SDF-1 Genotype Influences Insulin-Dependent Mobilization of Adult Progenitor Cells in Type 2 Diabetes

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CD34$^+$/CD133$^+$ circulating adult progenitor cells (PCs) play an important role in tissue repair in metabolic disease. PCs have a certain plasticity to differentiate into cells with tissue-specific phenotypes (1–3). In diabetic mice, therapeutic use of PCs in models of hindlimb ischemia and wound healing was particularly effective, suggesting a diabetes-dependent defect in PC function (4,5). Accordingly, in vitro outgrowth and angiogenic function of endothelial PCs differentiating from PCs (1) is diminished in patients with cardiovascular risk factors and in type 1 and type 2 diabetes (6–8). From clinical studies it is evident that insulin therapy is a factor determining cardiovascular complications and mortality in diabetes (9,10). It can therefore be speculated that insulin might influence mobilization of PCs in type 2 diabetes. In view of the strong genetic background for the development of late diabetes complications, we asked whether the SDF1-3′A/G genotype known to enhance PC mobilization (11) might be of influence.

RESEARCH DESIGN AND METHODS

— In the pilot study presented here, we quantified the number of PCs by flourescence-activated cell sorter (FACS) analysis in 23 patients with poorly controlled type 2 diabetes (HbA_{1c} 10.6 ± 1.6%, fasting glucose 15.9 ± 4.5 mmol/l) and 10 age-matched control subjects (59 ± 14 vs. 58 ± 10 years) without history of diabetes (HbA_{1c} 5.4 ± 0.5%). After 5.4 ± 1.6 weeks of treatment with (additional) insulin, 11 patients from this cohort had a follow-up measurement of PCs. Adequate insulin supplementation was documented by a lowering of HbA_{1c} of 1.5 ± 0.7%. All patients gave written consent, and the study was approved by the local ethics committee. All factors known to influence or possibly influencing the number of circulating PCs were excluded (i.e., infection, abnormal blood count, ischemia, anemia, hypoxia, statin [12] or erythropoietin [13] therapy, chemotherapy, immunosuppression). All patients who attended the follow-up examination were treated with insulin (7 of 11 on an intensified regimen); 5 received metformin and only 1 was treated with sulfonylurea.

FACS analysis and genotyping

Venous blood (30 ml) was drawn for detection of PCs and genotyping. Peripheral mononuclear cells (pBMCs) were isolated by density gradient centrifugation and incubated with anti–CD34-FITC (fluorescein isothiocyanate), anti–CD133-PE (phycoerythrin) (Miltenyi Biotech), and isotype controls (Becton Dickinson). The FACS analysis was normalized by single and double staining using isotype controls for each patient and measurement. PCs were counted and analyzed using FACS Calibur cell sorter (Becton Dickinson) and Cell Quest Pro Software (Becton Dickinson). SDF1 genotype was detected by PCR and restriction analysis as previously described in detail (14).

Statistical analysis

Student's *t* test, paired Student's *t* test, Pearson's correlation, and multivariate analysis were used for statistical validation (SPSS software). All data are given as means ± SD.

RESULTS

— Numbers of circulating CD34$^+$/133$^+$ cells (Fig. 1*A*) did not differ significantly between age-matched control subjects and patients with type 2 diabetes (0.071 ± 0.033 vs. 0.061 ± 0.035% of pBMC, $P = 0.48$). PCs did not correlate with micro- or macrovascular late complications in this small cohort with a diabetes duration of 9 ± 9 years (not shown). For methodological evaluation, numbers of PCs were detected in two healthy volunteers and four serial measurements over a period of 2 months, revealing a SD of 12% (not shown). After a medium time of 5.4 ± 1.6 weeks and adequate insulin supplementation, PCs increased in all 11 patients of the initial cohort attending the follow-up examination by 65.6 ± 62.7% ($P = 0.007$) (Fig. 1*B*). During this time, mean HbA_{1c} fell significantly from 10.2 ± 1.4 to 8.7 ± 0.6% ($P < 0.001$). Yet, neither fasting glucose levels in the initial cohort ($n = 23$, $R = 0.14$) (Fig. 1*C*) nor improvement of long-term glucose control as measured by change in HbA_{1c} ($R = 0.03$) (not shown) correlated with PC numbers or mobilization. However, the individual PC mobilization varied from 8% to a 176% increase and prompted us to screen the cohort for the SDF1-3′A/G genotype. Initial numbers of PCs in the cohort did not differ significantly between SDF-1 genotypes (0.08 vs. 0.05%, $P = 0.16$) (not shown).

