Interferon-Gamma Production by the Human Preimplantation Embryo

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PROBLEM: This study demonstrated that the human embryo produces interferon-gamma (IFNγ). It is important to know whether IFNγ can be produced before implantation. Therefore the aim of this study was to evaluate the profile of IFNγ production between days 2 and 5 after in vitro fertilization.

METHOD: Twenty embryos were cultured from day 2 to 5 after fertilization. The embryo stages were checked each day and the media refreshed. IFNγ levels were estimated by an enzyme-linked immunosorbent assay.

RESULTS: All embryos produced measurable IFNγ at least for 1 day. Yields of IFNγ were: 0.46 ± 0.45 (n = 4) on day 2, 0.69 ± 0.52 (n = 19) on day 3, 0.73 ± 0.52 (n = 15) on day 4, 0.55 ± 0.32 (n = 11) IU/ml on day 5, respectively. There was no significant difference in the IFNγ production between in vitro culture days or between the developmental stages of embryos.

CONCLUSION: IFNγ is produced by all the embryos and seems to peak between days 3 and 4, which is just before implantation.

CAPSULE:
Human embryos produced IFNγ during their in vitro development until day 5. This production may be the trigger of the HLA-G expression on the embryos.

INTRODUCTION

Some of the human embryos produce interferon-gamma (IFNγ) during the first 2 days of their in vitro development.1 In humans, trophoblasts do not express human leukocyte antigen (HLA) class II molecules and treatment with IFNγ fails to up-regulate class II expression on the cell surface.2 On the other hand, the extravillous trophoblast expresses class I molecules. However, the expression of class I is abnormal because HLA-G cell-surface proteins are expressed, not HLA-A or B.3 HLA class I can be induced by IFNγ treatment.4

The HLA-G is a nonclassical class I major histocompatibility complex molecule. It is expressed in fetal placental tissues at the maternofetal interface where classical HLA class I and II antigens are absent. This restricted expression suggests that HLA-G products may play an important role in the immunotolerance of the semi-allogeneic fetus by the mother.5
The aim of this study is to evaluate the profile of IFNγ production between days 2 and 5 after in vitro fertilization to assess whether IFNγ is still produced just before implantation which may trigger the HLA-G expression on embryos.

MATERIAL AND METHODS

Preimplantation embryo culture media (PECM) samples were collected from in vitro fertilization (IVF)-embryo transfer cycles. Follicular growth was stimulated with gonadotropin-releasing hormone analog/human menopausal gonadotropin/human chorionic gonadotropin (hCG) using a long protocol version. Oocyte retrieval was performed by ultrasound-guided transvaginal needle aspiration under general anesthesia, approximately 34–36 hr after hCG injection. After immediate collection the oocytes were placed in INRA Menezo B2 media (Laboratoire C.C.D., Paris, France), which were preincubated for one night (37°C, 5% CO₂, 95% air). One milliliter of INRA Menezo B2 medium without additional serum was used for oocyte or embryo culture and for sperm preparation. The sperm preparation was done by the swim-up method and oocytes were inseminated with a sperm concentration of 150,000/mL 4–5 hr after retrieval.

Cumulus and corona cells were removed mechanically 18–20 hr after insemination and the oocytes were checked for fertilization. Fertilized oocytes were placed in fresh INRA Menezo B2 media. The first PECM were collected 48 hr after ovum pick-up (day 2) when a check for cleavage also was done. Twenty embryos from IVF patients were further cultured in preincubated M3 Medium (MediCult, Copenhagen, Denmark) from day 2 until day 5 before embryo transfer. The media were refreshed daily and the cleavage stage of each embryo was noted. Eighty PECM samples were collected and stored at –70°C until measurement. INRA Menezo B2 and M3 media served as controls.

IFNγ levels were tested with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Medgenix, Brussels, Belgium). The minimum detectable level for IFNγ was 0.03 IU/mL. Data processing and statistical analysis were performed using SPSS 5.2 for Windows. Differences between groups were assessed by the Mann-Whitney U and Kruskal-Wallis test, with \( P < 0.05 \) as the level of statistical significance.

RESULTS

INRA Menezo B2 medium as well as M3 medium showed background IFNγ levels of <1.4 IU/mL, and this value was subtracted from all PECM measurements. Of the 80 PECM 49 had detectable IFNγ secretion. All 20 embryos produced measurable IFNγ at least for 1 day during their in vitro development between days 2 and 5. IFNγ was produced by four embryos at day 2, by 19 embryos at day 3, by 15 embryos at day 4 and by 11 embryos at day 5. Eighty percent of the embryos produced IFNγ at least for 2 days. Daily IFNγ production is shown in Figure 1. There was no significant difference in IFNγ production between in vitro culture days \( P = 0.66 \). The following IFNγ levels were found at the different embryo stages: 2–6 cells, 0.72 IU/mL \( n = 16 \); 7–9 cells, 0.58 IU/mL \( n = 16 \); 10 cell blastocyst, 0.65 IU/mL \( n = 17 \). No significant differences in IFNγ levels were found between the developmental stages of the embryos \( P = 0.79 \).

DISCUSSION

In the pig a clear-cut IFNγ production was found in the trophectoderm at implantation, whereas no IFNγ production could be shown in the mouse. Human trophoblasts also produce IFNγ. Recently we demonstrated IFNγ production by human embryos during the first 2 days of their in vitro development. This study showed that all embryos produced IFNγ during their in vitro development until day 5. IFNγ production seems to peak between days 3 and 4, which is just before implantation. As human trophoblasts express the IFNγ receptor, it is possible that one of the functional roles of IFNγ is exhibited via an autocrine way.

Looke and King suggested that HLA-G may be involved in the recognition of trophoblasts by maternal large granular lymphocytes in the decidua. Possibly the cells corresponding to HLA-G function in down regulating normal alloimmune responses, because the reduction or absence of class 1 antigen expression on a cell surface increases recognition and lysis of these cells by Natural Killer cells. It is shown that about 43% of human trophoblasts were positive for HLA-G. Patients who become pregnant have a significantly higher proportion of HLA-G

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Fig. 1. IFNγ levels in preimplantation embryo culture medium on days 2–5 of in vitro fertilization \( n = 4, 19, 15, 11, \) respectively.
positive sibling blastocysts than patients who do not conceive (57.2% versus 31.3%).

It is known that IFN-γ increases the expression of class I antigens approximately twofold on first trimester human trophoblast cells and on JEG-3 choriocarcinoma cells both at the cell surface and mRNA level. But in some cell lines derived from trophoblastic tumors (Jar) HLA-G expression was not augmented by IFN-γ. It appears that IFN-γ can only up-regulate class I expression in cells which normally produce these antigens.

In summary, the results of this study document the production of IFN-γ by human preimplantation embryos during day 2-5 in vitro development. This production of IFN-γ may be the trigger of the HLA-G expression on the human embryos which is important for the embryo in its resistance against the maternal immune system.

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REFERENCES