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Maternal Circulating CD34+VEGFR-2⁺ and CD133⁺ VEGFR-2⁺ Progenitor Cells Increase During Normal Pregnancy but Are Reduced in Women With Preeclampsia

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Abstract

Circulating endothelial progenitor cells (EPCs) may contribute to vascular endothelial cell homeostasis, and low levels of these cells are predictive of cardiovascular disease. We hypothesized that circulating EPCs increase in number during uncomplicated pregnancy but are reduced in women with preeclampsia. Peripheral blood was obtained from pregnant women and from nulligravidas in cross-sectional design. Cells expressing CD34 or CD133, in combination with vascular endothelial growth factor receptor-2 (VEGFR-2), were enumerated by flow cytometry. Both CD34⁺VEGFR-2⁺ (doubly positive) and CD133⁺VEGFR-2⁺ cells were significantly increased during the second and third trimesters of uncomplicated pregnancy compared to the first trimester. First trimester and nulligravida groups did not differ. Endothelial progenitor cells, quantified by flow cytometry or by circulating angiogenic cell (CAC) culture assay, were significantly reduced in women with preeclampsia compared to third trimester controls. Circulating EPCs appear to increase during normal pregnancy, and comparatively reduced numbers of these cells exist during preeclampsia.

Keywords

Endothelial progenitor cells; pregnancy; preeclampsia; angiogenic factors

INTRODUCTION

The normal maternal cardiovascular adaptation to pregnancy includes augmented endothelium-mediated relaxation and blunted vascular responsiveness to several vasoconstrictors, changes that contribute to a profound fall in peripheral vascular resistance. The pregnancy-specific syndrome preeclampsia is characterized by failure of these adaptations

and development of hypertension and proteinuria during the second half of pregnancy. Maternal endothelial cell dysfunction is a root cause of the peripheral vasoconstriction that characterizes preeclampsia and the multiorgan damage that often occurs in severe cases.¹ Morphologic evidence of endothelial damage can be seen in the glomerular capillaries (endotheliosis)² and frequently in the uteroplacental arteries (acute atherosclerosis).³ The mechanisms of endothelial dysfunction and damage in preeclampsia, however, remain unclear.

Endothelial progenitor cells (EPCs) comprise a heterogeneous group of circulating cells, derived from the bone marrow as well as the vascular wall, that are thought to play a role in endothelial homeostasis and vascular remodeling.^{4–11} Although no EPC-specific antigen has been identified to date, the combined cell surface expression of CD133 (whose expression is lost during further differentiation) and vascular endothelial growth factor receptor-2 (VEGFR-2, also known as kinase insert domain receptor, KDR) is considered to define subtype of circulating EPC whereas others coexpress CD34 and VEGFR-2, with declining or absent expression of CD133.^{11–15} Low numbers of circulating CD34⁺VEGFR-2⁺, CD133⁺VEGFR-2⁺, or CD34⁺CD133⁺VEGFR-2⁺ (triply positive) cells are reportedly predictive biomarkers of cardiovascular disease and other diseases in the nonpregnant state.^{11,13,16,17} Endothelial progenitor cells are mobilized to the circulation by nitric oxide–dependent pathways in response to vascular endothelial growth factor (VEGF), placental growth factor (PlGF), estrogen, and several other factors.^{18–20} Few studies, however, have examined the changes in circulating progenitor cells during pregnancy.

We used flow cytometry to test the hypothesis that numbers of circulating CD34⁺VEGFR-2⁺ and CD133⁺ VEGFR-2⁺ progenitor cells increase with advancing uncomplicated pregnancy and that women with preeclampsia manifest comparatively reduced numbers of these cells. We also compared preeclampsia cases and controls for the number of early outgrowth, hematopoietic-derived EPCs (commonly termed “circulating angiogenic cells” [CACs])^{10,12,21–23} forming in cell culture assay. Given the potential importance of VEGF and PlGF in mobilization of progenitor cells from bone marrow, we looked for correlations of progenitor cells with maternal plasma concentrations of free PlGF and soluble fms-like tyrosine kinase-1 (sFlt-1; also known as soluble VEGF receptor-1), the latter an anti-angiogenic decoy receptor that is increased in the circulation of most women with preeclampsia that binds and thereby neutralizes VEGF and PlGF.²⁴

