

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

PINGPING HUANG, MD^{1,2}
SHANGZHU LI, MSPH¹
MINGZHE HAN, PHD¹

ZHIJIAN XIAO, MD¹
RENCHI YANG, MD¹
ZHONG CHAO HAN, PHD, MD^{1,2}

OBJECTIVE — To assess the application of autologous transplantation of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cells (PBMNCs) in the treatment of critical limb ischemia (CLI) of diabetic patients and to evaluate the safety, efficacy, and feasibility of this novel therapeutic approach.

RESEARCH DESIGN AND METHODS — Twenty-eight diabetic patients with CLI were enrolled and randomized to either the transplant group or the control group. In the transplant group, the patients received subcutaneous injections of recombinant human G-CSF (600 $\mu\text{g}/\text{day}$) for 5 days to mobilize stem/progenitor cells, and their PBMNCs were collected and transplanted by multiple intramuscular injections into ischemic limbs. All of the patients were followed up after at least 3 months.

RESULTS — At the end of the 3-month follow-up, the main manifestations, including lower limb pain and ulcers, were significantly improved in the patients of the transplant group. Their laser Doppler blood perfusion of lower limbs increased from 0.44 ± 0.11 to 0.57 ± 0.14 perfusion units ($P < 0.001$). Mean ankle-brachial pressure index increased from 0.50 ± 0.21 to 0.63 ± 0.25 ($P < 0.001$). A total of 14 of 18 limb ulcers (77.8%) of transplanted patients were completely healed after cell transplantation, whereas only 38.9% of limb ulcers (7 of 18) were healed in the control patients ($P = 0.016$ vs. the transplant group). No adverse effects specifically due to cell transplantation were observed, and no lower limb amputation occurred in the transplanted patients. In contrast, five control patients had to receive a lower limb amputation ($P = 0.007$, transplant vs. control group). Angiographic scores were significantly improved in the transplant group when compared with the control group ($P = 0.003$).

CONCLUSIONS — These results provide pilot evidence indicating that the autologous transplantation of G-CSF-mobilized PBMNCs represents a simple, safe, effective, and novel therapeutic approach for diabetic CLI.

Diabetes Care 28:2155–2160, 2005

From the ¹National Research Center for Stem Cell Engineering and Technology, State Key Laboratory of Experimental Hematology, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College, Tianjin, China; and the ²TEDA Center of Life Science & Technology, Tianjin, China.

Address correspondence and reprint requests to Dr. Zhong Chao Han, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College, 288 Nanjing Rd., Tianjin, 300020, China. E-mail: tihzchan@public.tpt.tj.cn.

Received for publication 3 February 2005 and accepted in revised form 13 June 2005.

Abbreviations: ABI, ankle-brachial pressure index; CLI, critical limb ischemia; EPC, endothelial progenitor cell; G-CSF, granulocyte colony-stimulating factor; PAD, peripheral arterial disease; PBMNC, peripheral blood mononuclear cell.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Diabetes is a common chronic disease with significant morbidity and mortality. One devastating complication of diabetes is peripheral arterial disease (PAD) including critical limb ischemia (CLI), which may result in limb loss. There is no available permanent cure for diabetic CLI at present (1,2).

Several investigations have indicated that in patients with diabetes, the circulating endothelial progenitor cells (EPCs) exhibit impaired proliferation, adhesion, and incorporation into vascular structures. The adverse metabolic stress factors are associated with reduced number and dysfunction of EPCs (3,4). In response to tissue injury and remodeling, neovascularization usually occurs via the proliferation and migration of endothelial cells from preexisting vasculature (5). However, the EPCs resident within bone marrow and peripheral blood (6–8) can also contribute to injury-induced and pathology-induced neovascularization. In animal models of diabetes, transplantation of bone marrow- or blood-derived EPCs has been shown to accelerate blood flow restoration, neovascularization, and healing of diabetic mouse skin (9,10). Therefore, therapeutic angiogenesis induced by transplantation of functional EPCs into ischemic tissues may represent a novel approach for diabetic patients with CLI.

