Interleukin-1 system messenger ribonucleic acid and protein expression in human fallopian tube may be associated with ectopic pregnancy

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Objective: To investigate the interleukin-1 (IL-1) system mRNA and protein expression in human fallopian tubes with ectopic pregnancies.
Design: A controlled study.
Setting: Clinical and academic research setting in a university medical center.
Patient(s): Women undergoing salpingectomy for fallopian tube with ectopic pregnancy and women undergoing tubal ligation.
Intervention(s): Paired segments of human fallopian tubes containing an ectopic pregnancy and parafallopian tube segments adjacent to the ectopic pregnancy were collected from five women undergoing laparoscopic salpingectomy. Segments of fallopian tubes from four women undergoing tubal ligation were used as control tissues. Quantitative competitive polymerase chain reaction (QC-PCR) and immunohistochemistry were performed.
Main Outcome Measure(s): The differences of IL-1 system mRNA and the ratio of IL-1β to IL-1 receptor antagonist (IL-1ra) in both fallopian tubes with ectopic pregnancies and normal controls were analyzed.
Result(s): A complete IL-1 system mRNA and protein expression was identified in both fallopian tubes with ectopic pregnancies and normal controls. As QC-PCR demonstrated, IL-1β mRNA expression was decreased, and IL-1ra and IL-1 receptor type 1 were increased in fallopian tubes with ectopic pregnancies in comparison with normal control tubes. In para-ectopic tubes, IL-1 receptor type 1 mRNA was statistically significantly increased in comparison with normal controls. There was a lower ratio of IL-1β to IL-1ra at mRNA in fallopian tubes with ectopic pregnancies.
Conclusion(s): These results suggest that an inappropriate ratio of IL-1β to IL-1ra and a higher expression of its receptor in fallopian tubes may possibly be implicated to the implantation of an ectopic pregnancy in the oviduct. (Fertil Steril® 2005;84:1484–92. ©2005 by American Society for Reproductive Medicine.)

Key Words: Interleukin-1, ectopic pregnancy, embryo implantation, fallopian tube, PCR

The incidence of ectopic pregnancy, the implantation of a fertilized ovum outside the uterine cavity, has been increasing over the past several decades and now accounts for 2% of all pregnancies in the United States (1), although the factors responsible for early embryogenesis and ectopic implantation are incompletely understood. Risk factors for the development of ectopic pregnancy have been described (2), but fewer than 50% of women with ectopic pregnancy have a history of such risk factors. Surgically visualized tubal pathology, pelvic infection, endometriosis, or previous surgeries are the strongest risk factors. The risk is also increased in women who have had a previous ectopic pregnancy, and it increases further in direct proportion to the number of prior ectopic pregnancies (3).

The human fallopian tube is the site of oocyte capture and migration, sperm migration, fertilization, and early embryonic development. The fallopian tubes are regarded as being biologically active, providing an environment that sustains and enhances fertilization during early embryonic development as the embryo traverses the tubes and moves toward the uterine cavity (4, 5). The anatomy, histology, and physiology of the human fallopian tube all contribute important information to understanding the role of cytokines in the human fallopian tube as they impact embryonic development. Cytokines and growth factors have been detected in the preimplantation embryo, fallopian tube, and endometrium (6, 7). Moreover, it has been shown that improved embryo morphology, development, and hatching as well as better implantation rates are obtained after embryos are co-cultured on feeder layers of human oviduct cells (8). Therefore, oviduct cells as a feeder layer for co-cultured embryos might produce factors that possess direct or indirect embryotropic

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activity. However, these embryotropic factors, their regulation, and their potential function in vivo are not as yet understood, and their influence on the gametes/embryos has not yet been explored.

More specifically related to mechanisms of implantation, the interleukin-1 (IL-1) system may be one of the major candidates for these molecular regulators in local intercellular interactions during embryonic implantation. The IL-1 system is a family of polypeptides composed of two agonists, IL-1α and IL-1β, and an inhibitor, IL-1 receptor antagonist (IL-1ra), as well as two receptors (type 1 and type 2) (9–11). Both IL-1 agonist and receptor antagonist are recognized by IL-1 receptor type 1, and trigger signal responses in target cells (12, 13). Interleukin-1ra is a specific inhibitor to IL-1 agonist, which competes with IL-1 for the binding site of IL-1 receptor type 1 and blocks signal transduction (14, 15). Preimplantation individual human and mouse embryos are known to express IL-1 agonist, IL-1 receptor antagonist, and their common receptor, IL-1 receptor type 1 (16–18). Interleukin-1 is also expressed in human endometrium and has been shown to play an integral role in local cellular interactions during implantation (19–21).