METHODS

Study Population and Blood Collection

The Magee-Womens Hospital institutional review board approved the study. Written informed consent was obtained from all study participants who were recruited at the time of presentation to Magee-Womens Hospital for prenatal care. Blood samples for flow cytometric analysis were obtained in cross-sectional design from (1) 52 nulliparous controls with uncomplicated pregnancy outcome (11 first trimester [range 6 to 11 weeks], 21 second trimester [18 to 24 weeks], 20 third trimester [28 to 41 weeks]), (2) 14 nulliparous women with preeclampsia, and (3) 13 nulligravid women who were normotensive and not using oral contraceptives, the latter group studied during either the follicular (n = 5) or the luteal (n = 8) phase of the menstrual cycle. All of these women provided blood samples at a single time point. Table 1 lists clinical characteristics of these groups. One woman with preeclampsia and 4 controls were in labor at the time of venipuncture. The third trimester control and preeclampsia groups did not differ by mean gestational age at the time of venipuncture. A different set of 11 women with preeclampsia and 12 normotensive control pregnant women provided a third trimester blood sample for enumeration of early outgrowth, CACs in cell culture; Table 2 lists the clinical characteristics of these women.

Patients with chronic hypertension, diabetes, renal disease, or other significant preexisting metabolic disorders or with a recent history of cigarette smoking, illicit drug use, or with multifetal gestation were excluded. Pre-eclampsia was defined using the criteria of hypertension and proteinuria arising de novo after the 20th week of gestation, and hyperuricemia, with reversal of hypertension and proteinuria after delivery.²⁵ Criteria for gestational hypertension were an absolute blood pressure of >140 mm Hg systolic and/or >90 mm Hg diastolic. Proteinuria was defined as >300 mg of protein in a 24-hour urine collection, >2+ on a voided or >1+ on a catheterized random urine sample, or a random urine protein/creatinine ratio of >0.3. Hyperuricemia was defined as >1 standard deviation (SD) above normal for the given gestational age (at term >5.5 mg/dL [3.3 mmol/L]). Preeclampsia was considered severe if characterized by sustained systolic blood pressure of >160 mm Hg or sustained diastolic blood pressure of >110 mmHg or severe proteinuria (>3+ on dipstick or proteinuria >5 g/24 hour urine collection). Pregnancy controls had uncomplicated outcomes and were delivered of healthy babies at term of appropriate weight.

Blood Samples

Peripheral venous blood was withdrawn from the antecubital vein. After discarding the first 1 mL of blood to avoid vascular cells displaced by the venipuncture, the remainder of the sample was collected into 10 mL sterile tubes containing 4 mmol/L potassium-ethylenediaminetetraacetic acid (EDTA) and processed within 2 hours of collection for flow cytometry or cell culture. The plasma was stored at -80°C without thaw until further analysis.

Flow Cytometry

Vascular endothelial growth factor receptor-2-positive cells coexpressing either CD34 or CD133 were enumerated by methods similar to those described by Vasa et al.¹² Monoclonal antibodies (mAbs) against human VEGFR-2 (KDR; clone IMC-1121, ImClone Systems, Inc, New York) were conjugated with fluorescein isothiocyanate (FITC) using an antibody labeling kit (A-20181, Invitrogen/Molecular Probes, Eugene, Oregon). Within 2 hours of blood collection, duplicate samples of 100 µL of whole blood were incubated for 15 minutes in the dark with FITC-conjugated anti-KDR mAbs, along with peridinin chlorophyll protein (PerCp)-conjugated mAbs against human CD45 (leukocyte common antigen; clone 2D1, BD Pharmingen, San Diego, California) and either phycoerythrin (PE)-conjugated mAbs to CD34 (clone 581, BD Pharmingen) or allophycocyanin-(APC)-conjugated mAbs to CD133 (clone AC133, Miltenyi Biotec, Auburn, California). Each mAb was used at saturating concentrations optimized by initial titrations. Isotype-identical mAbs (BD Pharmingen), diluted to equivalent immunoglobulin concentration, served as negative controls. After incubation, erythrocytes were lysed with 2 mL PharM Lyse (BD Pharmingen) for 7 minutes at room temperature. The samples were then centrifuged and the cell pellets resuspended into 2 mL phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin and 0.1% sodium azide (Sigma Aldrich, St Louis, Missouri). The cells were centrifuged again and the pellets resuspended in 400 µL of 1% paraformaldehyde before analysis.

The cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, California) calibrated with CaliBRITE beads (BD Biosciences, San Jose, California). At least 100 000 events were collected for each antibody combination and the data analyzed using CELLQuest software (Becton Dickinson). Endothelial progenitor cells were enumerated as a percentage of total lymphomonocytic cells after setting tight gates around the lymphocyte and monocyte populations based on their side-light-scatter and pan-leukocyte CD45⁺ characteristics, setting lower limits on the basis of negative isotype controls.

Circulating Angiogenic Cell Culture Assay

Isolated PBMCs were cultured in supplemented endothelial growth media, resulting in an adherent population of spindle-shaped cells possessing both endothelial-like and monocyte/macrophage markers.^{21–23} In brief, 40 mL of peripheral blood was overlaid onto Ficoll-Paque Plus (Amersham Biosciences, New Jersey) and centrifuged at 400g for 40 minutes. The cells in the peripheral blood mononuclear cell fraction were collected, washed in Dulbecco phosphate-buffered saline (DPBS; Mediatech, Manassas, Virginia) containing 2% fetal bovine serum (FBS; Lonza, Walkersville, Maryland), and counted in a hemocytometer. Greater than 95% of the isolated cells excluded trypan blue in all cases, indicating viability. The cells were plated onto fibronectin-coated 4-well culture slides (BD Biosciences, Bedford, Massachusetts) in phenol red-free endothelial basal medium (EBM; Cambrex Bio Science, Walkersville, Maryland) supplemented with EGM-2 SingleQuot supplements plus 5% vol/vol FBS. Cells were maintained at 37°C in an atmosphere of 5% CO₂ for a total of 7 days, with media changed to remove nonadherent cells on days 4 and 6.²²

Cytoplasmic accumulation of acetylated low-density lipoprotein (LDL), lectin cell surface staining, and CD45 expression were assessed to confirm the typical phenotype of early-outgrowth cells.²⁶ The cells were incubated for 4 hours with 10 µg/mL of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled acetylated LDL (DiI-Ac-LDL, Biomedical Technologies Inc., Stoughton, Massachusetts), fixed in 2% paraformaldehyde for 20 minutes, and then counterstained for 1 hour with 10 µg/mL of FITC-labeled *Ulex europaeus* agglutinin I (lectin, Sigma-Aldrich). Nuclei were stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; Invitrogen). Circulating angiogenic cells were counted as the number of lectin, DiI-Ac-LDL, and DAPI-triply positive cells, averaged from at least 2 randomly selected, high-power fluorescence microscopic fields from each of 2 culture wells per patient, by a technician blinded to patient diagnosis. For assessment of CD45 positivity, separate cultures were fixed in 2% paraformaldehyde for 20 minutes, blocked with Image-iT FX signal enhancer (Invitrogen), and incubated overnight with 1 to 10 µg/mL of a murine mAb against human CD45 (#555480, BD Pharmingen) or mouse anti-human immunoglobulin G (IgG) isotype control (#555784, BD Pharmingen), in PBS at 4°C. Cells were incubated with secondary antibody, goat anti-mouse IgG (H + L) antibody directly conjugated to Alexa Fluor 568 (#A11031, Invitrogen), for 2 hours at room temperature and counterstained with DAPI. Human umbilical vein endothelial cells (Lonza), seeded at passages 4 to 6, were used as a control for immunoreactivity.

Plasma Total sFlt-1 and Free PlGF

Plasma concentrations of fms-like tyrosine kinase (sFlt-1, likely both soluble and Flt-1 bound to syncytiotrophoblast membrane fragments) and free (Flt-1 unbound) placental growth factor (PlGF) were measured in duplicate by enzyme-linked immunosorbent assays (ELISAs) according to manufacturers' instructions (R&D Systems, Inc, Minneapolis, Minnesota), as previously validated in our laboratory for pregnancy samples.²⁷ The sensitivities for sFlt-1 and PlGF were 5 and 7 pg/mL, respectively. Interassay and intraassay coefficients of variation were 7% and 3% for sFlt-1 and 11% and 5% for PlGF.