Recently, Tateishi-Yuyama et al. (11) reported that the autologous transplantation of bone marrow mononuclear cells was safe and effective for achievement of therapeutic angiogenesis in patients with ischemic limbs and could significantly improve the clinical status in patients whose legs received injections of bone marrow mononuclear cells. Numerous other investigations have also demonstrated that transplantation of stem/progenitor cells derived from other tissues can improve limb ischemia (5–10,12).

Here, we report an alternative, sim-

ple, and effective therapeutic approach for diabetic CLI by autologous transplantation of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cells (PBMNCs).

RESEARCH DESIGN AND METHODS

This clinical study was approved by the ethical committee board of the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College. All participants received written informed consent. Diabetic patients with proven CLI, but without hypercoagulable states or gangrene above the ankle and/or severe coronary, cerebral, and renal vascular disease, were eligible for participation in this trial. Twenty-eight volunteers who were eligible for this trial were admitted to our hospital from February 2003 to June 2004 and were consecutively enrolled in a prospective, controlled clinical trial aimed at assessing the safety, feasibility, and efficacy of transplantation of G-CSF-mobilized PBMNCs for the treatment of the diabetic patients with CLI. The patients were randomized (1:1) to either the transplant group or the control group. During the period of the trial, another 15 patients with diabetic CLI were invited to participate, but they declined to be enrolled into the study for various reasons.

CLI definitions

The definition of PAD was defined as ankle-brachial index (ABI) <0.90 . CLI was severe PAD and defined as previously described (13) with 1) persistent, recurring rest pain requiring analgesia and an ankle systolic pressure 50 mmHg and/or toe systolic pressure 30 mmHg, and/or 2) ulceration, gangrene, or nonhealing wounds of the foot with ankle systolic pressure 50 mmHg or toe systolic pressure 30 mmHg. The Fontaine classification stratified patients as class III (rest pain) or class IV (ulceration and/or gangrene).

Administration of therapy

The patients received conventional care for their ulcers. To remove extensive callus and necrotic tissue, wound debridement was performed. After wound dressing, pressure relief was provided. Broad spectrum antibiotics were prescribed if ulcers showed clinical signs of infection. Adjustments to the treatment were performed when indicated on the

basis of microbiologic cultures and sensitivity testing. The control patients received an intravenous injection of 90–200 $\mu\text{g/day}$ prostaglandin E1. In the transplant group, the patients received treatment with 600 $\mu\text{g/day}$ recombinant human G-CSF (Kirin Pharmaceuticals, Tokyo, Japan) by subcutaneous injection for 5 days to mobilize stem/progenitor cells. Meanwhile, a perfusion of 10,000 units/day heparin for 5 days by intravenous drip was used to avoid the possible risks of embolism because of a G-CSF-induced increase of circulating blood cells (14,15). Then, ~ 300 ml suspension of blood circulating PBMNCs were collected from patients treated with G-CSF, through a Version 4 blood-cells separator (Cobe, Lakewood, CO) and concentrated to 1×10^8 mononuclear cells/ml. Superfluous cells were frozen in liquid nitrogen for further use. Three hours later, each diseased lower limb was intramuscularly injected (40 sites, $\sim 3 \times 3$ cm distance, 1–1.5 cm deep, 7.5×10^8 mobilized PBMNCs per site) into thigh and leg with a total of 3×10^9 mobilized PBMNCs. Forty days after transplantation, the severely diseased lower limb was given an additional transplantation of the same number of the cells frozen in liquid nitrogen as the first.

Data collection and assessment guidelines

Clinical data, medication, and safety laboratory data were prospectively collected, and follow-up visits were performed in a minimum period of 3 months. Specific attention was paid to any potential adverse effects specifically because of transplantation during follow-up. The criteria of Tateishi-Yuyama (15) and Oyibo (16) were used to assess limb status. Rest pains on rating scales ranged from 0 points for the best (complete relief of pain with no use of analgesics) to 4 points for the worst result. Assessment of pain-free walking distance used a constant speed on the same road in our hospital. ABI and blood flow (height of wave amplitude) of 10 toes were measured for all patients before and after treatment by Medacord personal vascular laboratory (Medasonics, Mountain View, CA) and the blood perfusion of lower limbs of the patients in the transplant group by laser Doppler (Lisca Developments, Linköping, Sweden) in a room adjusted for 21°C.

