# Circulating Vascular Progenitor Cells in Patients With Type 1 Diabetes and Microalbuminuria

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**OBJECTIVE** — Patients with type 1 diabetes and microalbuminuria are at increased risk of cardiovascular disease (CVD). Abnormalities in vascular progenitor cells, which participate in vascular repair, may be implicated in this susceptibility.

**RESEARCH DESIGN AND METHODS** — We studied the number and function of vascular progenitor cells in 22 type 1 diabetic patients with history of microalbuminuria ($MA^+$) and 22 type 1 diabetic patients without history of microalbuminuria ($MA^-$), of similar age, diabetes duration, glycemic control, renal function, and no history of CVD.

**RESULTS** — $MA^+$ patients had lower circulating $CD34^+$ and $CD34^+/CD133^+$ cell numbers compared with $MA^-$ patients ($P < 0.006$). In in vitro functional assays, $MA^+$ patients had a significantly lower number of colony-forming units and impaired vascular endothelial growth factor (VEGF)-A–mediated tube formation, when compared with $MA^-$ patients ($P < 0.01$).

**CONCLUSIONS** — In type 1 diabetic patients with microalbuminuria, a marker of microvascular injury and a risk factor for CVD, circulating vascular progenitor cell number is reduced and function is impaired.

*Diabetes Care* 33:875–877, 2010

The number of circulating endothelial progenitor cells inversely relates to cardiovascular disease (CVD) (1–3); microalbuminuria is one of the earliest manifestations of diabetic nephropathy and a marker of CVD (4). A subset of patients with type 1 diabetes is susceptible to diabetic nephropathy, a condition characterized by a higher risk of cardiovascular morbidity and mortality (4,5). Type 1 diabetic patients without complications have a lower number of circulating progenitor cells than healthy control subjects (6,7). To gain insight into the susceptibility to CVD in type 1 diabetes, we studied circulating vascular progenitor cell number and function in type 1 diabetic patients with and without microalbuminuria.

## RESEARCH DESIGN AND METHODS

— Type 1 diabetic patients were recruited from Guy's and St Thomas' Hospital (London, U.K.). Patients with microalbuminuria ($MA^+$) had a positive history of early-morning urine albumin-to-creatinine ratio (ACR) ≥3.5 mg/mmol (in at least two of three consecutive measures), were on antihypertensive therapy, and had evidence of diabetic retinopathy.

The normoalbuminuric ($MA^-$) type 1 diabetic patients were defined as patients with ≥20 years' diabetes duration, ACR consistently <3.5 mg/mmol, and on no antihypertensive therapy.

Exclusion criteria were as follows: history of CVD, nondiabetic renal disease, and renal impairment defined as a serum creatinine >130 μmol/l. The study was approved by the local ethics committee.

Blood pressure, measured in the dominant arm with the patient seated after a 5-min rest using an automated sphygmomanometer (Dinamap-8100T; GE-Medical, Slough, U.K.), was calculated from the mean of three consecutive measurements. Fasting plasma glucose, serum total cholesterol, and creatinine were determined using a Cobas-Mira-Plus analyzer (Roche-Diagnostics, Basel, Switzerland). A1C was measured by liquid chromatography (Primus-CLC330; Primus Diagnostic, Kansas City, MO).

Circulating progenitor cells were investigated as described (3). Leukocytes were studied from peripheral blood after red cell lysis with ammonium chloride buffer. At least 500 $CD34^+/CD133^+$ events were collected for each patient and showed $>1 \times 10^6$ events in the lymphomonocyte gated area. Intra-assay coefficient of variation was <8%. Data are presented as events/$10^6$ lymphomonocytes (Fig. 1).

The cell colony–forming units–Hill (CFU-Hill) assay was performed as described (2). For the tube formation assay, early (7-day culture) and late (14-day culture) endothelial progenitor cells were studied in the presence and absence of vascular endothelial growth factor (VEGF)-A (3,8). Cells were characterized by immunofluorescence (supplementary Fig. 1, available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc09-1468/DC1).

Measurements and data analysis (SPSS-15) were performed blinded to group allocation. Not-normally and normally distributed variables were compared by Kruskall-Wallis and Student's *t* test (two-sided), respectively; when more than two groups were compared, ANOVA (least significance difference post hoc test) was used. $P \leq 0.05$ was considered statistically significant.