Statistical Analyses

Continuous demographic variables were normally distributed and are presented as mean ± SD; analysis of variance (ANOVA) with Bonferroni post hoc test was used to compare these data by trimester of normal pregnancy, and unpaired Student *t* test was used to compare third trimester control and preeclampsia groups. The distributions of progenitor cell counts and plasma concentrations of sFlt-1 and PlGF were skewed. These data are therefore given as the median (range). Nonparametric Kruskal-Wallis test followed by Dunn post hoc test was used to compare progenitor cell counts by each trimester of uncomplicated pregnancy. The

nonparametric Mann-Whitney U test was used to compare third trimester preeclampsia versus third trimester control progenitor cell numbers and sFlt-1 and PlGF values. Spearman rank correlation coefficient (r_s) was calculated to assess any relationship between progenitor counts and plasma factors. Statistical significance was accepted if the null hypothesis could be rejected at $P < .05$.

RESULTS

Figure 1 shows flow cytometry dot blots of third trimester circulating CD133⁺VEGFR-2⁺ and CD34⁺VEGFR-2⁺ cells. As illustrated in Figures 2 and 3, progenitor cells were increased during the second trimester [median percentage of lymphomonocytic cells; CD133⁺VEGFR-2⁺: 0.02 (range 0.000 to 0.09), $P < 0.03$] and third trimester [CD133⁺VEGFR-2⁺: 0.06 (0.00 to 0.86), $P < 0.001$; CD34⁺VEGFR-2⁺: 0.05 (0.00 to 1.00), $P < 0.001$] of uncomplicated pregnancy compared to the first trimester [CD133⁺VEGFR-2⁺: 0.00 (0.00 to 0.03); CD34⁺VEGFR-2⁺: 0.00 (0.00 to 0.03)]. Median second trimester levels of CD34⁺VEGFR-2⁺ cells (0.01 [0.00 to 0.06]) were marginally higher than first trimester ($P = .06$). CD34⁺VEGFR-2⁺ cells ($P < .03$), but not CD133⁺VEGFR-2⁺ cells ($P = .16$), increased significantly from second to third trimester (Figure 2). Significant differences persisted after excluding the third trimester women who were in labor ($n = 4$) at the time of venipuncture (CD133⁺VEGFR-2⁺: $P < 0.001$ third vs first trimester; $P < .02$ second vs first trimester; CD34⁺VEGFR-2⁺: $P < .001$ third vs first trimester; $P < .05$ third vs second trimester; $P = .05$ second vs first trimester). Circulating cell numbers in nonpregnant/nulligravid women (CD133⁺VEGFR-2⁺: 0.00 [0.00 to 0.09]; CD34⁺VEGFR-2⁺: 0.00 [0.00 to 0.07]) did not differ from first trimester of pregnancy ($P = .39$ and $.75$, respectively). No influence of menstrual cycle phase was detected (data not shown).

Significantly fewer circulating progenitor cells were detected in women with preeclampsia (CD133⁺VEGFR-2⁺: median 0.00 [0.00 to 0.18], $P < .03$; CD34⁺VEGFR-2⁺: 0.00 [0.00 to 0.19], $P < .01$) compared to the third trimester of uncomplicated pregnancy (Figures 2 and 3). Detectable levels of CD133⁺VEGFR-2⁺ cells and CD34⁺VEGFR-2⁺ cells, respectively, were present in 43% and 29% of women with preeclampsia compared to 75% and 80% of controls. Progenitor counts remained significantly lower in women with preeclampsia (CD133⁺VEGFR-2⁺: $P < .04$; CD34⁺VEGFR-2⁺: $P < .02$) after excluding women who were in labor (1 preeclamptic, 4 controls) at the time of sampling. CD133⁺VEGFR-2⁺ and CD34⁺VEGFR-2⁺ cells correlated significantly in pre-eclamptics ($r_s = .89$, $P < .01$) but not controls ($r_s = .35$, $P = .13$). Within each group, EPCs did not correlate with any of the demographic variables listed in Table 1.

