Association of the Leukemia Inhibitory Factor Gene Mutation and the Antiphospholipid Antibodies in the Peripheral Blood of Infertile Women


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ABSTRACT. To characterize the impact of the potentially functional mutation – the G to A transition at the position 3400 of the leukemia inhibitory factor (LIF; a pluripotent cytokine that plays a central role in the control of the embryo implantation) gene that leads to the exchange of valine with methionine at codon 64 we evaluated the association of the LIF gene mutation and the levels of antiphospholipid antibodies (aPLs) in the peripheral blood of infertile women (the aPLs examination was part of our routine immunological test during the infertility check-up). Eight infertile mutation-positive women were diagnosed with idiopathic infertility (n = 5) and endometriosis (n = 3) and their levels of aPLs in serum were compared with 115 infertile women without any LIF gene mutation. Enzyme-linked immunosorbent assay was used for the detection of seven antiphospholipid antibodies; the results were statistically assessed by the Fisher’s 2 by 2 exact test to evaluate the association of the LIF gene mutations and aPLs in serum of infertile patients. The presence of aPLs was significantly higher in our study group (100 %) than in 30 % of aPLs-positives in control infertile patients (p = 0.0035) which indicates that the aPLs are elevated in women with LIF gene mutations.

Abbreviations

aPLs antiphospholipid antibodies
ELISA enzyme-linked immunosorbent assay
FCS fetal calf serum
IVF in vitro fertilization
LIF leukemia inhibitory factor
LIFR LIF receptor
NK natural killer (cells)
ph-Ser l-phosphatidyl-serine
SD standard deviation
TGGE temperature gradient gel electrophoresis

Out of the many cytokines that take part in the endometrium–blastocyst cross talk, LIF has been found to be essential for the embryo implantation process (see, e.g., Kralíčková et al. 2005). In endometrium of healthy women, LIF and LIF mRNA are expressed throughout the menstrual cycle with a striking increase in the mid-secretory phase, coinciding with a supposed window of implantation. LIF acts on cells by binding to LIFR and gp130. Human blastocysts express mRNAs for LIFR and gp130, participating actively in establishing contact with the endometrium. In the endometrium, LIFR and gp130 are expressed in the epithelium throughout the cycle with strong increase in the mid-secretory phase (for review see Aghajanova 2004).

The lif knockout mice have doubled the percentage of uterine NK cells compared to the wild-type mice at the time of implantation window, indicating that LIF restricts the migration of NK cells to the uterus (Schofield and Kimber 2004).

It has been demonstrated that certain phospholipids are exposed on the endothelial surface and may interact with syncytiotrophoblast and cytotrophoblast layers and alter implantation during natural as well as in vitro fertilization as well as the feto–maternal interactions during the rest of pregnancy (Aron et al. 1995). aPLs have an established association with recurrent miscarriage, embryo implantation failure, growth retardation, placental abruption and stillbirth (Balasch et al. 1996; McIntyre 2003; Coulam et al. 1997). Because some investigators showed contrary results (Kowalik et al. 1997; Eldar-Geva et al. 1999), the relevance of
the presence of aPLs to embryo implantation, infertility and IVF outcome and their mechanism of action is still a matter of discussion.

The link between NK cells and aPLs has been established. Women with recurrent miscarriages and antiphospholipid antibodies showed significantly elevated NK cells than women without these antibodies. In addition, women with other infertility diagnoses and autoantibodies to phospholipids have significantly higher levels of NK cells than women without antiphospholipid antibodies (Kwak et al. 1995; Beer et al. 1996). A relationship between increased natural killer-cell activity, increased serum levels of aPLs and trophoblast cell apoptosis has been reported (Roussev et al. 1996; Kaider et al. 1999; Sher et al. 2000).

