Interferon Gamma and Interleukin 10 Levels in Preimplantation Embryo Culture Media


Submitted: May 31, 1995
Accepted: June 15, 1995

Purpose: The aim of our study is to elucidate whether human oocytes/embryos secrete IFNγ and/or IL-10 and whether the fertilization process depends on the balance between these cytokines.

Methods: A total of 142 embryo culture media from 24 patients were collected and the cytokine levels were tested with ELISA.

Results: IFNγ and IL-10 were detectable in 40.1% and 29.6% of culture media respectively. The difference of IFNγ and IL-10 levels in media from fertilized oocytes between day 1 and day 2 are significant (0.46 vs. 1.47 and 34.2 vs. 12.7, respectively). However, there was no significant difference between the IFNγ levels of the media from fertilized and nonfertilized oocytes 0.46 vs. 0.85 at day 1 and 1.47 vs. 1.49 at day 2, as well as IL-10 levels 34.2 vs. 30.9 at day 1 and 12.7 vs. 9.58 at day 2 respectively.

Conclusions: Human preimplantation embryos secrete the cytokines IFNγ and IL-10. No effect of these cytokines on fertilization process could be shown.

KEY WORDS: culture medium; embryo; fertilization; interferon γ; interleukin 10.

INTRODUCTION

In 1986 two types of T helper clones (Th1 and Th2) were defined on the basis of different patterns of cytokine secretion (1). Th1 cells secrete interleukin (IL)-2, tumor necrosis factor and interferon gamma (IFNγ) and are helper cells for cell-mediated immunity. Th2 cells secrete IL-4, IL-5, IL-6, and IL-10 and are helper cells for humoral immunity (2).

Pregnant women undergo immunological changes: weakening of cell mediated immunity and strengthening of humoral immunity. In 1993 Wegmann et al. postulated that the maternal immune state is maintained by the local secretion of Th2 cytokines, particularly IL-10 (3). In the same year Lin et al. showed that all Th2-type cytokines and IFNγ are synthesized by cells at the maternal-fetal interface (4).

The preimplantation embryo secretes some cytokines [e.g., IFNα (5), TNF (6), TGFβ, IL-1 (7)]. The aim of our study is to elucidate whether human oocytes/embryos secrete IFNγ and/or IL-10 and whether the fertilization process depends on the balance between IFNγ and IL-10. IFNγ and IL-10 levels in preembryo culture medium (PECM) were measured.

MATERIAL AND METHODS

The PECM samples were collected from 24 patients undergoing IVF-ET treatment at the Department of Obstetrics and Gynecology, University of Düsseldorf. All patients were stimulated with GnRH analoga/hMG/hCG using a long protocol version.

Oocyte retrieval was performed by ultrasound guided transvaginal needle aspiration under general anesthesia, approximately 34-36 h after hCG injection. The oocytes were collected immediately and the aspirated follicular fluid was replaced by Men-ezo B2 medium (Laboratoire C.C.D., Paris, France), which was preincubated for one night (37°C, 5% CO₂ + 95% air). One milliliter Menezzo
B2 medium without additional serum was used for oocyte or embryo culture and for sperm preparation. Single oocytes/embryos were cultured in B2 medium in test tubes (Falcon Nr. 2003, Meylan Cedex, France). The sperm preparation was done by the swim-up method and oocytes were inseminated with a sperm concentration of 150,000/ml 4–5 h after retrieval.

The PECM were collected 18–20 h after insemination (day 1). Cumulus and corona cells were removed mechanically and the oocytes were checked for fertilization. Fertilized/nonfertilized oocytes were placed in fresh B2 media. The second PECM were collected 48 h after ovum pickup (day 2) and a second check for cleavage was done. The cleavage stage of each embryo was noted. All the PECM from day 1 and day 2 were kept at -70°C until the cytokine measurements. A total of 142 PECM were collected, 71 from day 1 and 71 from day 2. PECM from fertilized oocytes, which did not cleave have not been investigated in this study. Menezo B2 media itself, from swim-up sperm and from oocyte-corona-cumulus complex only were included as controls.