As shown in Figure 4, fewer CACs, defined as adherent, spindle-shaped cells positive for DiI-Ac-LDL uptake and *Ulex europaeus* lectin surface staining were observed in cultures from women with preeclampsia compared to controls (median [range] cells/high power field: 24 [11 to 50] vs 44 [7 to 121]; $P < .03$). These cells were >90% positive for the hematopoietic surface antigen CD45. The number of CACs from women with preeclampsia showed a significant inverse (negative) correlation with gestational weeks at blood sampling ($r_s = -.73$, $P < .05$) and gestational weeks at delivery ($r_s = -.70$, $P < .05$), but there was no significant correlation of these variables among the controls. Birth weight percentile correlated inversely with the number of CACs in preeclamptics ($r_s = -.71$, $P < .005$) but not controls ($r_s = .16$, $P = .63$). Within each group, CACs did not correlate with any of the other demographic variables listed in Table 2. There was no difference in CAC counts between severe ($n = 7$) and nonsevere ($n = 5$) preeclampsia subgroups (median CACs/field: 26 vs 22, respectively; $P = .64$).

Plasma concentrations of sFlt-1 and free PlGF were significantly higher and lower, respectively, in women with preeclampsia compared to third trimester controls (Tables 1 and 2). There was no significant correlation of plasma sFlt-1 or free PlGF concentrations with

progenitor cells enumerated by flow cytometry or culture assay in either preeclampsia or control groups. The correlation of plasma free PlGF with average number of CACs per microscopic field approached significance in controls ($r_s = .51$, $P = .10$) but there was an opposite trend in the preeclampsia group ($r_s = -.40$, $P = .19$).

DISCUSSION

Endothelial progenitor cells constitute a pool of circulating cells that are thought to participate in angiogenesis and ongoing maintenance and repair of the vascular endothelium. Flow cytometry approaches to enumerate circulating EPCs most often use the hematopoietic stem cell markers CD34 or CD133 in combination with the endothelial marker VEGFR-2 (KDR).^{11–17, 28} CD133⁺VEGFR-2⁺ or CD34⁺VEGFR-2⁺ cells typically represent between 0.001% and 0.05% of mononuclear cells in peripheral blood, varying with health status of the individual and influenced by many inhibitory and stimulatory factors.^{12,22,28,29} In cross-sectional analysis of EPCs in the peripheral circulation during uncomplicated pregnancy, we found that CD133⁺VEGFR-2⁺ cells were increased during the second trimester of normal pregnancy compared to first trimester, without further increases during the third trimester, whereas CD34⁺VEGFR-2⁺ cells were significantly elevated during the third trimester compared to first or second trimester. The majority of first trimester values were below our detection threshold and were not different from nonpregnancy values. Although with median values of zero, the mean percentage $\pm$ SEM of circulating CD133⁺VEGFR-2⁺ and CD34⁺VEGFR-2⁺ cells in healthy nonpregnant women in our study (0.023 ± 0.01 and 0.01 ± 0.01 , respectively) are similar to mean values reported for young, healthy female or mixed gender controls by other investigators.^{12,22,28,29} Women with preeclampsia manifested significantly lower levels of both CD133⁺VEGFR-2⁺ and CD34⁺VEGFR-2⁺ cells compared to gestational age-matched normal pregnancy controls, the majority of the preeclampsia values falling below detection threshold. Due to these below threshold values, we are unable to draw inferences about the association of EPC numbers by flow cytometry and the severity of preeclampsia.

Consistent with our study, Buemi et al³⁰ reported a progressive increase in circulating EPCs (CD133⁺VEGFR-2⁺ cells) in the maternal circulation with advancing normal pregnancy. They also reported that CD133⁺VEGFR-2⁺ cells were more plentiful in gestational diabetics and in women with nonproteinuric gestational hypertension, relative to normal pregnancy controls. In contrast, Matsubara et al reported declining numbers of circulating CD133⁺CD34⁺VEGFR-2⁺ (triply) positive blood cells with advancing pregnancy and did not find differences in these cells in women with pre-eclampsia compared to normal pregnancy.³¹ Data from the later study were presented as absolute number of EPCs per milliliter of blood whereas we enumerated EPCs as a percentage of total lymphomononuclear cells. This is unlikely to explain differences, however, as lymphocyte and monocyte counts do not differ among non-pregnant, normal pregnant and preeclamptic groups of women.^{32,33} We likewise do not find a difference in the number of circulating lymphomononuclear cells between gestational age-matched normal and preeclamptic pregnancies (data not shown).