The role of lif gene mutations in embryo implantation failure and in infertility remains in general unclear. In infertile women, three heterozygous potentially functional point mutations have so far been identified (Giess et al. 1999; Steck et al. 2004); these studies conclude that heterozygosity for lif-gene mutations leading to decreased availability or specific biological activity of the LIF protein could act as genetic predisposition of infertility due to its impact on the efficacy of embryo implantation.

We proved that the LIF gene mutations are not restricted to nulli gravid patients or to other infertility diagnoses (i.e. to anovulation, tubal factor, endometriosis, male factor or idiopathic infertility); it is unfortunate that we were not able to characterize the detected mutations (Králíčková et al. 2006). While the significance of LIF gene alterations, their functional consequences and clinical impacts are still not fully understood, here we focus on their characterization by examining their association with increased levels of aPLs in the peripheral blood of infertile patients.

MATERIALS AND METHODS

Patients. Eight women (average age 35.2 ± 3 years) with potentially functional lif mutation, the G to A transition at the position 3400 leading to the exchange of valine with methionine at codon 64 (V64M) in the AB loop region of the LIF protein (study group) and 115 infertile women (average age 33.5 ± 6 years) without any LIF gene mutation (control group) were included.

The control group comprised 10 patients diagnosed with endometriosis and 32 patients diagnosed with unexplained infertility (group 1) plus 73 patients diagnosed with male factor (group 2). Endometriosis patients comprised all the stages of disease. Patients classified as idiopathic (unexplained) infertility were documented by laparoscopy to have patent tubes, were free of pelvic adhesions and endometriosis, and had a normal uterine cavity by hysteroscopy or hysterosalpingography. They had normal ovulation and there was no evidence of male factor, antisperm or zona pellucida antibodies.

The study was approved by the Charles University Ethics Committee and informed consent was obtained from all individuals.

DNA extraction, PCR and mutation status of the lif gene. Peripheral blood leukocytes were used for the DNA isolation in all cases; it was isolated by the DNeasy Tissue Kit (Qiagen, Germany) according to manufacturer’s protocol. The coding regions and the exon–intronic junctions were analyzed by TGGE. The exon 3 was divided into 3 parts and lif was screened and divided into 5 partly overlapping fragments. PCR was performed using five sets of primers (Table I) which were modified using Polish java script (www.biophys.uni-duesseldorf.de/POLAND/poland.html) by GC-clamp addition to create a thermostable domain suitable for TGGE. The reaction conditions were as follows: 12.5 μL of HotStart Taq PCR Master Mix (Qiagen), 10 pmol of each primer, 100 ng of DNA and distilled water up to 25 μL. The amplification program consisted of denaturation (15 min, 95 °C), 35 cycles of denaturation (30 s, 95 °C), annealing (30 s, 60 °C), extension (1 min, 72 °C) and final extension (7 min, 72 °C). The amplification program was the same for all analyzed exons except for exon 1 where the annealing temperature was 50 °C. The length and the quality of the PCR products were checked in standard agarose gels.

Screening of mutations was done by heteroduplex analysis on TGGE (Biometra, Germany) on 8 % denaturing acrylamide (AA) gel (AA–bis-AA 375: 10, 6 mol/L urea, 1× Mops, 2 % glycerol). The TGGE analysis was performed in two steps – electrophoresis conditions for parallel gels for each exon had to be at first optimized by perpendicular gels. Consequently, the parallel gels for patients’ samples were run. The running time was 1.5 h at temperature gradient (Table I). DNA bands were detected by silver-staining method according to standard protocol (Beranová et al. 2001; Králíčková et al. 2006).

The relevant DNA samples of all women positive in TGGE analysis were amplified and sequenced by automated sequencing using a Big Dye Terminator Sequencing Kit (PE/Applied Biosystems, USA). The samples were run on automated sequencer ABI Prism 3100 Avant (PE/Applied Biosystems) at a constant voltage of 12.2 kV for 18 min. In order to avoid errors, all PCR and sequencing experiments were repeated a minimum of two times in TGGE positive patients.