The cytokine levels were tested with a commercial ELISA kit (Medgenix, Brussel, Belgium). The minimum detectable level for IFNγ was 0.03 IU/ml and for IL-10 1 pg/ml.

All data processing and statistical analysis was performed using SPSS 5.2 for Windows. Differences between groups were assessed by the Mann-Whitney U and Kruskal-Wallis tests, with P < 0.05 as the level of statistical significance.

**RESULTS**

Menezo B2 medium as well as B2 medium from swim-up sperm showed baseline IFNγ levels of <1.4 IU/ml and this value was subtracted from all PECM measurements. B2 medium from oocyte-corona-cumulus complex only resulted in 0.9 IU/ml. Of the 142 PECM 57 had detectable IFNγ secretion (Table I). On day 1 22.5% of the cells produced IFNγ vs. 57.7% on day 2. The IFNγ secretions of fertilized oocytes increased from day 1 to day 2: 0.46 ± 0.25 and 1.47 ± 0.89 respectively, being statistically significant (P < 0.002). IFNγ levels from nonfertilized oocytes also increased from day 1 to day 2: 0.85 ± 0.79 and 1.49 ± 0.98 respectively, but the difference was not significant (Fig. 1). There was no statistically significant difference in IFNγ values between fertilized and nonfertilized cells on day 1 or day 2.

IFNγ levels were significantly higher (P < 0.015) in pathologically fertilized oocytes [≥3 pronuclei (PN)] than in physiologically fertilized oocytes on day 1 (0.92 ± 0.35 vs. 0.46 ± 0.25). There was no significant difference on day 2 (1.84 ± 0.81 vs. 1.47 ± 0.89) (Fig. 2).

Comparing the IFNγ levels from PN cells (day 1) to the different cleavage stage on day 2 revealed no significant difference. The values on day 1 for 2-, 3- and 4-cell embryos were 0.53 ± 0.34, 0.52 ± 0.31 and 0.36 ± 0.09 respectively, Day 2 values were 1.55 ± 0.52, 1.02 ± 0.78 and 1.74 ± 1.02 respectively.

There was no interindividual difference in IFNγ levels between individual on day 1 and on day 2. No difference was observed between those patients who achieved a pregnancy (n = 6) and those who did not, although the numbers of patients is too low to allow for statistical evaluation in this regard (data not shown).

We had 42 positive IL-10 samples in the 142 PECM (Table I). On day 1, 32.4% of the cells secreted IL-10 and on day 2, 26.7% did. The IL-10 concentrations decreased from day 1 to day 2 in fertilized oocytes (34.2 ± 39.6 vs. 12.7 ± 16.5) being statistically significant (P < 0.046). This was not the case in nonfertilized cells (30.9 ± 38.5 vs. 9.58 ± 1.2) (Fig. 3). There was no significant difference between fertilized and nonfertilized cells on day 1 or day 2.

**Table I. Distribution of Cytokine Positive Culture Media (%)**

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
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<tbody>
<tr>
<td></td>
<td>Nonfertilized</td>
<td>Fertilized</td>
</tr>
<tr>
<td>PECM</td>
<td>18 (100)</td>
<td>46 (100)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>2 (11.1)</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td>IL-10</td>
<td>7 (38.8)</td>
<td>13 (28.3)</td>
</tr>
<tr>
<td></td>
<td>Noncleaved</td>
<td>Cleaved</td>
</tr>
<tr>
<td>PECM</td>
<td>18 (100)</td>
<td>46 (100)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>10 (55.6)</td>
<td>24 (52.2)</td>
</tr>
<tr>
<td>IL-10</td>
<td>5 (27.8)</td>
<td>14 (30.4)</td>
</tr>
</tbody>
</table>

*Controls: Pure Menezo B2 (n = 3), swim-up sperm (n = 3), oocyte-corona-cumulus complex only (n = 3).*

Journal of Assisted Reproduction and Genetics, Vol. 12, No. 9, 1995
There was no interindividual difference in IL-10 values between patients (data not shown). The IL-10 level of PECM of culture medium from sperm, oocyte corona-cumulus complex only and culture media itself were lower than the minimum detectable level (<1 pg/ml).

