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Inhibition of pregnancy-associated granulocytic myeloid-derived suppressor cell expansion and arginase-1 production in preeclampsia

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ABSTRACT

Myeloid-derived suppressor cells (MDSCs) expand in maternal peripheral blood and cord blood during normal pregnancy to maintain maternal–fetal tolerance. Here we investigated the expansion and function of MDSCs in preeclampsia (PE) patients. Maternal peripheral blood mononuclear cells (PBMCs) and cord blood mononuclear cells (CBMCs) were sampled from healthy pregnant women and PE patients, and analyzed for the frequencies and phenotypes of MDSCs and T cells. Serum levels of key human MDSC effector enzymes were measured using appropriate detection kits. Peripheral blood samples of healthy non-pregnant women were used as controls. We found that normal pregnancy is associated with a significant increase of immunosuppressive MDSCs and regulatory T (Treg) cells. There was no significant difference in the frequency of Treg cells between normal pregnancies and PE patients, but the pregnancy-associated increase of granulocytic MDSCs (G-MDSCs), but not monocytic MDSCs (M-MDSCs), in both PBMCs and CBMCs was markedly inhibited in PE patients. Furthermore, serum levels of Arg-1, an important effector molecule for G-MDSC were significantly reduced in PE patients compared to healthy pregnant women. In conclusion, the lack of G-MDSC expansion is a most notable feature of PE-associated immune-cell alterations, suggesting that restoring G-MDSCs may have the potential to treat PE.

1. Introduction

Dysregulation of the immune system during pregnancy can lead to severe complications, such as abortion, preterm delivery and preeclampsia (PE)(Laresgoiti-Servitje, 2013; Perez-Sepulveda et al., 2014; Laresgoiti-Servitje et al., 2010), and maternal immune tolerance of the fetus is crucial for a healthy pregnancy process (Anon., 2017; Erlebacher, 2013). There is much evidence indicating that several cell subsets, such as decidual NK cells in the placenta, dendritic cells, helper T (Th) cells, regulatory T (Treg) cells, and immune regulatory molecules such as PD-1, PD-L1 and Tim-3 might be important players in the maintenance of maternal–fetal immune tolerance (Laresgoiti-Servitje, 2013; Hsu and Nanan, 2014; Saito et al., 2010; Darmochwal-Kolarz et al., 2012; Noman et al., 2014; D'Addio et al., 2011; Chabtini et al., 2013; La Rocca et al., 2014; Toldi et al., 2011). Myeloid-derived suppressor cells (MDSCs) have been reported to regulate both innate and adaptive immune responses. MDSCs have been shown to be involved in many clinical disorders, including autoimmune arthritis, skin inflammation, hematological and solid malignant tumors (Jeong Ryu et al., 2015; Crook et al., 2015; Fletcher et al., 2015; Skabytska et al., 2014; Cao et al., 2014; Talmadge and Gabrilovich, 2013). MDSCs are a heterogeneous population of myeloid progenitor cells, and can be subdivided into two main subsets: MDSCs with a granulocytic phenotype (G-MDSCs) and MDSCs with a monocytic phenotype (M-MDSCs) (Serafini, 2013). Recent studies in tumor and autoimmune disease models demonstrated that G-MDSCs and M-MDSCs differ in their ability to suppress T cell responses (Dietlin et al., 2007; Movahedi et al., 2008). Multiple mechanisms are implicated in the suppressive activity of MDSCs, and one of the major mechanisms is associated with the metabolism of L-arginine, which serves as the substrate for two kinds of enzymes: arginase-1 (Arg-1) and inducible nitric oxide synthase (iNOS). L-arginine depletion causes down-regulation of the ζ-chain in T cell receptor (TCR) complex and a proliferative arrest of T cells (Mundy-Bosse et al., 2011; Kim et al., 2011; Condamine and Gabrilovich, 2011). In this study, we investigated the changes in MDSCs and T cells that are associated with PE during pregnancy. Recent studies have shown that in

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healthy pregnancies MDSCs accumulate in both maternal peripheral blood and cord blood, suggesting a potential role for MDSCs in maintaining maternal–fetal tolerance (Gervassi et al., 2014; Rieber et al., 2013; Kostlin et al., 2014; Pan et al., 2016). In the present study, we show that, although there were some moderate differences in the pregnancy-associated T cell subset changes, the frequencies of Treg cells were comparable between normal pregnant women and PE patients. However, the expansion of normal pregnancy-associated G-MDSCs, but not of M-MDSCs, was significantly inhibited in PE patients. This was correlated with a reduction of Arg-1 production, implicating an important role for G-MDSCs and Arg-1 in maintaining maternal–fetal tolerance.

2. Materials and methods

2.1. Study population

Healthy non-pregnant women, and healthy and PE pregnant women in the third trimester of pregnancy were recruited from the department of obstetrics and gynecology at the First Hospital of Jilin University. PE was diagnosed as the new onset of hypertension after 20 weeks of pregnancy (blood pressure ≥140/90 mm Hg) and new proteinuria (≥300 mg in a 24 h urine collection in the proven absence of a urinary tract infection), with a return to normal postnatally. PE was regarded as severe if any of the following criteria was present: blood pressure 160 mmHg systolic or 110 mmHg diastolic, or proteinuria 5 g/24 h (or 3+ on dipstick). All the patients in our present study were severe preeclampsia. Early onset of PE was defined as onset of the disease before 34 weeks of gestation. There were three patients defined as early-onset subtype in our study and were given corticosteroid medications to promote fetus lung maturation. In addition, sixteen of the twenty patients had hypertension and were given antihypertensive medication before blood draw. Fetal growth restriction (IUGR) was diagnosed if the fetal birth weight was below the 10th percentile for gestational age and gender, based on Chinese birth weight percentiles. Clinical characteristics of the study participants are shown in Table 1. Patients suffering from other pregnancy complications (severe infections, gestational diabetes mellitus, preexisting renal disease and chronic hypertension) were excluded. All women had cesarean section deliveries. Peripheral blood samples from normal pregnancy and PE patients (age-matched) were collected before delivery, and cord blood samples were collected from umbilical vein immediately after delivery. The non-pregnant group consisted of age-matched healthy females. The local ethics committee approved this study and all women gave their written informed consent.