No consensus has yet been reached as to cell surface markers that distinguish EPCs from primitive hematopoietic progenitor cells.^{5,7,10} There are increasing indications that, unlike endothelial cells, a majority of circulating cells expressing CD133, CD34, and VEGFR-2 also express the pan-leukocyte surface marker CD45. These cells also do not form capillary-like structures in Matrigel or other tubule-forming assays and are more appropriately classified as primitive hematopoietic progenitors.^{5,7,10} Nevertheless, a reduction in circulating CD34⁺VEGFR-2⁺ cells measured by flow cytometry has been observed in several cardiovascular disease states.^{5,11–13,16} In both healthy populations and coronary artery disease patients, reduced levels of these cells have been shown to be robust predictors of endothelial

dysfunction independent of classical risk factors.^{13,15} The biological mechanisms underlying these observations, however, remain unclear.^{5,6,10,11}

A widely used cell culture–based approach to compare numbers of cells commonly referred to as “early outgrowth EPCs” or “CACs” is to plate peripheral blood mononuclear cells on fibronectin-coated culture dishes in media supplemented with endothelial growth factors and fetal calf serum and, several days later, count the number of spindle-shaped, adherent cells that display the ability to both take up acetylated LDL and bind the plant lectin *Ulex europaeus* agglutinin I.^{5,7,10,12,21,22} CACs are actually of monocyte/macrophage lineage and are correspondingly CD45 and CD14 positive.^{9,21,26} Although these cells do not differentiate to form endothelial cells or assemble into vascular networks, they nevertheless appear to participate in endothelial regeneration and homeostasis by enhancing the survival of other progenitor cells and endothelial cells through growth factor secretion and other paracrine pathways.^{9,21,23,34} We found reduced numbers of CACs in culture derived from blood samples from women with preeclampsia compared to normal pregnancy. Consistent with nonpregnancy studies, these cells were strongly CD45 positive. We were unable to assess the correlation of cells counted by flow cytometry and cell culture due to the different sets of patients providing blood samples for these assays. Endothelial progenitor cells quantified by flow cytometry often correlate poorly with EPCs in cell culture, however, suggesting that the cell populations are not identical.^{8,29}

The heterogeneity of EPCs is important to consider further in relation to our data. A relatively rare but potentially important EPC subtype termed “endothelial colony forming cells” (ECFCs) appear at days 7 to 14 of culture of peripheral blood mononuclear cells on collagen in endothelial-specific media. Unlike CACs, ECFC colonies are visually indistinguishable from (but more proliferative than) endothelial cells and can be clonally isolated and expanded.^{5,7,9,10,34} Endothelial colony forming cells are distinctly CD45 negative and may contribute structurally to neovessels. Importantly, when CACs and ECFCs are coinjected into mice with hind-limb ischemia vascular damage, they synergistically promote vascular repair/neovascularization, beyond that achieved by injection of the same total number of either cell type alone.³⁴ We did not attempt to determine the frequency of ECFC colonies in cell culture, however, because the large volume of adult peripheral blood necessary for reliable quantification (~ 1 ECFC in 20 mL blood)³⁵ was not available to us. Because no EPC-specific antigen has yet been discovered, no flow cytometry method exists that can reliably distinguish ECFCs from circulating endothelial cells sloughed from the vascular endothelium.^{6,7,10}

By binding VEGF and PlGF and thereby preventing their interaction with endothelial cell receptors, excess sFlt-1 may play a major role in development of endothelial dysfunction in preeclampsia.²⁴ As expected, sFlt-1 and free PlGF values were significantly higher and lower, respectively, in maternal plasma from women with preeclampsia compared to normal pregnancy in our study. Vascular endothelial growth factor and PlGF are key signaling factors promoting the mobilization of bone marrow–derived progenitor cells into the peripheral circulation^{18,19} and therefore an excess of sFlt-1 could adversely affect the prevalence and activity of these cells. Concentrations of CD133⁺VEGFR-2⁺ EPCs in fetal (umbilical cord) blood were previously found to be lower in preeclamptic compared to control pregnancies, with fetal EPCs inversely correlated with cord blood concentrations of sFlt-1.³⁶ In our study, however, progenitor cell counts did not correlate with sFlt-1 or free PlGF values in either pregnancy group. Other factors in addition to sFlt-1/PlGF imbalance may lead to reduction in EPCs in women with preeclampsia.