Angiographic analysis

The patients were subjected to analysis of digital subtraction angiography 1 week before and 12 weeks after treatment. The angiographic scores (11) for the formation of new collateral vessels were assessed as +0 (no collateral development), +1 (slight), +2 (moderate), and +3 (rich).

Analysis of CD34+ cells

The method of the International Society for Hemotherapy and Graft Engineering was applied to determine the percentage of CD34+ cells in peripheral blood cells using FACSCalibur and CellQuest Pro software (Becton Dickinson, San Jose, CA).

Statistical analysis

Continuous variables are presented as means \pm SD. Changes in variables from baseline to week 12 were analyzed by the paired or two-tailed Student's *t* and χ^2 tests. χ^2 Analysis with likelihood ratio was performed. Statistical significance was assumed at a value of $P < 0.05$. All statistical analysis was performed with SPSS version 11.5 for Windows (SPSS, Chicago, IL).

RESULTS

On admission to our hospital, all the volunteers had at least one ulcer with severe rest pain and had difficulty ambulating and sleeping (Table 1). In the transplanted patients, striking improvement of the main clinical manifestations was observed 3 months after injection of PBMNCs (Fig. 1A). The scale of rest pain decreased from 3.86 ± 0.36 to 1.07 ± 0.92 points ($P < 0.001$) with a pain-free walking distance from 0.0 ± 0.0 to 306.4 ± 289.1 m ($P = 0.001$). Eleven patients reported recovering normal sleep. In contrast, the scale of rest pain decreased from 3.79 ± 0.43 to 2.86 ± 1.17 points ($P = 0.013$) with a pain-free walking distance from 0.0 ± 0.0 to 78.6 ± 142.3 m ($P = 0.059$), whereas only six patients recovered normal sleep in the control group at 12 weeks. The number of ulcers healed at the end visit in the transplant group (14 of 18, 77.8%) was significantly higher than the control group (7 of 18, 38.9%, $P = 0.016$). No lower limb amputation occurred in the transplanted patients, but five control patients had to receive a lower limb amputation (0 of 23 vs. 5 of 24, $P = 0.007$).

Table 1—Baseline features and clinical characteristics of the patients enrolled

Characteristics	Implanted group	Control group	<i>t</i>
<i>n</i>	14	14	
Men/women	9/5	9/5	
Mean age [years (range)]	71.1 ± 5.9 (61–77)	70.9 ± 6.0 (59–81)	0.12*
Mean duration of diabetes [years (range)]	12.9 ± 8.9 (4–33)	11.6 ± 8.0 (3–31)	1.57*
Diabetic patients (type 1/type 2)	4/10	4/10	
Lower limbs with ABI <0.9	23	24	
ABI	0.50 ± 0.21	0.49 ± 0.25	0.08*
Lower limbs with ulcer	18	18	
Ulcer size of every patient (cm ²)	2.71 ± 1.32	2.39 ± 1.15	1.90*
Patients with type of ulcer (underlying factor)			
Ischemic	6	5	
Neuroischemic	8	9	
Patients with site of ulcer			
Forefoot	10	9	
Midfoot	3	4	
Hindfoot	1	1	
Patients with University of Texas grade and stage C			
Grade 1	4	5	
Grade 2	2	2	
Grade 3	0	0	
Patients with University of Texas grade and stage D			
Grade 1	5	4	
Grade 2	2	2	
Grade 3	1	1	

Data are *n* or means ± SD unless otherwise indicated. **P* > 0.05.

Blood flow restoration

Increased resting ABI was observed after cell transplantation. At week 12, the number of lower limbs with increased resting ABI (>0.1) was 15 of 23 limbs (65.2%) in the transplant group, and this was significantly higher than in the control group (4 of 24 limbs, 16.7%, *P* < 0.001). In the transplant group, mean ABI increased from 0.50 ± 0.21 at baseline to 0.63 ± 0.25 (*P* < 0.001), the blood flow of 10 toes increased from 0.92 ± 1.37 mm at baseline to 4.34 ± 3.84 mm (*P* < 0.001), and the laser Doppler blood perfusion of lower limbs increased from 0.44 ± 0.11 perfusion units at baseline to 0.57 ± 0.14 perfusion units (*P* < 0.001) (Fig. 1B). In contrast, mean ABI increased from 0.49 ± 0.25 at baseline to 0.51 ± 0.28 (*P* = 0.223) and blood flow of 10 toes increased from 0.94 ± 1.42 mm at baseline to 1.21 ± 1.54 mm (*P* = 0.104) in the control group.

