JEG-3 Cell Culture Supernatants Cause Reduced Interferon-γ and Interleukin-17 Production in Mixed-Lymphocyte Reactions

Sutatip Pongcharoen1, Pannika R. Niumsup2 Donruedee Sanguansermr1

1Department of Medicine, Faculty of Medicine, Naresuan University, Phitsanulok, Thailand; 2Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

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Correspondence
Dr. Sutatip Pongcharoen, Department of Medicine, Faculty of Medicine, Naresuan University, Phitsanulok, Thailand 65000.
E-mail: sutatipp@nu.ac.th

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Problem
Immunoregulatory effects of choriocarcinoma-derived factors on leukocytes have been documented. The present study was designed to investigate the effect of JEG-3 culture supernatants on interferon-γ (IFN-γ), interleukin-17 (IL-17) and IL-1β production in the mixed lymphocyte reactions (MLRs).

Method of study
A human choriocarcinoma cell line JEG-3 was used to test the effects of its culture supernatants on the proliferation and cytokine production in the MLRs. The cell proliferation was assessed using the BrdU incorporation and the amounts of cytokines were measured using enzyme-linked immunosorbent assays.

Results
The JEG-3 culture supernatants caused significantly reduced IFN-γ and IL-17 production in the MLRs. However, the supernatants did not influence MLR production of IL-1β.

Conclusion
IFN-γ and IL-17 are mainly produced by activated T cells but IL-1β is primarily produced by monocytes, thus suggesting that immunoregulatory factors of JEG-3 cells selectively inhibit cytokine production by activated T cells rather than that of the monocytes.

Introduction
Interferon-γ (IFN-γ) is produced by activated T lymphocytes. It has been shown that purified uterine natural killer cells and decidual T cells express IFN-γ,1,2 which can be stimulated by interleukin-2 (IL-2) and IL-12.3 IL-17 is a proinflammatory cytokine produced by a subset of activated CD4+ T cells distinct from Th1 and Th2 cells.4 We have recently reported the stimulatory effect of IL-17 on JEG-3 cell invasion5 as well as the expression of IL-17 in the human placenta.6 IL-17 receptor expression is distributed ubiquitously7 and we have also found that JEG-3 human choriocarcinoma cells express IL-17R mRNA and protein.8 IL-17 activities include stimulating T cells in allergen-specific cellular and humoral immunity, stimulating cytokine expression by different cell types8 and being associated with inflammatory disease and angiogenic process.9,10 IL-1β is produced by macrophages, promoting a wide range of cellular activities as well as inflammatory responses.11

Normally, an immune reaction to the conceptus does not cause its rejection, indicating a deviation of the maternal immune responses which favor pregnancy. Maternal T-cell population is partially and
transiently tolerant to fetal histocompatibility antigens during gestation. 12-14 Immunoregulatory factors produced by the trophoblast may facilitate the survival of the semi-allogeneic fetus in normal pregnancy. As pregnancy involves increased activated T cells but without compromising the trophoblast cells, 15 this study was aimed to investigate the immunoregulatory properties of culture supernatants from JEG choriocarcinoma cells as a model for human trophoblast on leukocyte cytokine production in the mixed lymphocyte reactions (MLRs). The cytokines tested are the representative of the T helper type 1 cytokine, the IFN-γ, the T helper II-17 cytokine and the monocyte cytokine II-1β.

Materials and methods

Cell Lines

The human choriocarcinoma cell line JEG-3 cells were obtained from the American Type Culture Collection (ATCC/number HTB-36) (University Boulevard, Manassas, VA, USA) and were grown as previously described. 5 A human endometrial adenocarcinoma cell line, obtained from the ATCC (number CRL-1622) and similarly grown, was used as an unrelated control supernatant.

MLRs and the Treatment with JEG-3 Culture Supernatants

Heparinized venous whole blood was obtained from healthy donors at the Blood Bank Centre of Naresuan University Hospital. This was approved by the Ethical Committee of Naresuan University. Peripheral blood leukocytes (PBLs) were isolated by density gradient centrifugation using Lymphoprep (Axis-Shield Poc AS, Oslo, Norway). The one-way MLRs assays were performed. Briefly, the PBLs used as stimulator cells were treated with 50 units/ml of mitomycin C (Sigma) for 20 min at 37°C in a CO2 incubator, washed twice in phosphate-buffered saline and then ready to be used. The responder cells are untreated PBLs.

