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Effects of anti-phospholipid antibodies on a human trophoblast cell line (HTR-8/SVneo)

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Summary

Antibodies to phospholipids (aPL) have been shown to adversely affect trophoblast invasion *in vivo* and *in vitro*. HTR-8/SVneo cells derived from first trimester of pregnancy extravillous trophoblast were studied. Matrigel invasion assay, cytochemistry and cell-based enzyme-linked immunosorbant assay (ELISA) with aPL or normal IgG was used. Our data show that aPL at 100 μg/ml decrease invasiveness of HTR-8/SVneo cells to 60% of control ($p<0.01$), and this was also shown for primary cytotrophoblast (to 15.5% of control, $p<0.001$). aPL treatment caused a significant decrease in integrin α_1, α_5, and β_1 proteins (86%, 84%, and 87%, respectively). We conclude that HTR-8/SVneo cell culture is a suitable model to study mechanisms of action of aPL on trophoblast, which in HTR-8/SVneo cells inhibit invasion by decreasing integrins α_5, α_1, and β_1.

Introduction

Several autoimmune disorders have been linked with infertility and adverse pregnancy outcomes (Gleicher and El-Roeiy, 1988; Rote and Stetzer, 2003). Anti-phospholipid syndrome (APS) is a clinical condition characterized by predisposition to thrombosis, the presence of autoantibodies reactive with cardiolipin or other negatively charged phospholipids and phospholipid-binding proteins such as β_2-glycoprotein I (β_2-GPI). Reproductive repercussions range from infertility and recurrent miscarriage to placental insuffciency, fetal growth restriction, and pregnancy-induced hypertension (Branch et al., 1985; Radojčić et al., 2004). It was originally assumed that the main reproductive complications in APS were caused by increased predisposition to thrombosis. However,

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more recent evidence suggests direct effects of aPL antibodies on trophoblast function.

Implantation of the blastocyst into the uterine wall and development of a functional placenta are crucial events for the establishment of pregnancy. In normal placentation, extravillous trophoblast (EVT) cells of the human placenta invade the uterine stroma and develop vascular connections between mother and fetus by replacing endothelial cells and mural vascular smooth muscle cells (Pijnenborg et al., 1983). It has been well established that for normal invasion to occur, integrin switch and adherence to a physiological pattern of expression of adhesion molecules is critical (Damsky et al., 1994). Integral membrane proteins – integrin $\alpha_5\beta_1$, acting as fibronectin receptor, and integrin $\alpha_1\beta_1$ as collagen/laminin receptor – of the invasive trophoblast have been found to be particularly relevant (Damsky et al., 1992). Deviations from the normal pattern have been linked to serious conditions such as pre-eclampsia (Zhou et al., 1993).

Direct binding of antibodies to phospholipids (aPL) to trophoblast cell membranes has been demonstrated (Chamley, 1997; Di Simone et al., 2005). This has been shown to induce inhibition of human chorionic gonadotropin and decrease trophoblast fusion (Quenby et al., 2005). Cell invasion was also inhibited by aPL (Katsuragawa et al., 1997; Di Simone et al., 1999), which in isolated term trophoblast was accompanied by modulation of cell adhesion molecules (Di Simone et al., 2002). aPL were recently hypothesized to stimulate premature onset of cytotrophoblast proliferation and syncytial fusion (Bose et al., 2006) which could adversely affect EVT invasion and plugging of spiral arteries, causing miscarriage.

This study was intended to explore the direct effects of aPL on first trimester human trophoblast using an immortalized extravillous first trimester cell line HTR-8/SVneo (Graham et al., 1993). Effects of aPL on *in vitro* trophoblast invasion, cell proliferation, and expression of integrins α_1, α_5, and β_5 by HTR-8/SVneo cell are documented here indicating that integrins may be involved in the inhibition of trophoblast invasion induced by aPL. This demonstrates the suitability of this cell line for further studies of aPL-induced pathophysiology of the human trophoblast.

Material and methods

Reagents and antibodies

RPMI 1640 medium and fetal calf serum (FCS) were purchased from PAA Laboratories (Linz, Austria). Matrigel was purchased from BD Biosciences (USA). The following mouse monoclonal antibodies were used: anti-integrin α_1 and anti-integrin β_1 (Santa Cruz, CA); anti-integrin α_5 antibody (R&D Systems, UK & Europe); anti-cytokeratin-18 (Sigma-Aldrich, St. Louis, MO, USA); anti-proliferation marker Ki-67 (Dako, Denmark), anti-cytokeratin 18 fragment M30, as marker of apoptosis (Roche, Germany). Medium DMEM/F12, trypan blue, thiazolyl blue (MTT), gentamycin, were from Sigma-Aldrich, St. Louis, MO, USA. Biotinylated horse anti-mouse IgG, avidin–biotin-peroxidase complex (ABC) and diaminobenzidine (DAB) substrate kit for peroxidase were from Vector Laboratories, Burlingame, CA, USA. All other reagents were of the highest commercial grade available. IgG antibodies were isolated in our laboratory from plasma of 13 patients with APS positive for anti-cardiolipin IgG as determined by solid-phase enzyme-linked immunosorbant assay (ELISA) and 10 healthy normal non-pregnant subjects. This was achieved by ammonium-sulfate precipitation and affinity chromatography on a Sepharose 4B column (Pharmacia, Uppsala, Sweden), as previously described (Bollag and Edelstein, 1991).

Cells

The effects of aPL were studied on primary cytotrophoblast and/or HTR-8/SVneo cells. HTR-8/SVneo cells were kindly provided by Dr. Charles H. Graham (Queen's University, Kingston, ON, Canada). This cell line was obtained from human first trimester placenta explant cultures immortalized by SV40 large T antigen (Graham et al., 1993; Irving et al., 1995). Cytotrophoblast cells were isolated from first trimester of pregnancy placentas from legal abortions undertaken for non-medical reason at Institute of Obstetrics and Gynaecology, Clinical Center of Serbia, Belgrade in accordance with the local ethical standards. Primary cytotrophoblast cells were isolated from first trimester human placentas (6–12 weeks) by trypsin/DNAse digestion, purified on a Percoll gradient, characterized by immunolabelling for cytokeratin-18 and cultured as previously reported (Vićovac et al., 2007). HTR-8/SVneo cells were cultured on glass coverslips for immunocytochemistry, in 96-well plates for cell-based ELISA and on Matrigel-coated inserts for invasion assay. In each experiment, cells were treated with 100 μg/ml aPL or control IgG in DMEM/F12 for primary cytotrophoblast and RPMI 1640 for HTR-8/SVneo cells, all with 10% FCS.