Angiographic analysis

Analysis by digital subtraction angiography revealed a significant formation of new vessels after cell transplantation (Fig. 2). At the end of visit, the number of ischemic limbs with rich new collateral vessels (+3) in the transplant patients (10 of 13, 76.9%) was significantly higher than that in the control patients (2 of 11, 18.2%, *P* = 0.003 compared with the transplant group).

Analysis of CD34+ cells

In the transplant group, the percentage of CD34+ cells in PBMNCs before and 5 days after recombinant human G-CSF mobilization was 0.013 ± 0.005 and 0.134 ± 0.026% (*n* = 14, *P* < 0.001), respectively. In the final suspension of G-CSF-mobilized PBMNCs, the percentage of CD34+ cells was 0.408 ± 0.049%, as shown by FACS analysis.

Fasting plasma glucose level

During a 12-week follow-up period, all patients in the two groups received a constant fixed-dose insulin administration every day. After 12 weeks of treatment, the mean fasting plasma glucose level significantly decreased from 9.00 ± 0.95 mmol/l at baseline to 6.12 ± 0.97 mmol/l (*P* < 0.001) in the transplant group but slightly decreased from 8.42 ± 1.20 mmol/l at baseline to 7.82 ± 1.59 mmol/l (*P* = 0.065) in the control group.

No side effects specifically due to transplantation were observed by measurement of ECG or dynamic ECG, ultrasound cardiogram, function of liver and kidney, or routine blood and urine parameters, etc., during a 12-week follow-up period. To date, four patients in the transplant group have been followed up over a 14-month period, and none have had a relapse.

CONCLUSIONS— Previous studies have shown that the EPCs can be isolated from the peripheral blood of adult humans, mice, and rabbits and that G-CSF can mobilize peripheral blood CD34⁺, CD133⁺, and KDR⁺ cells with the capacity to differentiate into EPCs that are further able to incorporate into newly forming blood vessels in pathological and nonpathological conditions (16–20). G-CSF mobilization is thus an alternative approach to collect a large number of stem/progenitor cells from peripheral blood for autologous transplantation in many diseases such as leukemia and solid cancer (21).

We have recently reported that transplantation of G-CSF-mobilized autologous PBMNCs improves limb ischemia in patients with arteriosclerosis obliterans of lower extremities (22). Therefore, we believed that the autologous transplantation of mobilized PBMNCs may be effective in the treatment of diabetic CLI. In this pilot clinical trial, we observed that many clinical manifestations in the transplanted patients were significantly improved after autologous transplantation of PBMNCs, including outcome of lower limb pain, pain-free walking distance, diabetic foot ulcers, API, and angiographic scores. The angina pectoris and embolism (13,14) because of G-CSF mobilization and adverse effects specifically as a result of transplantation had not been found during a 12-week follow-up period. These results indicate that the autologous transplanta-