Cytokines have recently been identified within human fallopian tubes, including endothelial growth factor (EGF), transforming growth factor α (TGF-α), insulin-like growth factor (IGF), TGF-β, granulocyte-macrophage colony-stimulating factor (GM-CSF), gonadotropin-releasing hormone (GnRH) and leukemia inhibitory factor (LIF) (22, 23). Little information is available about the presence and regulation of the IL-1 system in the pathogenesis of fallopian tube ectopic implantation. The purpose of this study was to investigate the presence of the IL-1 system of fallopian tubes with ectopic pregnancies, representing "abnormal" embryo implantation. We examined human fallopian tubal ectopic pregnancies by polymerase chain reaction (PCR) to determine the presence of IL-1β as well as IL-1ra and IL-1 receptor type 1 mRNA expression. We also examined the quantitative amount of human fallopian tubal cell IL-1 mRNA expression by quantitative competitive PCR (QC-PCR) to examine IL-1 regulation in abnormal embryonic implantation. The protein expression of the IL-1 system components in fallopian tubes was demonstrated by immunohistochemistry.

MATERIALS AND METHODS

Tissue Collection

Paired segments of human fallopian tubes containing an ectopic pregnancy and parafallopian tube segments adjacent to the ectopic pregnancy after removal of intraluminal gestations (n = 5) were collected from women undergoing laparoscopic salpingectomy with approval by the Chang Gung Memorial Hospital institutional review board after informed consent (CGMG IRB#90-144). All of the tissues were documented to be ectopic pregnancies by pathologic examination. Segments of fallopian tubes from women undergoing tubal ligation (n = 4) were used as control tissues. Human luteal phase endometrium obtained from surgical specimens of normally cycling women undergoing hysterectomy for nonmalignant indications including uterine fibroids or urinary incontinence, known to express IL-1 system transcripts, were used as a positive control for PCR and immunohistochemistry experiments.

Tissue Processing

Total RNA was extracted from tissues with RNAzol reagent (Tel-test, Inc., Friendswood, TX), as described previously elsewhere (24). Briefly, tissue samples were washed three times in phosphate buffered saline (PBS) (GIBCO BRL, Grand Island, NY) to remove blood contamination. The RNA concentration was quantified by measuring optical density with a Spectronic 601 spectrophotometer (Milton Roy Co., Rochester, NY), then the RNA was diluted to 1 μg/μL for reverse transcriptase PCR (RT-PCR).

For immunohistochemistry, tissue was embedded in optimal cutting temperature (OCT; Shakura Finetek, U.S.A., Inc. Torrance, CA) and frozen in liquid nitrogen until sectioned. Twelve serial sections (8 μm) from each sample were then prepared for immunohistochemistry to detect tissue macrophages and the IL-1 system. The first and last sections were stained with hematoxylin and eosin and examined with a Nikon microphot-FXA microscope (Nikon Instruments, Garden City, NY).

Preparation of Oligonucleotide Primers for RT-PCR

Specific sequences of oligonucleotide primers for detecting tubal cell expression of human IL-1β (25), IL-1ra (26), and IL-1 receptor type 1 (27) were obtained from the Genebank Database of the National Center for Biotechnology Information (NCBI) of the National Institutes of Health (NIH), as described previously elsewhere (28). To ensure that the product detected resulted from amplification of specific cDNA in question rather than contamination of other cDNAs, all primers were designed to span the exon and intron regions. As a control molecule, β-actin message was amplified. Human luteal endometrium from endometrial biopsy specimens of normal cycling women, known to express all these transcripts, was used as a positive control to identify cDNA fragments generated using the various different primers. Total extracted RNA was reverse transcribed into cDNA and amplified by PCR using specific primers for β-actin (838 bp), IL-1β (203 bp), IL-1ra (179 bp), and IL-1 receptor type 1 (284 bp) target cDNA. The summary of oligonucleotide primer sequences is listed in Table 1.

Reverse Transcription

For RT-PCR, the GeneAmp RNA PCR kit (Perkin-Elmer, Foster City, CA) was used. For each sample, the 19 μl RT master mix was prepared containing 5 mmol/L $MgCl_2$, 1X PCR-Buffer II, 1 mmol/L of each dNTP, 2.5μmol/L Oligod

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