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Monoclonal antibody 26 cross-reactive with β_2-glycoprotein I affects human trophoblast invasion in vitro

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ABSTRACT

Objective: Monoclonal antibody 26 (MAb 26) raised against tetanus toxoid has documented cross-reactivity with β_2-glycoprotein I. Passive introduction of this antibody in mice results in an antiphospholipid syndrome-like condition. We investigated the effects of MAb 26 on first trimester human trophoblast in vitro.

Study design: Binding of MAb 26 to placental tissue trophoblast, isolated cytotrophoblast and HTR-8/SVneo cells was analyzed by immunohisto(cyto)chemistry. Possible effects on cell invasion in vitro were assessed by Matrigel assay. Effects on cell viability were assessed by MTT test. A possibility that MAb 26 induces change in levels of effector molecules important for cell invasion was investigated. Integrin subunits α_1, α_5 and β_1, and galectin-1, were analyzed by qPCR and Western blot. Metalloproteinases -2 and -9 were assessed by gelatin zymography.

Results: Immunohisto(cyto)chemistry showed binding of MAb 26 to placental tissue trophoblast, isolated cytotrophoblast and HTR-8/SVneo cells. The antibody had a significant inhibitory effect on cell invasion by both isolated cytotrophoblast and HTR-8/SVneo. The antibody induced significant decrease in protein levels of metalloproteinases, integrin subunit α_1 and galectin-1. Cell viability was not affected.

Conclusion: MAb 26 reduces trophoblast invasion in vitro through decreased levels of metalloproteinases-2 and -9, integrin α_1 and galectin-1.

1. Introduction

Antiphospholipid antibodies (aPL) comprise a heterogeneous group of autoantibodies that are a hallmark of the systemic autoimmune disease known as antiphospholipid syndrome (APS). They are associated with poor obstetric outcomes and thrombotic events. Other aPL-related reproductive disorders range from placental insufficiency, fetal growth restriction, and pre-eclampsia to infertility [1].

Increased predisposition to thrombosis is one of the main complications in APS, but thrombotic events alone cannot account for all of the pathological findings [2]. Recent studies have provided evidence that aPL have a direct effect on trophoblast function, and found it to be a likely mechanism of recurrent pregnancy loss. aPL has been reported to inhibit trophoblast differentiation [3–5] and invasiveness [5–7], and to affect cell viability and proliferation [8,9], as well as syncytialisation of villous trophoblast [9].

Antibodies found in APS have diverse specificities. They target various membrane anionic phospholipids, phospholipid-binding plasma proteins and/or phospholipid–protein complexes. The most extensively studied phospholipid-binding protein recognized by aPL is β_2-glycoprotein I (β_2GPI), as both an autoantigen and an indispensible binding co-factor [10]. It is an abundant plasma glycoprotein with high homology between different mammalian species. Heterologous β_2GPI is a potent immunogen. It has been reported that even exposure to β_2GPI -like epitopes can lead to autoimmunity directed to autologous β_2GPI, and subsequent development of APS [11].

Inić-Kanada et al. [12] have recently documented anti-β_2GPI specificity of murine MAb 26 raised against tetanus toxoid (TTd). The antibody was produced by fusion of TTd-immunized mice spleen cells with partner myeloma cells [13]. Immunoblot analysis demonstrated MAb 26 binding to β_2GPI purified from human plasma. In addition, MAb 26 bound β_2GPI immobilized on the surface of UV-irradiated plates, directly, as well as complexed with cardiolipin or other anionic phospholipids [12]. In an in vivo mice

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model, MAb 26 exerted effects comparable to those reported for other anti-β_2GPI antibodies [14,15], including fetal loss, growth restriction and pregnancy-related morbidity [12,16]. Therefore, MAb 26 was used here to model anti- β_2GPI effects on human trophoblast cells.

2. Material and methods

2.1. Cell culture

The human extravillous trophoblast cell line HTR-8/SVneo [17,18] was kindly provided by Dr. Charles H. Graham (Queen's University, Kingston, Ontario, Canada). Primary cytotrophoblast (CT) cells were isolated from first trimester placentas (6–12 weeks) from elective pregnancy terminations at the Military Medical Academy, Belgrade, with patient consent, and upon study approval by the Institutional Ethical Board, INEP. CT cells were isolated by trypsin/DNAse digestion, followed by purification on a Percoll gradient as described previously [19]. Purity of the isolated cells was determined by cytokeratin-7 (CK-7) staining and cells were used if purity was >90%. CT cells were cultured in DMEM/F12 with 10% FBS (v/v), and HTR-8/SVneo cells in RPMI 1640 with 5% FBS (v/v) (PAA Laboratories, Linz, Austria), both containing antibiotic–antimycotic solution (Sigma, St. Louis, MO, USA), at 37 °C in a moist atmosphere of air with 5% CO_2.

2.2. Treatments

At a designated time point, medium was replaced with FBS supplemented medium (except for the zymography assay where serum-free medium was used), containing MAb 26 or commercial non-immune mouse IgG (mIgG), as suitable control. Both immunoglobulins were added to cell culture at 5 μg/ml, followed by 24 h incubation.

