



Placental Release of Distinct DNA-associated Micro-particles into Maternal Circulation: Reflective of Gestation Time and Preeclampsia

A.F. Orozco^a, C.J. Jorgez^b, W.D. Ramos-Perez^a, E.J. Popek^c, X. Yu^d, C.A. Kozinetz^d, F.Z. Bischoff^e, D.E. Lewis^{a,f,g,h,*}

^a Department of Immunology, Baylor College of Medicine, Houston, TX, USA

^b Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA

^c Department of Pathology, Baylor College of Medicine, Houston, TX, USA

^d Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

^e Biocept Inc., San Diego, CA, USA

^f Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA

^g Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA

^h Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA

ARTICLE INFO

Article history:

Accepted 29 June 2009

Keywords:

Apoptotic micro-particles
Fetal DNA
DNA-associated micro-particles
HLA-G
PLAP
Preeclampsia
Syncytiotrophoblast
Extravillous cytotrophoblast

ABSTRACT

Background: The aim of this study was to determine whether DNA-associated micro-particles (MPs) in maternal plasma express fetal-derived human leukocyte antigen-G (HLA-G) or placental alkaline phosphatase (PLAP) and whether the levels differ between women with normotensive pregnancies and preeclampsia.

Methods: DNA-associated MPs expressing HLA-G or PLAP were examined in the plasma of normal pregnant women and preeclamptic patients using flow cytometric analysis.

Results: DNA-associated HLA-G⁺ MPs were significantly increased in maternal plasma compared to plasma from non-pregnant controls ($p < 0.005$), with highest levels found in the first and second trimesters. DNA-associated PLAP⁺ MPs were also increased in maternal plasma compared to plasma from non-pregnant controls ($p < 0.006$), with highest levels in the second and third trimesters. Term preeclamptic women had higher levels of DNA-associated MPs than control pregnant women. HLA-G⁺ MPs from the plasma of preeclamptic women had more DNA per MP than HLA-G⁺ MPs from the plasma of normal pregnant women ($p < 0.03$).

Conclusions: HLA-G⁺ and PLAP⁺ MPs increase in maternal circulation at different times during gestation. DNA amounts per HLA-G⁺ MP increase in preeclamptic women which might indicate dysfunctional extravillous cytotrophoblasts.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Circulating placenta-derived micro-particles (MPs) in maternal plasma are likely released from apoptotic fetal cells, suggesting potential use as predictive biomarkers of pathogenic pregnancies and fetal development. Previous studies, using a sandwich ELISA that detects the fetal protein, placental alkaline phosphatase (PLAP), or its enzymatic activity, suggest an increase in syncytiotrophoblast MPs (STBMs) in plasma of women with early-onset

preeclampsia compared to normal pregnant women [1,2]. Although these studies indirectly found higher levels of STBMs in the second or third trimester of preeclamptic women compared to normal pregnant women [1–3], the prediction of preeclampsia earlier in gestation might allow speedier medical intervention for improved clinical outcome.

As opposed to the later gestational detection of STBMs, fetal cell-free DNA (cfDNA) can be found as early as the 5th week of gestation [4,5]. In addition, increased amounts of circulating fetal DNA correlate with pregnancy complications such as preterm labor [6], polyhydramnios [7], hyperemesis gravidarum [8], aneuploidies [9], and preeclampsia [10]. Several groups have also reported an increase in fetal cfDNA prior to the onset of preeclampsia [10–12], suggesting that fetal cfDNA could be useful to monitor or predict preeclampsia.

* Corresponding author. Department of Internal Medicine, Infectious Diseases Division, University of Texas Medical Branch, 301 University Boulevard, Mail Route 0435, Galveston, TX 77555, USA. Tel.: +1 409 747 0240; fax: +1 409 772 6527.
E-mail address: dlewis@bcm.edu (D.E. Lewis).