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Received 7 August 2009 and accepted 27 December 2009. Published ahead of print at http://care.diabetesjournals.org on 12 January 2010. DOI: 10.2337/dc09-1468.
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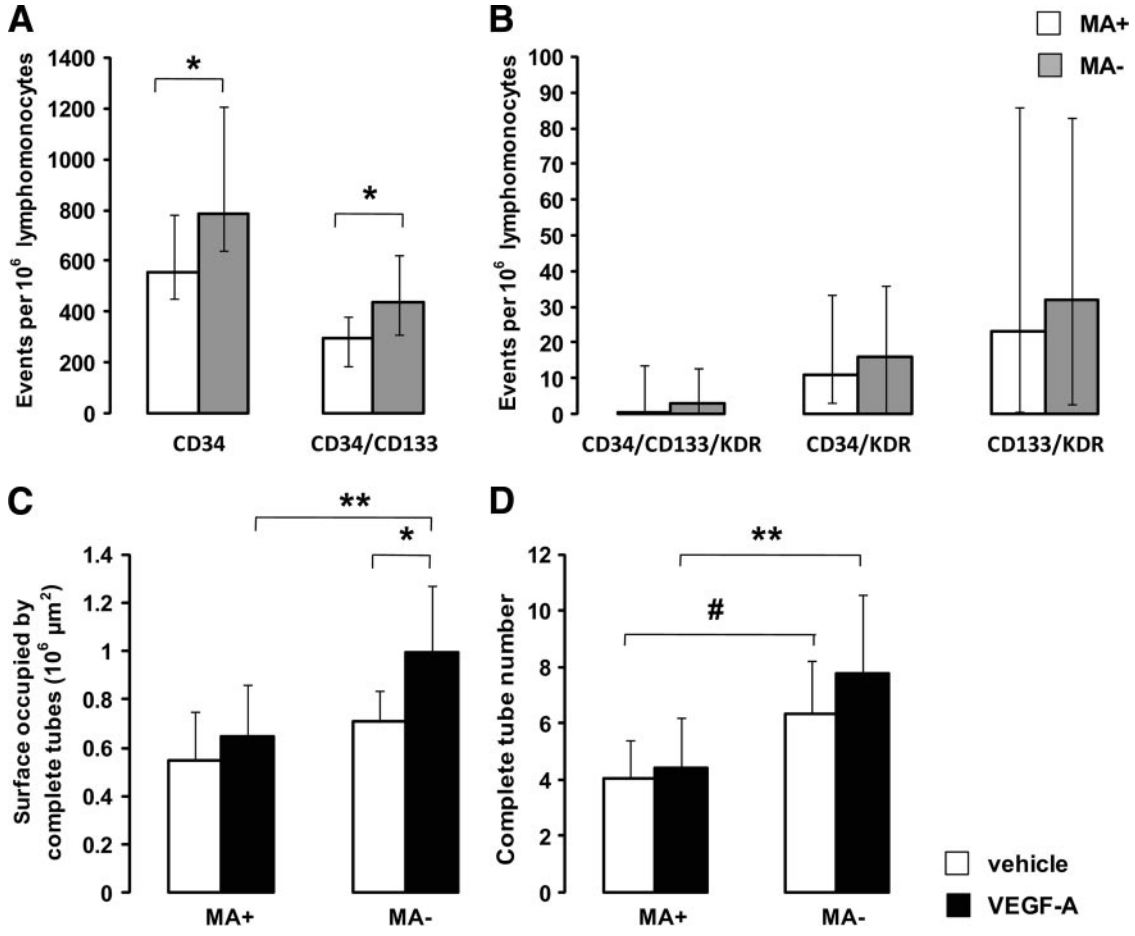


**Figure 1**—*Circulating vascular progenitor cell* (A *and* B) *and tube formation* (C *and* D) *assay in type 1 diabetic patients with and without microalbuminuria. Circulating CD34$^+$ and CD34$^+$/CD133$^+$ progenitor cell number was lower in MA$^+$ (□) versus MA$^-$ (■) (*P < 0.006) (*A*) patients. No difference was seen in CD34$^+$/CD133$^+$/KDR$^+$, CD34$^+$/KDR$^+$, and CD133$^+$/KDR$^+$ cells* (B) (n = *22 for MA$^+$;* n = *22 for MA$^-$, data are presented as median and interquartile range). In experiments conducted with late endothelial progenitor cultured cells* (C *and* D)*, surface area occupied by complete tube per field was similar in MA$^+$ and MA$^-$ in vehicle-treated cells (□). VEGF-A (■) increased tube surface formation only in MA$^-$* (C) *patients (MA$^-$/vehicle vs. MA$^-$/VEGF-A, *P = 0.016; MA$^+$/VEGF-A vs. MA$^-$/VEGF-A, **P = 0.003). Tube number was similar between vehicle (□) and VEGF-A–treated (■) conditions within MA$^+$ and MA$^-$ groups. MA$^+$ patients had a significant lower tube number than MA$^-$ patients both in vehicle and VEGF-A–treated cells (MA$^+$/vehicle vs. MA$^-$/vehicle, #P = 0.02; MA$^+$/VEGF-A vs. MA$^-$/VEGF-A, **P = 0.01)* (D)*. All experiments were conducted in triplicate, and the average obtained for each patient was used for statistical analysis* (n = *9 for MA$^+$;* n = *7 for MA$^-$, data are presented as mean ± SD).*

**RESULTS** — A total of 22 MA$^+$ (17 males/5 females) and 22 MA$^-$ (13 males/9 females) subjects of Caucasian origin were studied ($P = 0.23$ for sex between groups). There were no differences between the two groups (MA$^+$ vs. MA$^-$) in age (mean ± SD) 50.3 ± 11.9 vs. 49.8 ± 6.6 years, diabetes duration 35.2 ± 10.2 vs. 30.9 ± 7.7 years, BMI 25.2 ± 3.4 vs. 27.9 ± 6.3 kg/m$^2$, A1C 8.1 ± 1.6 vs. 8.3 ± 1.3%, total cholesterol 4.8 ± 0.8 vs. 4.6 ± 0.6 mmol/l, estimated glomerular filtration rate (eGFR–Modification of Diet in Renal Disease formula) 79.3 ± 23.6 vs. 86.0 ± 27.8 ml/min, systolic 127.5 ± 15.7 vs. 125.3 ± 9.5 mmHg, and diastolic blood pressure 74.7 ± 8 vs. 72.1 ± 7.5 mmHg. All patients had evidence of diabetic retinopathy and eGFR >50 ml/min.

