T-Helper 2 and 3 type immunity to trophoblast in successful in vitro fertilization–embryo transfer

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Objective: To determine whether peripheral blood mononuclear cell (PBMC) secretion of T-helper (Th)-1 type cytokines and Th-2 and Th-3 type cytokines in women undergoing in vitro fertilization-embryo transfer (IVF-ET) is associated with therapeutic failure and success, respectively.
Design: Cohort study.
Setting: Academic medical center.
Patient(s): One hundred one women undergoing IVF-ET and 19 fertile controls.
Intervention(s): Peripheral blood was obtained from women undergoing IVF-ET before oocyte retrieval and from 19 nonpregnant fertile controls. The PBMCs were cultured in the presence or absence of a protein extract from either a trophoblast cell line or sperm membrane.
Main Outcome Measure(s): Supernatants from PBMC cultures were tested by enzyme-linked immunoabsorbent assay (ELISA) for the Th-1 type cytokines tumor necrosis factor-alpha (TNF)-α) and interferon-gamma (IFN)-γ), the Th-2 type cytokines interleukin (IL)-6 and IL-10, and the Th-3 type cytokine transforming growth factor (TGF)-β1.
Result(s): Levels of IL-6 and IL-10 were significantly higher in controls than in infertile women with endometriosis, and levels of IL-10 were higher in controls than in women with unexplained infertility. No differences were found in unstimulated levels of TNF-α, IFN-γ, or TGF-β1 between infertile patients and controls. In trophoblast-stimulated PBMC cultures, levels of TGF-β1 were significantly lower in subjects who experienced failed compared with ongoing pregnancies.
Conclusion(s): Baseline PBMC secretion of IL-6 and IL-10 is higher in fertile controls than in women with endometriosis, and IL-10 secretion is also higher than in women with unexplained infertility. Trophoblast-stimulated PBMC secretion of TGF-β1 is positively associated with the establishment of successful pregnancy in women undergoing IVF-ET. Our study provides novel evidence to support a faciliatory role of Th-2 and Th-3 type responses to trophoblast in early pregnancy. (Fertil Steril® 2005;83:1659–64. ©2005 by American Society for Reproductive Medicine.)

Key Words: IVF-ET, infertility, endometriosis, T-helper lymphocytes, cytokines

In human IVF, although apparently viable embryos are transferred into an adequately prepared endometrium, only a fraction of them will implant. This supports the concept that human pregnancy is an intrinsically inefficient process, with a high incidence of spontaneous loss occurring even before the expected onset of menses (1, 2). A large number of such losses are due to chromosomal aneuploidy of the embryo (3, 4). However, increasing evidence suggests that an unbalanced maternal immune response toward the conceptus may also cause its untimely rejection (5–8). Humans are viviparous mammals with a hemochorial placenta, a unique situation that allows trophoblastic antigens to first contact maternal lymphocytes 7 to 10 days after the ovulatory luteinizing hormone (LH) surge (9). It is known that the embryo expresses paternal antigens and that these are recognized by maternal T-lymphocytes (10, 11). Maternal decidual immune cells, like those of the periphery, coordinate immune responsiveness patterned cytokine secretion.

Studies in mice have described the secretion of distinctive subsets of cytokines by T-helper (Th) cells in response to antigen activation (12). The Th-1 type cytokine profile is characterized by the secretion of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-alpha;), and interleukin (IL)-2 and induces cell-mediated, inflammatory immune responses. Cytokines in the Th-2 type response include IL-4, IL-5, IL-6, and IL-10. These Th-2 products prevent the release of Th-1 cytokines, while inducing antibody production through B-cell activation (12, 13). Additional cytokine secretory profiles and lymphocyte subsets have also been

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characterized. For instance, specific T lymphocyte subpopulations may primarily secrete TGF-β-1 in response to antigen recognition, a profile described first in mice and termed a Th-3 type response (12, 14, 15). Transforming growth factor-beta-1 (TGF-β-1) has well-described roles in immunomodulation and immune tolerance. Recent reports have demonstrated analogous human Th-3 cytokine profiles (6, 14, 15). Our laboratory was the first to note a dichotomous Th response to trophoblast antigens in vitro among women with unexplained recurrent pregnancy loss (RPL) (5). When compared with normal fertile counterparts, women with a history of unexplained RPL exhibit an increased inflammatory Th-1 response and a decreased Th-2 response to trophoblast antigens.

