

## Endothelial progenitor cells and pregnancy

TO THE EDITORS: Savvidou et al<sup>1</sup> reported a decline in endothelial progenitor cells (EPCs) in the maternal circulation with advancing gestation. The available literature on EPCs during pregnancy is conflicting, however, as appropriately acknowledged by the authors.

The approach used by Savvidou et al was to culture unfractionated peripheral blood mononuclear cells (PBMCs) in supplemented endothelial growth media for 3 days, whereupon the non-adherent fraction of cells was removed, resulting in a fibronectin-adherent subpopulation. EPCs were enumerated as a percentage of the adhered PBMCs, according to double positivity for surface binding of the *Ulex europaeus* agglutinin (lectin) and uptake of acetylated low-density lipoprotein. It should be noted, however, that the cells collected in this manner comprise as much as 2% of the whole PBMC population, are poorly proliferative, and display features of monocytes and macrophages throughout the culture period despite expression of endothelial markers.<sup>2,3</sup> Although these circulating cells may be critically important to endothelial health, they are perhaps better described as proangiogenic myeloid cells or circulating angiogenic cells.<sup>2</sup>

These cells often correlate poorly with EPCs enumerated on the basis of stem cell antigens (CD133 and/or CD34) in combination with vascular endothelial growth factor receptor-2. The latter subtype of EPC represents between 0.001% and 0.03% of total PBMCs from normal peripheral blood.<sup>2</sup> Other methodologies such as the colony-forming unit (Hill) assay and the late outgrowth assay select for different cells that are also referred to as EPCs.<sup>2</sup>

Given this heterogeneity, it may be premature to conclude that normal pregnancy is associated with a progressive overall decline in maternal circulating EPCs. Nevertheless, the study by Savvidou et al is informative and should stimulate further investigation regarding the role of EPCs in normal and abnormal pregnancy. ■

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### REFERENCES

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### REPLY

We thank Dr Hubel for his comments. The methodology to identify circulating endothelial progenitor cells (EPCs) used in our study has been described in detail.<sup>1</sup> In many previous studies, EPCs are described as a peripheral blood mononuclear cell (PBMC) population expressing CD34, kinase insert domain receptor (KDR)/vascular endothelial growth factor receptor-2, and CD133/AC133 with adherent growth characteristics.

Whereas the function and the clonogenic capacity of EPC should be evaluated using colony-forming unit assays, their number is usually assessed by flow cytometry using either antibodies against CD34 and KDR or CD133. However, very strict and rigorous technology in flow cytometry analysis is needed, and a completed negative/positive control should be included in this analysis. This is because it is very difficult to reproduce the data of EPC numbers because of the limitation of nonspecific background of this measurement and because of the fact that just a very small fraction (0.001% to 0.03% of PBMCs or less than 5 per mL of blood) of CD34-AC133-KDR-triple positive EPCs exist in the circulation.

Alternatively, phenotypes of human EPCs can be confirmed by the uptake of 1, 1'-dioctadecyl-3, 3'-tetramethylindocarbocyanine-labeled acetyl low-density lipoprotein (DiI-Ac-LDL) and binding of *Ulex europaeus* agglutinin (lectin).

Our previous study<sup>2</sup> demonstrated that a large proportion (from 25% to 75%) of DiI/lectin double-positive cells were endothelial cell-specific markers positive, under our culture conditions, such as CD31, CD144, von Willebrand factor, KDR, and endothelial nitric oxide synthase, but conducting all the experiments to check the endothelial-specific marker expression in DiI/lectin double-positive cells was not feasible. Therefore, double-positive cells for DiI-Ac-LDL and lectin were counted as circulating EPCs and used in the present study.

We acknowledge that all termed EPCs have recently been reclassified as 3 kinds of cells: colony-forming unit-endothelial cells, circulating angiogenic cells, and endothelial colony-forming cells.<sup>3</sup> Although we recognize the controversy in the EPC definition, based on the previously cited information, we can conclude that our measurements reflect the changes of termed EPCs during the different stages of pregnancy. Undoubtedly, a consensus among scientists would be desirable to help further the research in this field. ■

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