These free insulin levels (resulting from both the constant intravenous insulin infusion that was maintained throughout the in-patient study days as well as any residual concentration remaining from the previous dose of subcutaneous or inhaled insulin) did not show changes from baseline to posttreatment values (mean of weeks 12 and 24) with either treatment (median baseline 16.6, 16.4; posttreatment 18.0, 18.4 uU/ml; inhaled and subcutaneous insulin, respectively).

Free insulin concentrations would not be expected to reconcile the lack of glucose changes observed in this study with pharmacokinetic changes observed in previous studies cited by Prof. Chantelau. In fact, the referenced investigations not only showed relationships between antibodies and free insulin levels, but also with postprandial glucose and other pharmacodynamic parameters (3–6). If insulin levels showed changes in the absence of glucodynamic consequences, the significance and/or accuracy of the insulin data would necessarily be called into question.

We also do not agree that use of the euglycemic clamp technique is the reason no glucodynamic correlates with insulin antibodies were demonstrated in our study. Importantly, the primary end point of the study, postprandial glucose, was not measured with the glucose clamp technique. Furthermore, the clamp technique is precise enough to determine glucodynamic changes secondary to insulin antibodies as was in fact demonstrated in one of the studies cited by Prof. Chantelau (6). We agree with others that the euglycemic clamp technique is the gold standard for assessment of pharmacodynamic responses to insulin (7,8).

The difference in glucodynamic results from this study compared with others may be related to methodologic differences (including prospective study design and optimization of test drug doses for test meal), as well as the ranges of insulin antibody levels achieved (as discussed in our article). Nevertheless, the results of this study show that insulin antibody levels measured prospectively during treatment with inhaled insulin are not associated with relevant changes in insulin pharmacodynamics or with adverse clinical effects.

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Autologous Transplantation of Granulocyte Colony–Stimulating Factor–Mobilized Peripheral Blood Mononuclear Cells Improves Critical Limb Ischemia in Diabetes

Response to Huang et al.

Huang et al. (1), in their small seminal clinical trial of cellular therapy for critical limb ischemia in diabetic patients, did not include presence of diabetic retinopathy among their exclusion criteria. It is known that individuals with diabetes are subjected to poor blood vessel growth in ischemic hearts and limbs and increased angiogenesis in retinal complications. This so-called "diabetic paradox" has been attributed to the differential regulation of angiogenic factors in the retina versus the systemic circulation (2). While decreased levels of endothelial progenitor cells are seen in diabetic patients with peripheral arterial disease (3), a role for endothelial progenitor cells in the development of proliferative diabetic retinopathy (PDR) also has been demonstrated (4). Possible harmful side effects of progenitor cell transplantation may include pathologic neoangiogenesis favoring the development or progression of cancer or PDR (5). Moreover, although data on blood cells are not presented, the increased blood viscosity due to the leukemoid response to granulocyte colony–stimulating factor may favor retinal vessel occlusion. Therefore, it was desirable that patients were screened for PDR before and after cell transplantation, in order to identify or exclude such an undesirable effect.

Finally, the achievement of a better metabolic control in the transplant group than in the control group is not unexpected and can be explained without postulating β-cell regeneration, since wound healing per se and reduced inflammation may have improved insulin sensitivity. The authors correctly cite the hypothesis that circulating progenitor cells may have a role in rescue of pancreatic endocrine function (6), but this has not been demonstrated thus far. Transplanted cells are

recruited in the pancreas of streptozotocin-induced diabetic animals, but neither sign of endocrine transdifferentiation nor improvement in blood glucose metabolism have been shown (7). Moreover, this claimed mechanism would clearly interest only those forms of diabetes due to primitive β-cell failure, while only 40% of the study patients had type 1 diabetes.

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Autologous Transplantation of Granulocyte Colony–Stimulating Factor–Mobilized Peripheral Blood Mononuclear Cells Improves Critical Limb Ischemia in Diabetes

Response to Fadini and Avogaro

Recently, our pilot study provided evidence that autologous transplantation of granulocyte colony–stimulating factor–mobilized peripheral blood mononuclear cells (PBMNCs) may represent a simple, safe, effective, and novel therapeutic approach for diabetic critical limb ischemia (CLI) (1). In our study, we chose diabetic patients with proven CLI, but without hypercoagulable states and/or severe coronary, cerebral, and renal vascular disease. As pointed out by Fadini and Avogaro (2), poor blood vessel growth in ischemic hearts and limbs and increased angiogenesis in retinal complications are paradoxical vascular complications in diabetic patients. This so-called "diabetic paradox" has been attributed to the differential regulation of angiogenic factors in the retina versus the systemic circulation (3). Thus, we may have to choose a compromised approach to balance these two divergent complications. For patients with mild or absent retinal complications but very severe limb ischemia that manifests ulceration, gangrene, or nonhealing wounds, we may give priority to improving CLI. We agree that we must be cognizant of a treatment approach that focuses on improving CLI, as well as remain aware of the potential risk for worsening diabetic retinopathy. In addition, we must monitor undesirable retinal vascular changes.

Dysfunctional endothelial progenitor cells (EPCs) from diabetes (4) may attenuate the effectiveness of our approach for CLI. However, we have observed that mobilized PBMNCs yielded more EPCs from diabetic individuals than nonmobilized ones, partially compensating for the fewer number of EPCs in diabetes. In addition, our results revealed that the mechanism in vivo is not limited to EPCs. Proangiogenic factors secreted by mononuclear cells played an equally important role in vivo (S. Li, B.Z., Z.C.H., unpublished data). Clinically, allogenic transplantation of normal mobilized PBMNCs may be more effective, but such transplanted cells may encounter rejection. Therefore, autologous transplantation of mobilized PBMNCs is still a good, albeit compromised and imperfect, approach.

As for decreased plasma glucose, we proposed that mobilization resulted in more circulating EPCs that could be recruited to the pancreas and that EPC-mediated neovascularization of the pancreas could in principle facilitate the recovery of non–terminally injured cells (5). The precise mechanism of decreased plasma glucose after mobilization awaits further investigation, for which a much higher number of patients will need to be involved.

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