In summary, preeclampsia appears to be characterized by a relative reduction in endothelial-like, CD45-positive cells of the type first reported by Asahara and colleagues⁴ that are commonly referred to^{21,34} as “early EPCs” or CACs. Our data indicate that these cells increase

with advancing normal pregnancy but not preeclampsia, consistent with their having a role in the vascular adaptation to pregnancy that fails in preeclampsia. However, reductions in the number of these cells with preeclampsia may reflect a common underlying etiology or an effect of the disease, rather than a causal role. For example, a defect in the bioavailability of nitric oxide, which negatively affects both EPC mobilization from bone marrow^{37,38} and arterial function, might contribute to reduction in these EPCs and independently the development of preeclampsia. Longitudinal studies will be necessary to determine whether diminution of EPCs precedes development of the preeclampsia syndrome. Additionally, it is presently unknown whether the apparent reduction in circulating EPCs in women with preeclampsia is caused by accelerated cell death, decreased mobilization from bone marrow stores, altered differentiation, or increased utilization.

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Figure 1.

CD34⁺VEGFR-2⁺ cells (left panel) and CD133⁺VEGFR-2⁺ cells (right panel) detected in peripheral blood by flow cytometry. Whole blood was stained with antihuman monoclonal antibodies and processed as described in the text. Gates were drawn on the lymphocyte population based on side-scatter and CD45 characteristics. Within these gates, CD34⁺ and CD133⁺ elements expressing VEGFR-2 (KDR) were analyzed. Percentage positive cells were defined by setting lower limits on the basis of negative isotype controls. CD34⁺VEGFR-2⁺ and CD133⁺VEGFR-2⁺ cells are located in the upper right quadrants. VEGFR-2 indicates vascular endothelial growth factor receptor-2; KDR = kinase insert domain receptor.

Figure 2.

Scatterplot showing the quantity (number) of CD34/VEGFR-2-doubly positive endothelial progenitor cells (EPCs) in maternal peripheral blood during normal pregnancy and preeclampsia. Endothelial progenitor cells increased significantly over time during normal pregnancy, with highest levels during the third trimester. Endothelial progenitor cells were significantly lower on average during preeclampsia compared to third trimester controls. Triangles indicate women who were in labor at the time of blood sampling. VEGFR-2 indicates vascular endothelial growth factor receptor-2.

Figure 3.

Scatterplot showing the quantity (number) of CD133/VEGFR-2-doubly positive endothelial progenitor cells (EPCs) in maternal peripheral blood during normal pregnancy and preeclampsia. Endothelial progenitor cells increased significantly over time during normal pregnancy, with highest levels during the third trimester. Endothelial progenitor cells were significantly lower on average during preeclampsia compared to third trimester controls. Triangles indicate women who were in labor at the time of blood sampling. VEGFR-2 indicates vascular endothelial growth factor receptor-2.

Figure 4.

Scatterplot showing the quantity (number) of adherent, Ulex lectin, 1,1'-dioctadecyl-3,3,3,3'- β -tetramethylindo-carbocyanine perchlorate (DiI)-labeled acetylated low-density lipo-protein (DiI-Ac-LDL), and dihydrochloride (DAPI)-triply positive cells in culture (circulating angiogenic cells, CACs) during the third trimester. Each symbol corresponds to the average of at least 2 randomly selected, high-power fluorescence microscopic fields from each of 2 culture wells per patient. The thick horizontal bars denote the median value for each group.