2.3. Invasion assay

The transwell invasion assay was performed as previously described [20]. Isolated CT cells (2×10^5) or HTR-8/SVneo cells (1×10^5) were seeded into the upper chamber of transwell inserts (6.4 mm filters, 8 μm pore size, Falcon, BD Labware, USA) coated with 10 μl (at 5 mg/ml) of Matrigel (BD Biosciences, Bedford, MA, USA) in 200 μl of treatment containing media. Corresponding media (500 μl) were added to the lower chambers. After 24 h cells on the upper side of the filters were gently removed with a cotton swab. The remaining cells were fixed with ice-cold acetone-methanol (1:1) and stained using anti-CK-7 antibody (isolated CT cells) or Giemsa (HTR-8/SVneo cells). Cells on the underside of the filters and the occupied pores were counted in at least 50 randomly selected non-overlapping fields of the membranes under a light microscope (Reichert-Jung, Germany).

2.4. Determination of viable cell number (MTT test)

The MTT test was used as an indicator of HTR-8/SVneo cell viability upon treatment [21] and was performed as described by Jovanovic et al. [7]. Absorbance was measured at 540 nm using a micro plate reader (LKB, Austria).

2.5. Immunohistochemistry

Immunohistochemistry was performed on paraffin-embedded placental tissue from the first trimester of pregnancy. Sections (7 μm) were de-waxed, rehydrated and microwaved in 10 mM citrate buffer (pH 6.0). Non-specific binding was blocked with 1% casein, sections were incubated with MAb 26 (10 μg/ml), mIgG (10 μg/ml) or anti-CK-18 antibody (Table 1) for 1 h at RT. Endogenous peroxidase activity was quenched with 1% (v/v) H_2O_2 for 30 min. Corresponding biotinylated secondary antibody was administered for 30 min and staining was visualized as indicated in Table 1. The sections were counterstained with hematoxylin (Vector laboratories, Burlingame, CA, USA), dehydrated, and mounted using Vecta Mount medium (Vector laboratories, Burlingame, CA, USA). Slides were examined using a Carl Zeiss Axio Imager 1.0 microscope (Jena, Germany), with a Canon A640 Digital Camera System (Tokyo, Japan).

2.6. Immunocytochemistry

Isolated CT and HTR-8/SVneo cells were cultured on glass cover slips, for 24 h (CT), or until 80% confluence was reached (HTR-8/SVneo). Cells were fixed with ice-cold acetone methanol (1:1) and

Table 1
Primary and secondary antibodies and detection methods used in the study.

Antibody	Manufacturer	Host animal	Application	Dilution	Detection
Primary antibodies					
Anti-integrin α1	R&D (Abingdon, UK)	Mouse	Western blot	1:3000	ECL*
Anti-integrin α5	Santa Cruz (Santa Cruz, CA, USA)	Rabbit	Western blot	1:500	ABC**/DAB***
Anti-integrin β1	Chemicon (Temecula, CA, USA)	Rabbit	Western blot	1:3000	ECL
Anti-galectin-1	R&D (Abingdon, UK)	Goat	Western blot	1:1000	ABC/DAB
Anti-cytokeratin-7	Santa Cruz (Santa Cruz, CA, USA)	Rabbit	Immunocytochemistry	1:200	ABC/DAB Fluorescence
Anti-cytokeratin-18	Sigma (St. Louis, CA, USA)	Mouse	Immunohistochemistry	1:6000	ABC/DAB
Anti-β-actin	Sigma (St. Louis, CA, USA)	Rabbit	Western blot	1:400 1:3000	ABC/DAB ECL
Secondary antibodies					
HRP-linked anti-mouse IgG	Cell Signaling Technology (Beverly, MA, USA)	Horse	Western blot	1:2000	ECL
HRP-linked anti-rabbit IgG	Cell Signaling Technology (Beverly, MA, USA)	Goat	Western blot	1:2000	ECL
Alexa Fluor 488 anti-mouse IgG	Life Technologies (Carlsbad, CA, USA)	Rat	Immunocytochemistry	1:1500	Fluorescence
Alexa Fluor 555 anti-rabbit IgG	Life Technologies (Carlsbad, CA, USA)	Goat	Immunocytochemistry	1:1500	Fluorescence
Byotinilated anti-mouse IgG	Vector Laboratories (Burlingame, CA, USA)	Horse	Immunohistochemistry	1:200	ABC/DAB
Byotinilated anti-rabbit IgG	Vector Laboratories (Burlingame, CA, USA)	Goat	Western blot Immunocytochemistry	1:750 1:200	ABC/DAB ABC/DAB
Byotinilated anti-goat IgG	Vector Laboratories (Burlingame, CA, USA)	Rabbit	Western blot	1:750	ABC/DAB
*Enhanced chemiluminescence	Pierce Biotechnology (Rockford, IL, USA)				
**Avidin-biotinylated peroxidase complex	Vector Laboratories (Burlingame, CA, USA)				
***Diaminobenzidine substrate kit for peroxidase	Vector Laboratories (Burlingame, CA, USA)				