-based structures positioned at the apical surface of the epithelial cells. They are vital in controlling fallopian tubal function including rapid movement of tubal fluid, fast cell motility, and induction of cellular signaling. IL-6Rα is specifically located in the cilia in both human and mouse fallopian tubes in vivo, suggesting that it is involved in functions specific to ciliated epithelial cells, such as ciliary beating. Because coordinated ciliary beating (28) and tubal peristalsis (14, 15) are regarded as the leading factors responsible for propelling the gametes and embryo through the fallopian tube when muscular activity is blocked and because ciliary beating frequency can be modulated by IL-6 in vitro (39), we propose that IL-6Rα may be involved in the process of transporting gametes and embryo(s) by regulating cilia.
activity in the fallopian tube. In superovulated mice, WB data show that IL-6Rα protein levels are significantly decreased in the fallopian tube between 24 and 36 h, following E2 stimulation, which coincides with the time that ovulated OCC begin to move toward the uterus. Subsequently, the levels of IL-6Rα return to normal by 48 h following E2 injection, when the OCC transport process is complete. Besides the well-established role of a functional IL-6Rα that binds IL-6 in inflammation and immune responses, the temporal pattern of IL-6Rα expression supports the idea that IL-6Rα has an additional role in reproductive physiology (5, 8, 29). However, from the present study, it is not possible to establish whether IL-6Rα contributes directly or indirectly to fallopian tubal transport. In addition to genetically disrupted IL-6 in vivo, tissue-selective ablation of directly or indirectly to fallopian tubal transport. In addition to have shown that low levels of E2 and high levels of IL-6 are fallopian tube cellular function. Indeed, several clinical studies immune system response, which are reflected in alterations in both structural and/or functional defects in cilia are linked to human reproductive disorders (11, 28). Because normal fallopian tubal transport is a prerequisite for implantation, retention of gametes/embryo(s) resulting from tubal damage may lead to tubal ectopic pregnancy, which remains a potentially life-threatening condition (10). Tubal ectopic pregnancy in women can be caused by changes in both steroid hormone levels and immune system response, which are reflected in alterations in fallopian tube cellular function. Indeed, several clinical studies have shown that low levels of E2 and high levels of IL-6 are coupled to an increased incidence of tubal ectopic pregnancy (2, 10, 34). Furthermore, an exuberant or inappropriate expression and production of IL-6 has been shown to correlate with tissue damage, infection, inflammation, and reproductive pathology (16, 21, 31, 60). Because IL-6 is present in the human fallopian tubal fluids (54), and because the OCC can be another source of IL-6 after ovulation (29), our investigation raises the question of whether regulation of ciliated IL-6Rα via IL-6 directly results in the changes in OCC transport in vivo. IL-6 treatment of animals could be contemplated, however, adverse systemic effects of such a treatment must be considered. Certainly, local administration of IL-6 or IL-6R antibody in vivo deserves consideration even though technically challenging, since rodent fallopian tube is integrated in the bursa ovarica (3). Moreover, the time-window for OCC transport in the fallopian tubes using the immature mouse model is limited to 48 h following hCG injection, which makes determination of IL-6 action locally in the fallopian tubes much more difficult, if not impossible. Furthermore, two different signaling pathways of IL-6R, through membrane-bound IL-6 receptor or soluble IL-6 receptor, has been proposed (44). Although our data strongly suggest that the IL-6/IL-6R signaling, at least in part, contributes to functional E2-enhancement of OCC transport, the physiological relevance of our findings must be interpreted with caution, because IL-6 knockout female mice are fertile (23). Whereas the biological basis of acute and/or chronic fallopian tubal inflammation is poorly understood (5), these studies point toward an involvement of the IL-6/IL-6Rs signaling pathway in the regulation of OCC transport that may be more complicated than our current understanding. Although the exact mechanisms by which IL-6 affects tubal ciliated epithelial cells in vivo remains to be clearly dissected, it is tempting to speculate that systemic pathogenic IL-6 causes the abnormal expression of IL-6Rα in the fallopian tube leading to cilia damage or to increased susceptibility to tubal ectopic pregnancy. Given the possible role of IL-6Rα in fallopian tubal transport in mice under physiological conditions, it will be important in the future to examine its possible association with human tubal ectopic pregnancy. This association is under investigation in our laboratory.

In conclusion, this investigation reveals for the first time that proteins for the two IL-6 receptors show distinct cellular localization in mouse and human fallopian tube and that IL-6Rα is restricted to the cilia of epithelial cells, which play an essential role in fallopian tube transport (11, 28). Our results also indicate that estrogen selectively downregulates IL-6Rα expression in an IL-6-dependent manner and that ERβ, but not ERα, mediates the E2 signal, thereby influencing IL-6Rα in mouse fallopian tubes. Although additional studies are needed to determine a cause-effect relationship between the functional IL-6Rα and the tubal transport process, our experiments show that estrogenic regulation of tubal IL-6Rα is associated with the estrogen-induced OCC transport process in vivo. The results suggest that if the tubal transport process in humans and mice is similar (6), then modulation of IL-6Rα may be a possible therapeutic intervention to improve outcomes in patients with fallopian tubal dysfunction.

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