**DISCUSSION**

IFNγ has been reported to have a variety of stimulatory activities including activation of macrophages, induction of surface expression of MHC molecules and enhancing of natural killer cell activity. IFNγ can also mediate inhibitory effects such as antiviral activity and antiproliferative activity on tumors (8). We showed that some of the human preimplantation embryos secrete IFNγ during the first 48 h after *in vitro* fertilization. In the pig a clear-cut IFNγ production was found in the trophoblast at implantation (9), whereas no IFNγ production could be shown in the mouse (10).

A total of 22.5% of the human preimplantation embryos produced IFNγ on day 1 and 57.7% on day 2 respectively. The IFNγ concentrations are not different in fertilized and nonfertilized oocytes. From our data we cannot show any clear effect of IFNγ on the fertilization process. It is known that HLA class I expression on human trophoblast can be up regulated by IFNγ (11). HLA G is a nonclassical monomorphic class I antigen which is only found on trophoblasts (12). HLA G may be involved in the recognition of trophoblasts by maternal NK large granular lymphocytes (LGL) cells in the decidua. These cells may function in limiting the invasion of trophoblasts into the decidua (13). IFNγ may produce an increase of HLA G antigens on trophoblasts before or during implantation period and therefore protect the preimplantation embryo against the LGLs. We did not differentiate the source of the cytokines on day 1. They might originate from oocyte or corona-cumulus complex.

Pathologically fertilized oocytes (≥3 PN) secrete significantly more IFNγ on the first day after insemination. But there is no difference on day 2 between the cleaved cells from pathologically or physiologically fertilized oocytes. This difference may be a result of the high metabolic activities of the pathologically fertilized oocytes.

IL-10 is a potent suppressor of the effector functions of macrophages, T-cells and NK cells. IL-10 is
regulating proliferation and differentiation of B-cells (14). HL-10 has been shown to inhibit cytokine production, especially IFN-γ. High levels of production of Th2 cytokines was shown in placental tissues, in particularly IL-10 (2). We showed an IL-10 production of 32.4% of the oocytes on day 1 and 26.7% on day 2 after in vitro fertilization. The IL-10 levels decreased significantly in fertilized oocytes from day 1 to day 2. This decrease may be explained by the increase of IFN-γ, because IFN-γ strongly reduces IL-10 synthesis (15). Austuglen et al. recently showed also a decrease of IL-6 levels during the first 2 days after in vitro fertilization, which is another Th2-type cytokine (7). This is parallel to our IL-10 results. We could not show any relationship between IL-10 levels and the fertilization process. However, IL-10 may be produced in increasing quantities at later stages of embryo development, as is known for IL-6, and therefore may play an important role at implantation (6).

In conclusion only a part of the human preimplantation embryos secrete the cytokines IFN-γ and IL-10 during the first 2 days after in vitro fertilization. The other embryos may produce cytokines below the detectable levels of our test kits. The decrease of IL-10 concentrations from day 1 to day 2 after in vitro fertilization is paralleled by an increase in IFN-γ secretion and vice versa. It could not be shown any effect of the balance of these cytokines on fertilization process. Maybe this early cytokine production is the prephase of the implantation.

These are preliminary results, further investigations on later stages of embryo development are necessary in order to get more information about the effect of Th1/Th2 balance on fertilization and implantation.

ACKNOWLEDGMENTS

We thank Ingeborg Deepke and Gertrud Fenkes for their kind help with conducting the ELISA assays.

REFERENCES