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Microparticle-Free Placentally Derived Soluble Factors Downmodulate the Response of Activated T Cells

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ABSTRACT: Soluble placental factors may have immunoregulatory properties and have been demonstrated to inhibit T-lymphocyte proliferation *in vitro.* On the other hand, placentally derived syncytiotrophoblast microparticles and crude placental homogenates have been demonstrated to inhibit proliferation of mixed lymphocytes *in vitro.* Because previous studies on placentally derived soluble factors may have been contaminated by the presence of trophoblast-derived microparticles, we prepared microparticle-free placental supernatants. Such supernatants reduced the activation response of T cells, as well as their proliferation and the production of cytokines such as interleukin-2 and interferon gamma, in a dose-dependent manner. This reduction in T-cell proliferation does not appear to be caused by indoleamine 2,3-dioxygenase (IDO) because it was not reversed by the addition of L-tryptophan or an inhibitor of IDO (1-methyl-DL-tryptophan). No evidence was found for the presence of IDO in these supernatants when we used a biochemical assay measuring tryptophan catabolism. We conclude that the placenta produces currently unknown soluble factors that reduce T-cell activation, proliferation, and cytokine production. *Human Immunology 66, 977–984 (2005).* © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: IDO; CD69; T-cell proliferation; Th1 cytokine

ABBREVIATIONS

IFN-γ	interferon gamma
IDO	indoleamine 2,3-dioxygenase
IL-2	interleukin-2
1-MT	1-methyl-DL-tryptophan
PBL	peripheral blood lymphocyte
PBS	phosphate-buffered saline
PHA	phytohemagglutinin
PMA	phorbol 12-myristate 13-acetate
STBM	syncytiotrophoblast microparticles
L-trp	L-tryptophan

INTRODUCTION

During pregnancy, survival of the semiallogenic fetus depends on processes that suppress the maternal immune response to fetal alloantigens during gestation [1]. Abnormal maternal immune tolerance to the fetal semiallograft have been implicated in several common disease processes of pregnancy, including recurrent early miscarriage and preeclampsia [2–4]. It has been proposed that local immunoregulatory factors present at the maternal-fetal interface may cause enhancement of a Th2-type response (humoral) with a relative decrease in the Th1-type response (cell mediated) [5, 6]. An increase in the ratio of Th2 cytokines to Th1 cytokines is associated with successful pregnancy, and conversely, a decrease in this ratio is associated with recurrent pregnancy loss and preeclampsia [7–11]. One possible factor that could mediate such an effect is progesterone, which has been demonstrated to promote the production of Th2 cytokines, thereby contributing to the maintenance of pregnancy [12].

Several studies have indicated that the placenta can exhibit immunoregulatory properties [13]. In this regard, placentally derived syncytiotrophoblast microparticles (STBM), which were generated by mechanical disruption of the placenta, have been demonstrated to inhibit proliferation of peripheral blood lymphocytes (PBLs) *in vitro,* a feature that was associated with reduced interleukin-2 (IL-2) receptor expression [14, 15]. Fur-

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Received April 27, 2005.

0198-8859/05/$–see front matter
doi:10.1016/j.humimm.2005.07.006

thermore, STBM have been demonstrated to reduce expression of the activation markers HLA-DR, IL-2R, TfR, and CD69 on PBLs, which had been stimulated by mitogen phytohemagglutinin (PHA), and to induce apoptosis of the Jurkat T-cell line [15–17]. In addition, it has also been demonstrated that crude placental extracts obtained after mechanical dissection of villous tissue can inhibit the proliferation of PHA-stimulated peripheral blood mononuclear cells *in vitro* [13, 18]. These findings suggest that placental factors and/or particles are immunoregulatory in nature. However, it remains unclear whether placentally derived soluble factors or microparticles present in placental homogenates are responsible for this reduction in T lymphocyte responsiveness.

In order to understand the possible effects of placental soluble factors, we prepared microparticle-free placentally derived conditioned supernatants (VE-CM) from villous explant cultures. Furthermore, in contrast to previous studies in which mixed lymphocytes were used, we used purified T cells and monitored several parameters such as T-cell activation, proliferation, viability, apoptosis, and cytokine production after incubation with VE-CM.