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Abbreviations: pBMC, peripheral mononuclear cell; PC, progenitor cell.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
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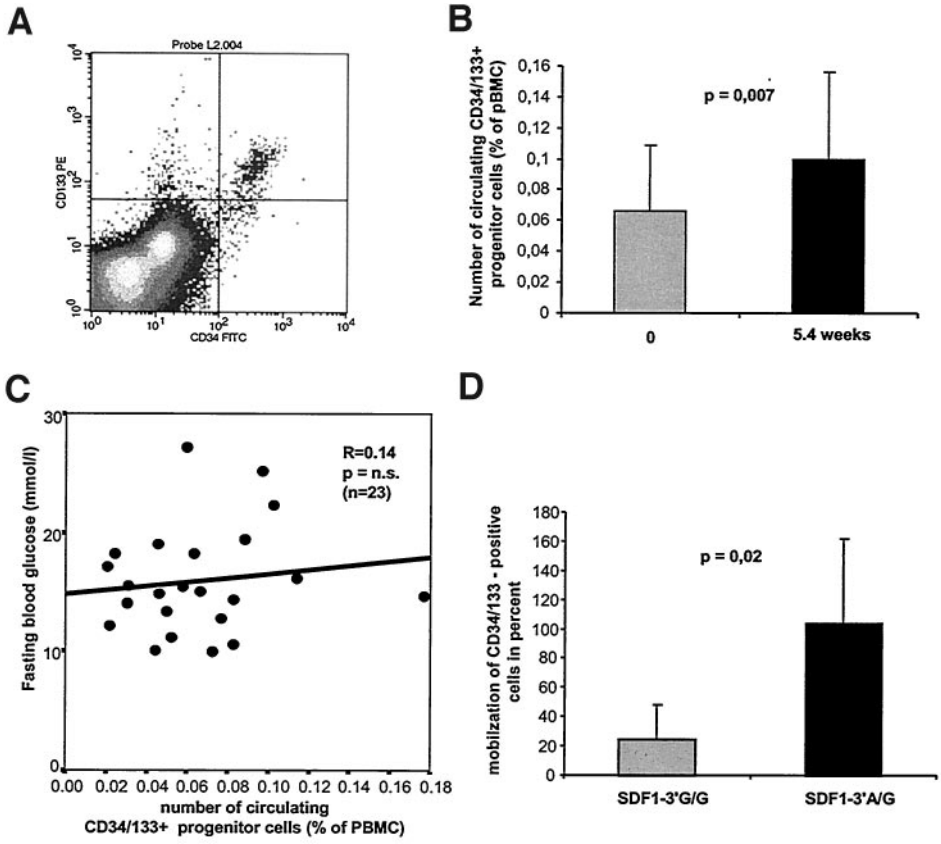

Figure 1—A*: FACS analysis. Double staining of CD34 and CD133 antigens on pBMCs. Fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-coupled isotype controls were used for normalization. CD34$^+$/133$^+$ adult PCs are shown in the* upper right quadrant. B*: Number of PCs (% of pBMCs) in patients with poorly controlled type 2 diabetes before (gray bar) and after 5.4 weeks of insulin therapy* (n = *11, black bar).* (P *value as given by paired* t *test for normally distributed values.)* C*: Correlation of CD34$^+$/133$^+$ in pBMCs (%) with fasting blood glucose (mmol/l).* (R*, Pearson's correlation coefficient;* P *value as given for Pearson's correlation.)* D*: Mobilization of CD34$^+$/133$^+$ cells in pBMCs (%) after 5.4 weeks of treatment with additional insulin for carriers of SDF1-3′G/G allele (gray bar) and carriers of SDF1-3′A/G allele (black bar).* P *value as given by Student's* t *test.*

Although the effectiveness of insulin therapy was similar in both groups (decrease of HbA_{1c} 1.5 ± 1.0 vs. 1.4 ± 0.4%, P = 0.8), carriers of the SDF1-3′A/G allele presented with a significantly enhanced mobilization of PCs compared with carriers of the SDF1-3′G/G allele (103.5 ± 48.4 vs. 23.6 ± 23.7%, P = 0.02) (Fig. 1*D*). Multivariate analysis for age, sex, change in HbA_{1c}, BMI, and SDF1 genotype in this small cohort identified the SDF1-3′A/G variant to be the only independent predictor of PC mobilization upon insulin therapy (P = 0.027).

CONCLUSIONS— The data presented in this pilot study have two major implications. First, this is the first study showing an effect of insulin therapy on mobilization of circulating CD34/133-positive pluripotent PCs in patients with type 2 diabetes. Second, the SDF1-3′A/G genotype leads to enhanced recruitment of PCs upon insulin therapy. Multivariate analysis revealed the SDF1-3′A/G variant to be an independent predictor of enhanced PC mobilization after adequate insulin supplementation. Consistent with this, it was just recently shown that SDF-1 regulates mobilization of PC (15). A change in SDF-1 expression in the SDF1-3′A/G genotype might thus enhance mobilization of PCs after insulin therapy.

Recruitment of suitable patients, excluding all factors known to influence PCs, was difficult; hence, the impact of this study is clearly limited due to small patient numbers. Nevertheless, the results obtained in the well-defined cohort that was followed up after insulin treatment are highly significant. It is beyond the scope of this pilot study to elucidate the molecular mechanisms by which insulin interacts with PC mobilization. However, influences of insulin on chemotaxis and inflammatory reactions (16–18), as well as proliferation of PCs (19), should be considered, and ongoing studies should aim to answer some of the questions addressed. This pilot study is not sufficient to rule out minor effects of hyperglycemia and oxidative stress on PC mobilization and demands for larger clinical trials to study the effects of different insulin preparations (insulin analogs) on PC mobilization and development of diabetes complications. This is especially true for diabetic retinopathy, since PCs seem to play a crucial role in the retinal neoangiogenesis and could possibly influence the progression of this complication (20).

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