Maternal Insulin Therapy Increases Fetal Endothelial Progenitor Cells During Diabetic Pregnancy

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Endothelial progenitor cells (EPCs) are bone marrow–derived cells involved in endothelial homeostasis and angiogenesis. Reduction and dysfunction of EPCs have been associated with the development of atherosclerosis and diabetic complications (1,2).

Recent studies in human pregnancies suggest that a mother's EPCs are involved in the physiologic vascular remodeling of systemic and utero-placental circulation (3,4). Hyperglycemia induces dysfunction and apoptosis of EPCs (5); this may impair the development or maturation of the utero-placental circulation, causing maladaptive responses during diabetic pregnancies. Indeed, EPCs have been shown to be dysregulated in pregnant women with diabetes (4).

Little is known about the effects of maternal factors on fetal EPCs. One study showed reduced cord blood EPCs in severe preeclampsia (6), but there were no data on diabetes. This study was undertaken to evaluate quantitative alterations of cord blood progenitor cells during diabetic pregnancies.

RESEARCH DESIGN AND METHODS

We enrolled 24 nondiabetic and 17 diabetic pregnant women: two had pregravidic type 1 diabetes and 15 had gestational diabetes mellitus (GDM). GDM was diagnosed according to the American Diabetes Association guidelines (7). Provided that informed consent was obtained from the mother, at time of delivery 5 ml of umbilical vein cord blood was collected into heparin tubes for progenitor cell count. The following data about the mother were recorded: age, pregravidic BMI, weight increase, smoking habit, family history of cardiovascular disease, arterial blood pressure, and type of antidiabetic treatment. Before labor, blood samples were collected for determination of plasma glucose levels, leukocyte count, fibrinogen levels, and total cholesterol and triglycerides concentrations. After delivery, gestational age, neonatal weight and sex, and 1–5 min Apgar scores were recorded.

Progenitor cell count was performed using direct two- or three-color flow cytometry as previously described (8). Briefly, after red cell lysis, blood cells in the mononuclear cell gate were analyzed for the expression of surface antigen using FITC-conjugated anti-CD34 (Becton Dickinson), phycoerythrin-conjugated anti-KDR (R&D Systems), and allophycocyanin-conjugated anti-CD133 (Miltenyi Biotech) monoclonal antibodies. $CD34^+$, $CD133^+$, and $CD34^+CD133^+$ cells were considered generic circulating progenitor cells, while $CD34^+KDR^+$, $CD133^+KDR^+$, and $CD34^+CD133^+KDR^+$ cells were considered endothelial progenitor cells (EPCs). Cell counts were expressed per 10^6 cytometric events.

Data are expressed as means ± SEM. Differences between two groups were assessed using Student's t test and χ^2 analysis, where appropriate. Linear correlations between continuous variables were assessed using Pearsons's r coefficient, while multiple linear regression analyses were used to correct for potential confounders. SPSS version 13.0, was used, and statistical significance was accepted at $P < 0.05$.

RESULTS

Diabetic pregnant women had a higher prevalence of arterial hypertension (29.4 vs. 4%, $P = 0.02$) and family history of cardiovascular disease (53 vs. 16%, $P = 0.01$) than nondiabetic women. There was a nonsignificant trend toward higher plasma glucose (92.5 ± 6.9 vs. 79.9 ± 3.3 mg/dl, $P = 0.07$) and higher birth weight (3,453 ± 91 vs. 3,214 ± 77 g, $P = 0.06$) in diabetic versus nondiabetic pregnancies, indicating overall good metabolic control, as also shown by a near-normal A1C (6.1 ± 0.2%). There were no significant differences in maternal age, pregravidic BMI, weight increase, smoking habit, gestational age, total cholesterol, triglycerides, leukocyte count, fibrinogen levels, neonatal weight and sex, and Apgar scores. None of the participants had diabetic retinopathy at the time of the study.

We found a trend toward reduction of cord blood $CD34^+$, $CD133^+$, $CD34^+CD133^+$, and $CD34^+KDR^+$ cell levels in diabetic versus nondiabetic pregnancies, which was nearly significant for $CD34^+$ cell count ($P = 0.052$). No significant differences were present in progenitor cell counts between type 1 diabetes and GDM. These data indicate that fetal EPCs are not significantly reduced in the presence of well-controlled maternal diabetes (Fig. 1*A*).

When diabetic women were divided according to the antidiabeties treatment (diet alone vs. diet plus insulin), we found higher levels of all progenitor cell phenotypes in women treated with insulin than in women on diet alone, which was largely significant for $CD34^+KDR^+$ cell count (172 ± 50 vs. 44 ± 8, $P = 0.02$) (Fig. 1*B*). The association between mater-

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Abbreviations: EPC, endothelial progenitor cell; GDM, gestational diabetes mellitus.