In vitro sperm stimulation of peripheral blood mononuclear cells (PBMCs) has been used as a model for the effect of sperm on female immunity. In contrast to natural intercourse, females are not directly exposed to high concentrations of sperm following in vitro fertilization, although small numbers of sperm bound to the zona pellucida may be present on embryos transferred into the uterine cavity. In the present study, we sought to test the hypotheses that [1] women experiencing pregnancy loss after in vitro fertilization-embryo transfer (IVF-ET) were more likely to display a Th-1 type response to reproductive antigens, and [2] women achieving an ongoing pregnancy through IVF-ET were more likely to display a Th-2 or Th-3 type response to reproductive antigens.

MATERIALS AND METHODS

Subjects

The Institutional Review Board of the Brigham and Women's Hospital approved this study. All subjects signed informed consents. Peripheral blood mononuclear cells (PBMCs) were isolated from blood obtained from 101 patients undergoing IVF at Brigham and Women's Hospital 1 hour before oocyte retrieval. Samples were collected prospectively and results from all patients were included in the final analysis. The PBMCs were also obtained from 19 fertile controls with 1 or more children and no history of spontaneous abortion, allergies, or recent infection. No attempt was made to control for the time in the menstrual cycle when blood was collected from fertile controls because our own previous studies have not found this to be a significant factor in antigen presentation status.

Assessment of Cytokine Levels

Cytokine assessments were performed in cell culture supernatants of PBMCs (10×10^6/10 mL), following 4 days in culture with and without a protein extract derived from [1] a human trophoblast cell line, JEG-3 (30 ug/mL), and [2] sperm membrane extracts (1ug/mL) as previously described (16, 17). Briefly, for sperm antigens, fresh ejaculates from donors were obtained by masturbation and allowed to liquefy for 30 minutes at room temperature. Semen was subjected to a discontinuous Percoll (Amersham Biosciences AB, Uppsala, Sweden) gradient (2 mL of 47% layered on 2 mL 90% Percoll) at $600 \times g$ for 30 minutes. Percoll-isolated sperm pellets preparation were pooled, washed twice in Ham's F-10 medium and adjusted to 50×10^6 spermatozoa/mL. Whole spermatozoa were disrupted with four sonic bursts of 20 seconds each on ice (20% maximum output using a Braunsonic 220 sonicater; Braunson Cleaning Equipment Company, Shetton, CT), centrifuged at $10{,}000 \times g$ at 4°C for 10 minutes, and the supernatants were stored at –70°C before use (16). For trophoblast antigen prepatration, JEG-3 cells (American Tissue type Collection, Manassas, VA) were cultured to 80% confluence in media supplemented with 10% fetal bovine serum. Cells were harvested without trypsinization using a cell scraper and washed in phosphate-buffered saline, resuspended in hypotonic buffer and homogenized using 35 strokes of a Dounce homogenizer. Tonicity was restored, and the lysate was subjected to serial centrifugation to isolate nuclear, organelle, and membrane-enriched fractions. These were solubilized in a 0.2% sodium dodeclysulfate solution, sterilized using gamma radiation and stored at −70°C until use (17).

Levels of the Th-1 type (TNF-α and IFN-γ), Th-2 type (IL-6 and IL-10), and Th-3 type (TGFβ-1) cytokines in antigen-stimulated and unstimulated PBMC culture supernatants were determined by quantitative enzyme-linked immunosorbent assay (ELISA) after a media change 24 hours before collection. For all determinations, commercially available ELISA kits were employed according to the manufacturers' directions. We purchased kits for TNF-α and IFN-γ determinations from Endogen (Boston, MA; lowest limit of sensitivity, 10 pg/mL for both cytokines), and kits for IL-6, IL-10, and TGF-β-1 determinations from R&D Systems, Inc., (Minneapolis, MN; lower limit of sensitivity, 1 pg/mL, 4 pg/mL, and 7 pg/mL, respectively). All kits were solid-phase sandwich ELISAs: 96 well plates with attached specific monoclonal antibodies, to which a secondary enzyme-conjugated specific antibody was added together with substrate. Substrate turnover was monitored spectrophotometrically at the recommended wavelength using an automated microplate reader. Cytokine concentrations were calculated from a standard curve generated using control standards provided with each kit. All values below the lowest limit of sensitivity were considered undetectable. Samples were collected and stored to allow all ELISA testing to be performed within a short temporal period and within a limited number of assay runs.

Statistical Analysis

Normality tests demonstrated that the cytokine levels in the supernatants were not normally distributed. Therefore, Kruskall–Wallis nonparametric analysis of variance (ANOVA) followed by Dunn's multiple comparison test was used to compare cytokine levels between patients with different infertility diagnoses (i.e., unexplained infertility, en-