MATERIALS AND METHODS

Preparation of VE-CM

This study was approved by the Cantonal Institutional Review Board of Basel, Switzerland, and written informed consent was obtained in all instances. Placentas were obtained after normal term delivery or elective cesarean section from uncomplicated pregnancies and transported in a sterile manner. Collected placentas were processed within 30–90 minutes of collection. After removal of decidua, villous explants 1–2 mm in size were cut and washed in sterile phosphate-buffered saline (PBS). Explants were cultured in Dulbecco modified Eagle medium (DMEM):F-12 nutrient mixture (1:1) (Gibco Invitrogen Life Technologies, Grand Island, NY, USA) supplemented with 1% antimycotic/antibiotics (Gibco), 10% fetal calf serum (FCS), 25 U/ml heparin (Roche Diagnostics, Mannheim, Germany), 50 U/ml aprotinin (Fluka Chemicals, Buchs, Switzerland) and 2 mM $MgSO_4$ for 72 hours at 37°C in 5% CO_2. After incubation the culture supernatant was collected and stored at −70°C. VE-CM was obtained from stored culture supernatants after high speed ultra centrifugation to deplete STBM as described previously [19].

Isolation of T Lymphocytes

Blood samples (40 ml) were obtained from healthy donors at the blood donation center, Swiss Red Cross, Basel. PBLs were isolated from whole blood by centrifugation over a Ficoll Paque plus gradient (Amersham Biosciences, Uppsala, Sweden). $CD4^+$ and $CD8^+$ T cells were enriched together with specific magnetic microbeads (Miltenyi Biotech, Gladbach, Germany) with MACS mini columns (Miltenyi Biotech) according to the manufacturer's instructions. The purity of the enriched T lymphocytes ($1.2 \times 10^7$to 1.8×10^7cells) routinely reached >90%, as confirmed by flow cytometry.

FACS Analysis

Fluorescence-activated cell sorter (FACS) analysis was performed to examine CD69 expression and apoptosis of T cells after coincubation with VE-CM. Analysis of CD69 expression was performed with directly labeled monoclonal antibodies to CD3 and CD69 (BD Biosciences, Basel, Switzerland). In brief, 1×10^6 T cells were harvested, washed and incubated with the antibodies for 30 minutes at 4°C, after which they were washed again before two-color flow cytometry with a Becton Dickinson FACScan flow cytometer (BD Biosciences). A total of 20,000 gated events were acquired and data were analyzed by Cell Quest Pro software (BD Biosciences). Apoptosis of T cells after the treatments with VE-CM was measured with an Annexin-V-FLUOS kit (Roche Diagnostics, Mannheim, Germany) in combination with propidium iodide according to the manufacturer's instructions.

T-Cell Proliferation Assay

Cell proliferation was assessed with the Cell Proliferation kit (Roche Diagnostics, Basel, Switzerland), which measures 5′-Bromo-2′-deoxy-Uridine (BrdU) incorporation during DNA synthesis. T cells (1×10^5) were cultured in triplicates in 96 wells plate (Nunc, Kamstrupvej, Denmark) for 72 hours at 37°C in 5% CO_2 in the presence or absence of VE-CM. The culture medium used was RPMI 1640 medium (Gibco) supplemented with 10% FCS, 2 mM L-glutamine (Gibco), and 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco). A total of 50 ng/ml PMA (phorbol 12-myristate 13-acetate; Sigma Chemical Company, St. Louis, MO, USA) and 1μM ionomycin (Sigma) were used as stimuli. In the last 18 hours of culture 10 μM of BrdU was added to the cells. Additions of L-tryptophan (L-trp) (Sigma) or 1-methyl-DL-tryptophan (1-MT) (Sigma) were also made.

Interferon Gamma and IL-2 ELISA

Interferon gamma (IFN-γ) and IL-2 levels produced by T cells after incubation with VE-CM were measured with commercial human IFN-γ and IL-2 enzyme-linked immunosorbent assay (ELISA) kits (Ebioscience, San Diego, CA, USA) according to the manufacturer's instructions. The ELISA plate was read at 450 nm in an ELISA reader (Molecular Devices, Sunnyvale, CA, USA), and the data were analyzed by Softmax Pro software (Molecular Devices).