The effects of the JEG-3 culture supernatants on PBL proliferation in the MLRs were performed. Briefly, 1 x 10^5 cells/well of mitomycin C-treated stimulator PBLs and 1 x 10^5 cells/well of responder PBLs in complete medium were plated in a final volume of 200 µl in each well of 96-well flat-bottomed tissue culture plates. Normal MLR control wells contained responders and mitomycin C-treated stimulators without the supernatant. The JEG culture supernatants were added into the MLR wells at 20%, 10%, and 5% concentrations, each in triplicate wells. An unrelated control was culture supernatants from a human endometrial adenocarcinoma cell line used at the same range of concentrations. Negative controls were responders or stimulators alone. The viability of the PBLs was tested with Trypan blue solution before the cultures were harvested and the results showed that the cells were 90-96% viable in all experiments.

The MLR cultures were incubated for 5 days before the supernatants were collected and the cell proliferation was assessed using the Cell Proliferation ELISA, BrdU (Roche Applied Science, IN, USA) according to the manufacturer’s instruction. Statistical differences on the cell proliferation between the JEG-3 supernatant treated and untreated MLR cultures were determined using the one-way ANOVA with P < 0.05 as a significant difference. The MLR assays were performed on three different occasions using all different blood donors for all experiments.

Measurement of IFN-γ, IL-17, and IL-1β

The IFN-γ, IL-17 and IL-1β in culture medium of the MLR were measured using enzyme-linked immunosorbent assay kits (R&D System, Minneapolis, MN, USA) according to the manufacturer’s instructions. Statistical differences between the levels of cytokines produced in JEG-3 supernatant treated and untreated MLR culture medium were determined using the one-way ANOVA with P < 0.05 as a significant difference.

Results

In the absence of JEG-3 supernatants the PBLs in the MLRs greatly proliferated. At all concentrations used, the JEG-3 supernatants significantly suppressed (P < 0.05) the proliferation of PBLs in the MLRs, whereas the endometrial adenocarcinoma supernatants did not have any effect (Fig. 1). The JEG-3 supernatants significantly decreased (P < 0.05) the production of IFN-γ by the MLR (Fig. 2a). The endometrial adenocarcinoma supernatants did not influence IFN-γ production. At the highest concentration, JEG-3 supernatants significantly decreased (P < 0.05) IL-17 production by the MLRs (Fig. 2b), while there was no change in the
MLRs treated with endometrial adenocarcinoma supernatants. However, like endometrial carcinoma supernatants, JEG-3 supernatants did not influence IL-1β production in the MLRs (Fig. 2c). JEG-3 and endometrial carcinoma culture supernatants as well as the complete medium alone contained only small amounts of these cytokines (<10 pg/mL).

Discussion

The JEG-3 culture supernatants down-regulated IFN-γ and IL-17 production but did not affect IL-1β production in the MLRs, indicating selective inhibition of T cell cytokines rather than macrophage cytokines. In addition, the expression of IL-1β can be considered non-specific and not directly associated with the allogeneic T-cell responses,16 thus the JEG-3 supernatants are likely to specifically influence the allogeneic T-cell response rather than the inflammatory process per se. IFN-γ may cause detrimental pregnancy outcomes,17 thus reduced local IFN-γ production may favor successful pregnancy. IL-17 is present at the fetomaternal interface6,18 which can regulate JEG-3 cell invasion.5 IL-17 is produced by a unique subset of helper T cells,4 thus unlike the Th1 and Th2 cytokines, IL-17 may be differentially regulated at the fetomaternal interface.

Suppressive effects of trophoblast cell line-derived factors on lymphocyte proliferation have been reported.19–22 Our unpublished observations showed that the expression of IL-2Rα (CD25) by phytohemagglutinin-activated lymphocytes exposed to JEG-3 culture supernatants did not differ from that of control unstimulated lymphocytes. Thus, the immunoregulatory factors in these supernatants may not involve the lymphocyte activation pathway. Reduced IFN-γ and IL-17 production found in the
present study may reflect the decreased proliferation of the T cells. It has been well documented that IL-17 participates in the allogeneic T-cell proliferation as well as proinflammatory process via induction of proinflammatory cytokines. Therefore, reduced IL-17 production in the MLR treated with JEG-3 supernatants may be involved in less T-cell activity and reduced production of other proinflammatory cytokines.

As IL-17 is produced by activated helper T cells, which have important roles in various pathophysiological responses, the reduced IL-17 production caused by JEG-3 supernatants may demonstrate one of the regulatory properties of trophoblast factors on potential helper T cells. Immunoregulatory factors in JEG-3 supernatants may also be prostaglandins and progesterone as these substances can cause immune suppression associated with changes in Th1/Th2 cytokine profile such as decreased IFN-γ and TNF-α and increased IL-4. However, the immunoregulatory pathways remain to be defined. In summary, the present study showed that immunoregulatory effect of culture supernatants generated from JEG-3 choriocarcinoma cells on the MLR is involved in reduced T-cell cytokine production.

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