Table 1
Clinical Characteristics of Normal pregnant women (NP) and Preeclamptic women (PE).

	Normal pregnant women (NP; n = 21)	Preeclamptic women (PE; n = 20)
Age (years)	29.1 (20-35)	29.3 (27-42)
Primiparas (%)	13 (61.9)	19 (95) [a]
Systolic blood pressure (mmHg)	123.5 (110-139)	168.3 (132-195) [b]
Diastolic blood pressure (mmHg)	79.1 (70-93)	114.3 (110-139) [b]
Gestational age at birth (weeks)	38.6 (36-40)	31.8 (26-39) [a]
Fetal birth weight (g)	3372 (3000-4270)	2463 (1300-3750) [a]
Intrauterine growth restriction (%)	0 (0)	4 (20) [c]

Data are presented as median (interquartile range) for continuous variables and as number (%) for categorical variables.

[a] P < 0.01 versus normal pregnant women.

[b] P < 0.001 versus normal pregnant women.

[c] P < 0.0001 versus normal pregnant women.

2.2. Cell isolation and flow cytometry

Fresh peripheral blood mononuclear cells (PBMCs) and cord blood mononuclear cells (CBMCs) were prepared from heparinized blood samples by Ficoll density gradient sedimentation. Trypan blue staining confirmed a cell viability of > 95% for all samples, and all assays were performed within 4 h of sample collection. Fluorescence-conjugated antibodies against the following surface antigens (BD Biosciences) were used to identify MDSCs populations: CD11b, HLA-DR, CD33, CD66b, and CD14. Fluorescence-conjugated antibodies used to characterize T cells and their subsets included CD3, CD4, CD8, CD45RA, CD45RO, CD25, CD127, and $Foxp3^+$ (intracellular staining). Corresponding isotype controls were applied and data were analyzed using a LSR Fortessa flow cytometer (BD Biosciences, San Jose, CA, USA).

2.3. Detection of serum NO, iNOS and Arg-1

Serum NO and iNOS levels were detected using appropriate detection kits (A012 and A014-1, Nanjing Jiancheng Bioengineering Institute), and serum Arg-1 levels were examined by a quantitative colorimetric arginase determination assay (Quanti Chrom Arginase Assay Kit, DARG-200, Bioassay Systems, CA, USA) according to the manufacturers' instructions.

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0. Differences between group means were analyzed by unpaired two-tailed Student's t-test. Where there were more than two groups, differences among group means were analyzed by ANOVA test with post hoc analysis by Tukey's test. A P value of < 0.05 was considered to be significant.

3. Results

3.1. Inhibition of pregnancy-associated G-MDSC expansion in PE patients

We first measured the frequencies and numbers of MDSCs and their subsets (G-MDSCs and M-MDSCs) in maternal peripheral blood mononuclear cells (PBMCs). MDSCs were defined by flow cytometry as $CD11b^+HLA\text{-}DR^-CD33^+$ cells, which were further divided into $CD66b^+CD14^-$ G-MDSCs and $CD14^+$ $CD66b^-$ M-MDSCs (Fig. 1A). Although significant MDSC expansion was detected in both healthy pregnant women and PE patients (24.63 ± 16.61% and 13.62 ± 8.5%, respectively) compared to non-pregnant controls (4.81 ± 2.81%), the percentage of total MDSCs in PBMCs was significantly lower in PE than in healthy pregnant women ($P < 0.01$; Fig. 1B). Further analysis showed that healthy and PE pregnant women had a similar increase of the M-MDSC subset, but only the former group had increased G-MDSCs compared to non-pregnant controls (Fig. 1B). There was no detectable difference in the percentages of G-MDSCs between PE and non-pregnant control women. The absolute numbers of MDSCs and their subsets showed a same trend with the frequencies (Fig. 1C). These results indicate that the pregnancy-associated expansion of G-MDSCs, but not of M-MDSCs, in the maternal blood is completely inhibited in PE.

We also measured the frequencies of fetal MDSCs and their subsets (G-MDSCs and M-MDSCs) in CBMCs. Although the frequencies of MDSCs in CBMCs of both healthy pregnant (26.86 ± 14.13%) and PE (15.68 ± 8.8%) women were significantly higher than those in PBMCs from non-pregnant controls (4.81 ± 2.81%; Fig. 1B), there was a significant reduction in PE patients compared to healthy pregnant women ($P < 0.01$; Fig. 2A and B). Similar to the maternal MDSCs, only the G-MDSC (7.29% ± 6.94% vs. 16.84% ± 11.21%; $P < 0.001$), but not M-MDSC (6.01% ± 4.97% vs. 7.08% ± 5.78%; $P = 0.689$) subset was reduced in PE patients (Fig. 2B). The absolute numbers of MDSC and their subsets showed a same trend with the frequencies (Fig. 2C). These