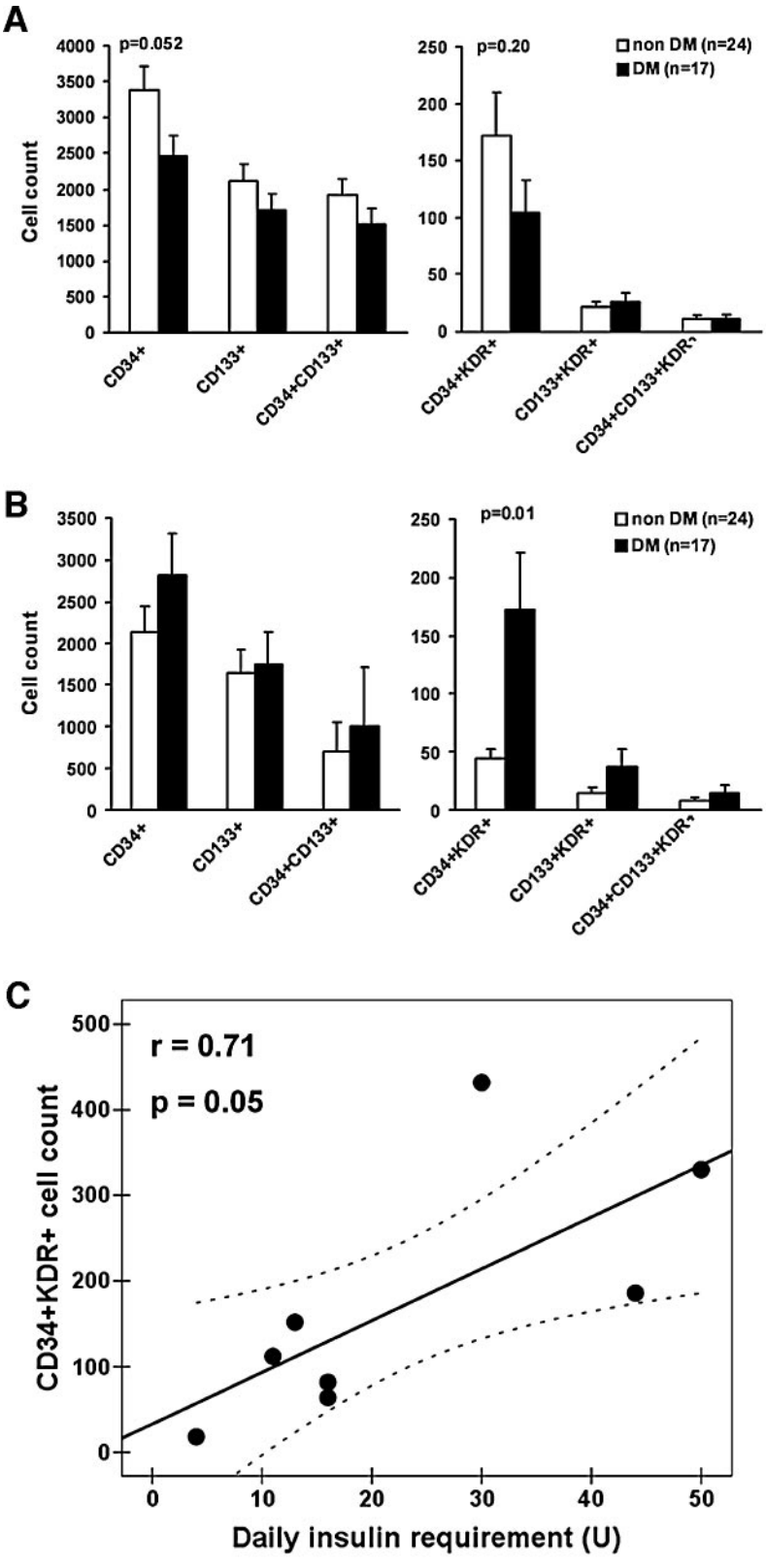

Figure 1—A: *Levels of cord blood (fetal) progenitor cells in the presence and in the absence of maternal diabetes.* B: *Levels of cord blood (fetal) progenitor cells in pregnant diabetic women divided according to the antidiabetes treatment (diet alone vs. diet plus insulin). C: Significant direct linear correlation between fetal CD34$^+$KDR$^+$ cells and maternal daily insulin requirement.*

nal insulin therapy and higher fetal CD34$^+$KDR$^+$ cell count remained significant after correction for maternal age, hypertension, plasma glucose, family history for cardiovascular disease, cholesterol and triglyceride values, and pregravidic BMI. These results suggest that insulin increases EPCs in the fetal circulation. In support of this hypothesis, we also show that the levels of fetal CD34$^+$KDR$^+$ EPCs directly correlated with maternal daily insulin requirements (Fig. 1*C*). This correlation remained significant after correction for maternal age, hypertension, plasma glucose, and pregravidic BMI. On the contrary, no significant correlation was seen between fetal EPCs and maternal A1C.