Our lab reported a 10-fold enrichment of fetal cfDNA using a flow sorting technique based on forward light scatter and acridine orange fluorescence of MPs found in maternal plasma [13], suggesting that plasma MPs contain DNA. An apoptotic trophoblastic cell line released PCR-amplifiable, DNase protected cfDNA in association with membranous apoptotic MPs, which we termed micro-particle DNA (mpDNA) [14]. Because maternal plasma mpDNA generated *in vivo* displayed light scatter and DNA fluorescence similar to apoptotic mpDNA generated *in vitro*, mpDNA found in maternal circulation is likely of apoptotic origin. However, there are no studies addressing the potential physical association between circulating fetal MPs and fetal cfDNA. Therefore, the aims of this study were to investigate whether MPs from pregnant women expressed the placental cell markers HLA-G and PLAP over gestation, compare levels of MPs between normal pregnancies and those with late-onset preeclampsia and to test the hypothesis that quantitative abnormalities of HLA-G⁺ and PLAP⁺ MPs are reflective of preeclampsia.

2. Materials and methods

2.1. Antibodies and reagents

Monoclonal antibody (mAb) MEM-G/1 (IgG₁), which recognizes the $\alpha 1$ domain of HLA-G, isotype matched control mAb IgG₁ mAb PLAP (H17E2, IgG₁), which recognizes human placental alkaline phosphatase, and isotype matched control mAb were purchased from Serotec (Raleigh, NC). Phycoerythrin-labeled goat-anti-mouse immunoglobulin polyclonal Ab (PE-GAMIG) was purchased from BD Pharmingen (San Diego, CA). PicoGreen, which preferentially binds to dsDNA, was purchased from Invitrogen (Carlsbad, CA).

2.2. Isolation and quantification of JEG-3 apoptotic MPs

JEG-3 apoptotic MPs were prepared as previously described [15] using double filtered (0.25 μ m) PBS (dPBS). MPs were centrifuged at room temperature, unless otherwise stated. MPs from 24 h apoptotic supernatants were separated from detached cells by two centrifugation steps (300 $\times$ g, 5 min; 800 $\times$ g, 5 min). MP concentration was determined as previously described [16]. Briefly, 100 μ L of fluorescent beads (counted at 500/ μ L with a hemocytometer) were added to 400 μ L of each MP supernatant plus 300 μ L of dPBS for a total volume of 800 μ L (1:2 dilution of MPs) and the bead count was stopped at 10,000. To rule out intrinsic “MP contamination” induced by the beads, a control bead count (100 μ L beads + 700 μ L of dPBS = 800 μ L) was determined prior to the MPs plus beads count (total MP count). After subtracting the number of background fluorescent beads (~1000 beads), the specific MP count was obtained. The concentration of MPs in 800 μ L was determined as follows: (MP concentration) = [(specific MP count) $\times$ (bead concentration)]/(bead count) and the appropriate number of MPs was centrifuged (25,000 $\times$ g, 1 h at 4 °C) in an SW 40 Ti Swinging-Bucket Rotor in a Beckman L8-M, class H, ultracentrifuge (Beckman Coulter, Miami Lakes, FL) prior to flow cytometric staining.

2.3. Assessment of HLA-G expression on JEG-3 MPs by flow cytometry

Mild acid treatment of freshly prepared MPs was performed as previously described with slight modifications [17]. Briefly, 2×10^6 apoptotic MPs were centrifuged (10,000 rpm, 10 min) using an Eppendorf Centrifuge 5415c (Eppendorf, Westbury, NY, USA). The MP pellets were re-suspended in 70 μ L of 0.2 M citrate-phosphate buffer, pH 3.0, supplemented with 0.1% BSA on ice. After 1 min incubation, the sample was neutralized by adding a 15-fold excess (1 mL) of cold 0.1% BSA in PBS, pH 7.2, centrifuged and washed with PBS (pH 7.2). MPs were incubated on ice for 20 min with MEM-G/1 diluted in PBS, pH 7.2, containing 1% BSA. An irrelevant mAb was used as IgG₁ isotype control. After washing MPs once with 0.1% BSA in PBS (pH 7.2), MPs were labeled with PE-conjugated goat-anti-mouse IgG for 20 min at 4 °C, counter-stained with PicoGreen (1:15,000 in PBS) and analyzed using a Beckman Coulter EPICS XL2 (Beckman Coulter). Data were analyzed using EXPO 32 software (Beckman Coulter).