A total of 100% of the MA$^+$ patients were on ACE inhibitors compared with 0% in MA$^-$ ($P = 0.005$). Twenty MA$^+$ patients were on statins compared with 5 MA$^-$ patients ($P = 0.05$); 5 MA$^+$ and 4 MA$^-$ patients were smokers.

MA$^+$ patients had significantly lower number of CD34$^+$ and CD34$^+$/CD133$^+$ cells when compared MA$^-$; CD34$^+$/KDR$^+$, CD34$^+$/CD133$^+$/KDR$^+$, and CD133$^+$/KDR$^+$ cell number was similar between groups (Fig. 1*A* and *B*).

The CFU-Hill assay was conducted in 10 MA$^+$ (6 males/4 females) and 8 MA$^-$ (5 males/3 females) consecutive patients from the population described comparable for all characteristics. Colony formation was lower in MA$^+$ (MA$^+$ median [interquartile range], 43 [35–56] vs. MA$^-$ 93 [52–103], $P = 0.01$).

Tube formation experiments were conducted in 9 MA$^+$ (5 males/4 females) and 7 MA$^-$ (4 males/3 females) type 1 diabetic consecutive patients as above. In experiments with early endothelial progenitor cells, we did not observe differences between groups. In experiments with late endothelial progenitor cells, we observed a VEGF-A–mediated increase in tube surface area only in MA$^-$ patients; MA$^+$ patients had a significantly lower tube number than MA$^-$ patients (Fig. 1*C* and *D*).

**CONCLUSIONS** — We demonstrated a lower number of circulating CD34$^+$ and CD34$^+$/CD133$^+$ cells and CFU-Hill in type 1 diabetic/MA$^+$ patients compared with type 1 diabetic/MA$^-$ patients. VEGF-A–mediated in vitro tube formation was observed only in cells derived from MA$^-$

patients, suggesting impaired vascular repair processes in $MA^+$ (3).

Microalbuminuria is a strong predictor for CVD in longstanding type 1 diabetic patients (5), and in our study, microalbuminuria associates with a low number of circulating progenitor cells, a recognized marker of CVD.

ACE inhibitors increase the number and function of vascular progenitor cells (9); despite a more prevalent use of these medications in the $MA^+$ group, we still observe a significantly lower number of $CD34^+$, $CD34^+/CD133^+$, and impaired tube formation in $MA^+$ patients. Conversely, the effect of statins on progenitor cells has been controversial (9,10), and this may represent a confounder in our study.

The number of $CD34^+/KDR^+$ and $CD34^+/CD133^+$ cells is reduced in hypertensive patients (11); normalization of blood pressure with renin-angiotensin system inhibitors is paralleled by normalization of these cells (11). This suggests that in our type 1 diabetic/$MA^+$ population (100% on ACE inhibitors), the observed reduction in $CD34^+$ and $CD34^+/CD133^+$ cells is independent of blood pressure. Further, we found no correlation between systolic or diastolic blood pressure and $CD34^+$ and $CD34^+/CD133^+$ within the $MA^+$ or $MA^-$ groups.

Our observations are in line with the described inverse relationship between $CD34^+$ cells and progression of nephropathy in type 2 diabetes (12). Indeed, significant renal impairment affects progenitor cell number and function (13); however, both our groups had relatively preserved and comparable renal function, and we did not observe a correlation between progenitor cell number and eGFR.

All our patients had retinopathy; however, its severity was not measured. The observation that microalbuminuric diabetic patients have more severe retinopathy (14), a condition paralleled by higher circulating progenitor cells (15), could have underestimated the observed differences.

In conclusion, in a relatively "protected" population of type 1 diabetic patients, with or without microalbuminuria, circulating vascular progenitor cells may be a mean by which individuals respond to the vascular risk linked with diabetes and improve their long-term vascular health, while others, unable to respond to insults, are at higher risk for renal and vascular diseases.

**Acknowledgments**— This work was funded by the "Start up grant scheme" from Diabetes U.K. (grant RD04/0002818) and by the European Union, 6th Framework, Multi-Knowledge (reference: IST-2004-027106). M.H.-F. acknowledges financial support from the National Institute for Health and Research, Biomedical Research Centre award to Guy's and St Thomas Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust.

No other potential conflicts of interest relevant to this article were reported.

We are grateful to the patients who participated in this study.

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