CONCLUSIONS — EPCs are deputed to the maintenance of vascular integrity, and their level reflects cardiovascular health (1,8). Moreover, EPC defects promote vascular disease, and emerging data indicate that dysregulation of maternal EPCs is associated with abnormalities of pregnancy. Given the comprehensive role played by EPCs in vascular development and homeostasis, the effects of maternal diabetes on fetal EPCs may have important consequences.

Herein, we show that well-controlled maternal diabetes is associated with a nonsignificant reduction of EPCs circulating in the fetal blood. Interestingly, insulin therapy undertaken to achieve a near-normal glycemic control was dose-dependently associated with higher fetal CD34$^+$KDR$^+$ EPC levels. It seems that the CD34$^+$KDR$^+$ phenotype was selectively modulated by maternal insulin therapy. Although there is considerable uncertainty regarding the exact EPC definition, this observation is in compliance with the notion that CD34$^+$KDR$^+$ may represent one of the most suited antigenic combinations to define EPCs in the clinical setting (9,10).

Insulin has been previously shown to promote mobilization of EPCs from the bone marrow to peripheral circulation in diabetic animals and in adult diabetic humans (11,12). Our present data suggest that maternal insulin therapy increases EPCs in the fetal circulation. As insulin should not pass the placental barrier, an indirect effect on the fetal environment, possibly through the modulation of maternal and/or placental metabolism, must be postulated. In any case, this effect seems independent of glycemic control because EPC and A1C levels were not

closely correlated. Alternatively, insulin may induce placental vascular endothelial growth factor, one potent stimulator of EPC mobilization and differentiation (2).

Currently, we don't know whether EPCs are consistently modulated in the maternal circulation as they are in the fetal circulation. Emerging data in animal models indicate that fetal EPCs pass the placental barrier and participate in maternal angiogenesis during pregnancy (13), whereas trafficking of EPCs in the opposite direction has not been substantiated. A simultaneous study of fetal and maternal EPCs may provide additional insights into the regulation of these cells during normal and diabetic pregnancy. Although the origin of fetal EPCs and the clinical significance of their increase remain unclear, it is possible that insulin therapy exerts protective effects on the fetal vascular system through the stimulation of EPCs. Assessing whether this influences long-term neonatal outcomes is a major challenge to pursue.

References

1. Fadini GP, Agostini C, Sartore S, Avogaro A: Endothelial progenitor cells in the natural history of atherosclerosis. *Atherosclerosis* 194:46–54, 2007
2. Fadini GP, Sartore S, Agostini C, Avogaro A: Significance of endothelial progenitor cells in subjects with diabetes. *Diabetes Care* 30:1305–1313, 2007
3. Matsubara K, Abe E, Matsubara Y, Kameda K, Ito M: Circulating endothelial progenitor cells during normal pregnancy and pre-eclampsia. *Am J Reprod Immunol* 56:79–85, 2006
4. Buemi M, Allegra A, D'Anna R, Coppolino G, Crasci E, Giordano D, Loddo S, Cucinotta M, Musolino C, Teti D: Concentration of circulating endothelial progenitor cells (EPC) in normal pregnancy and in pregnant women with diabetes and hypertension. *Am J Obstet Gynecol* 196: 68.e1–68.e6, 2007
5. Krankel N, Adams V, Linke A, Gielen S, Erbs S, Lenk K, Schuler G, Hambrecht R: Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. *Arterioscler Thromb Vasc Biol* 25:698–703, 2005
6. Kwon JY, Maeng YS, Kwon YG, Kim YH, Kang MH, Park YW: Decreased endothelial progenitor cells in umbilical cord blood in severe preeclampsia. *Gynecol Obstet Invest* 64:103–108, 2007
7. American Diabetes Association: Standards of medical care in diabetes. *Diabetes Care* 27 (Suppl. 1):S15–S35, 2004
8. Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A: Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. *Eur Heart J* 27:2247–2255, 2006
9. Fadini GP, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A, de Kreutzenberg SV: Peripheral blood CD34+KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke* 37:2277–2282, 2006
10. Fadini GP, Avogaro A, Agostini C: Critical assessment of putative endothelial progenitor phenotypes. *Exp Hematol* 35: 1479–1480, 2007
11. Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A, Agostini C, Avogaro A: Diabetes impairs progenitor cell mobilisation after hindlimb ischaemia-reperfusion injury in rats. *Diabetologia* 49:3075–3084, 2006
12. Humpert PM, Neuwirth R, Battista MJ, Voronko O, von Eynatten M, Konrade I, Rudofsky G Jr, Wendt T, Hamann A, Morcos M, Nawroth PP, Bierhaus A: SDF-1 genotype influences insulin-dependent mobilization of adult progenitor cells in type 2 diabetes. *Diabetes Care* 28:934–936, 2005
13. Huu SN, Oster M, Uzan S, Chareyre F, Aractingi S, Khosrotehrani K: Maternal neoangiogenesis during pregnancy partly derives from fetal endothelial progenitor cells. *Proc Natl Acad Sci U S A* 104:1871–1876, 2007