2.4. Plasma sample collection

2.4.1. Normal pregnancies

After obtaining Institutional Review Board approval from Baylor College of Medicine and written consent from the human subjects, 5–10 mL of peripheral blood was collected in vacutainer tubes containing 1.5 mL of ACD solution A (trisodium citrate, 22.0 g/L; citric acid, 8.0 g/L; and dextrose 24.5 g/L) and processed within 24 h. Plasma from pregnant women and non-pregnant controls was

separated from whole blood by centrifugation at 800 $\times$ g for 10 min. Recovered plasma was centrifuged for an additional 10 min at 1600 $\times$ g to remove residual cells. Finally, the cell-free supernatant was removed and stored in a –80 °C freezer.

2.4.2. Preeclamptic and normotensive controls

Preeclampsia was defined as blood pressure 140/90 or greater plus proteinuria of 300 mg or greater in 24 h or 100 mg/dL or more in at least two random urine specimens collected 6 or more hours apart. Frozen (–80 °C) plasma samples (3 min at 1000 $\times$ g) from term preeclamptic and matched normal pregnancies were provided by Dr. Popek.

2.5. Labeling of plasma micro-particles

MP concentration was determined as described in Section 2. Fluorescent beads (20,000) were added to 15 μ L plasma in double filtered (0.25 μ m) PBS (dPBS) to a total volume of 800 μ L and a final concentration of 25 beads/ μ L. The number of beads counted by the flow cytometer was stopped at 1000. All labeling procedures were performed at room temperature. One million MPs were re-suspended in dPBS for a final volume of 66 μ L, labeled with 3 μ g MEM-G/1 or 1 μ g PLAP and isotype control mAbs and mixed on a BD ADAMS Nutator (Aria Medical Equipment) for 6 min. Secondary PE-GAMIG (0.8 μ g) was added directly to the labeled MP samples and mixed for 5 min. MPs were then labeled with 2 μ L PicoGreen for 10 min in the dark. Unlabeled MPs were also used as negative controls. Both labeled and unlabeled MPs were re-suspended in 350 μ L dPBS and analyzed by an EPICS XL2 flow cytometer. The number of events was stopped at 10,000. Data were analyzed using EXPO 32 software (Beckman Coulter).

2.6. Statistical analysis

Because the variables were highly skewed, comparisons between two groups (control pregnancy vs. preeclamptic pregnancy) were performed using a two-sample Wilcoxon rank-sum test. When more than two groups (non-pregnant controls, first, second, and third trimester plasma samples) were simultaneously considered, the Kruskal Wallis test was applied and two-sample Wilcoxon rank-sum tests adjusted using Bonferroni multiple comparisons tested inter-group differences. A two-sided *p* value less than 0.05 was considered statistically significant. All statistical analyses were performed using SAS[®] version 9.2.

3. Results

3.1. Concentration of total MPs during pregnancy

The number of total plasma MPs/mL from normal pregnant women over gestation and non-pregnant controls was quantitated by flow cytometry using a fluorescence bead assay [16]. Although the number of total MPs/mL increased from 9.3×10^6 MPs/mL during the first trimester to 18.3×10^6 MPs/mL during the second trimester and finally to 23.0×10^6 MPs/mL during the third trimester, no significant difference in the total number of MPs/mL for each trimester (or total pregnancy) was observed between normal pregnancies and non-pregnant controls (data not shown), indicating that the actual numbers of MPs are not reflective of *in vivo* status during normal pregnancy, and likely represent normal homeostasis of dying cells.

3.2. Characteristics of placenta-derived MPs in maternal plasma

To determine the concentration of placenta-derived MPs in the plasma of normal pregnant women, plasma MPs were labeled with MEM-G/1 (anti-HLA-G mAb), anti-PLAP or appropriate isotype control mAbs and stained with PicoGreen for flow cytometry. Supernatants from isolated JEG-3 apoptotic MPs were used as a positive control for HLA-G⁺ MPs containing DNA (Fig. 1a). Dot plots show the percentage of HLA-G⁺ MPs and PLAP⁺ MPs containing DNA in the plasma of normal pregnant women (Fig. 1b–e), indicating that both types of fetal MPs contain DNA.

3.3. Number of HLA-G⁺ MPs in normal pregnancy

Concentrations of HLA-G⁺ MPs containing DNA in the plasma of the pregnant women are shown in Fig. 2a, which ranged from

Download English Version:

<https://daneshyari.com/en/article/2789284>

Download Persian Version:

<https://daneshyari.com/article/2789284>

[Daneshyari.com](https://daneshyari.com)