Table 1
Clinical and Angiogenic Factor Data of Groups of Women (n = 79) Who Provided Blood for Flow Cytometry^a

	Nulligravid (n = 13)	CTRL, First Trimester (n = 11)	CTRL, Second Trimester (n = 21)	CTRL, Third Trimester (n = 20)	Preeclampsia (n = 14)
Age (years)	25.5 ± 6.5	23.5 ± 6.0	25.0 ± 5.1	25.2 ± 4.2	27.7 ± 5.8
BMI prepregnancy (kg/m ²)	23.1 ± 4.0 ^b	26.9 ± 3.5	25.3 ± 6.3	24.3 ± 5.0	26.4 ± 4.0
Gestational weeks at delivery		39.2 ± 2.0	39.0 ± 1.5	39.0 ± 2.0	35.3 ± 3.4 ^c
Gestational weeks at venipuncture		8.3 ± 1.7	20.4 ± 1.8	35.7 ± 3.3	34.5 ± 2.9
Early pregnancy (or nulligravid) blood pressure ^d					
Systolic (mm Hg)	107 ± 8	110 ± 7	110 ± 8	112 ± 9	119 ± 10
Diastolic (mm Hg)	69 ± 9	66 ± 4	67 ± 8	70 ± 6	71 ± 6
Predelivery blood pressure					
Systolic (mm Hg)		115 ± 13	123 ± 9	123 ± 10	153 ± 15 ^c
Diastolic (mm Hg)		65 ± 7	73 ± 9	72 ± 7	96 ± 9 ^c
Uric acid (mg/dL)	NM	NM	NM	4.7 ± 1.3	6.1 ± 1.3 ^c
Birth weight (g)		3339 ± 290	3243 ± 423	3409 ± 676	2219 ± 827 ^c
Birth weight centile		51 ± 19	53 ± 27	57 ± 29	23 ± 25 ^c
In labor during venipuncture (%)				20% (4/20)	7% (1/14)
Race (% black)	8% (1/13)	36% (4/11)	24% (5/21)	15% (3/20)	21% (3/14)
Plasma angiogenic factors					
sFlt-1 (pg/mL)	NM	NM	NM	3957 (1686–12 211)	27 154 ^e (5631–570 989)
PlGF (pg/mL)	NM	NM	NM	169 (937–728)	46 ^e (3–103)

Abbreviations: BMI, body mass index; CTRL, control (uncomplicated) pregnancy; NM, not measured.

^aContinuous variables are given as mean (standard deviation). Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) concentrations are given as median (range).

^b*P* < .04 versus CTRL first trimester.

^c*P* < .01 versus CTRL third trimester.

^dAverage of gestational blood pressure recorded before 20 weeks of gestation or at the time of blood sampling/venipuncture for nullgravidas.

^e*P* < .0001 versus CTRL third trimester.

Table 2Clinical and Angiogenic Factor Data of Groups Who Provided Blood for Cell Culture Analyses^a

	Normotensive Controls (n = 12)	Preeclampsia (n = 11)	P Value
Age (years)	25.9 ± 4.7	26.8 ± 6.6	.71
BMI prepregnancy (kg/m ²)	28.0 ± 7.9	28.0 ± 5.0	.99
Gestational weeks at delivery	39.3 ± 2.1	35.5 ± 3.8	.03
Gestational weeks at venipuncture	35.7 ± 5.1	35.0 ± 3.9	.52
Early pregnancy blood pressure ^b			
Systolic (mm Hg)	110 ± 10	118 ± 6	.07
Diastolic (mm Hg)	69 ± 8	73 ± 7	.30
Predelivery blood pressure			
Systolic (mm Hg)	117 ± 13	156 ± 10	<.0001
Diastolic (mm Hg)	74 ± 8	96 ± 11	<.0001
Uric acid (mg/dL)	4.8 ± 1.0	6.4 ± 1.3	.003
Birth weight (g)	3445 ± 540	2674 ± 1138	.06
Birth weight centile	59 ± 23	46 ± 37	.35
In labor during venipuncture (%)	0%	0%	1.0
Race (% black)	25% (3/12)	36% (4/11)	.67
Plasma angiogenic factors			
sFlt-1 (pg/mL)	3120 (1462–11 372)	26 629 (11 472–68 912)	<.001
PlGF (pg/mL)	106 (34–890)	40 (3–199)	<.005

Abbreviation: BMI, body mass index.

^a Continuous variables are given as mean (standard deviation). Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) concentrations are given as median (range).

^b Average of gestational blood pressures recorded before 20 weeks of gestation.