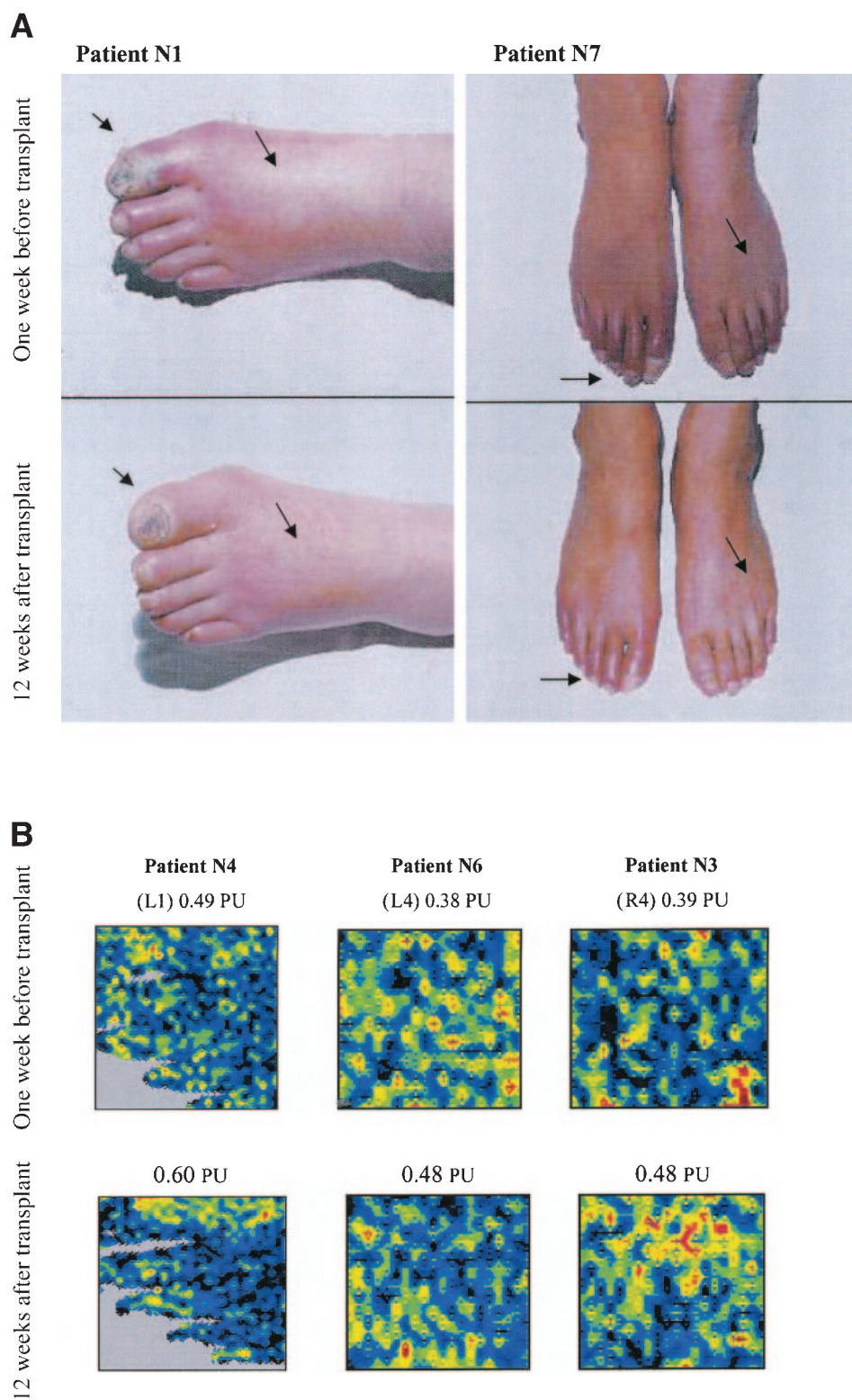


Figure 1—A: Change in clinical characteristics of the feet in patients N1 and N7 of the transplant group before and after cell transplantation. The ulcers, edema, and colors have significantly improved. Arrows direct to the same place of the feet before and after cell transplantation. B: Change in laser doppler blood perfusion of lower limbs in three patients (N3, N4, and N6) of the transplant group before and after cell transplantation. L1 and L4, four fixed examination areas of left foot; PU, perfusion unit; R4, four fixed examination areas of right foot.

tion of mobilized PBMNCs is an effective and safe therapeutic approach for CLI in diabetes. In comparison with the method of Tateishi-Yuyama et al. (11), which requires a general anesthesia and an aspira-

tion of a large amount of marrow (~500 ml), the autologous transplantation of G-CSF–mobilized PBMNCs reported here is an alternative and simple therapy strategy for diabetic CLI.

The role of G-CSF administration in the healing process in diabetic patients with CLI is still unclear at present. It has been suggested that G-CSF by itself is possible to improve neovascularization

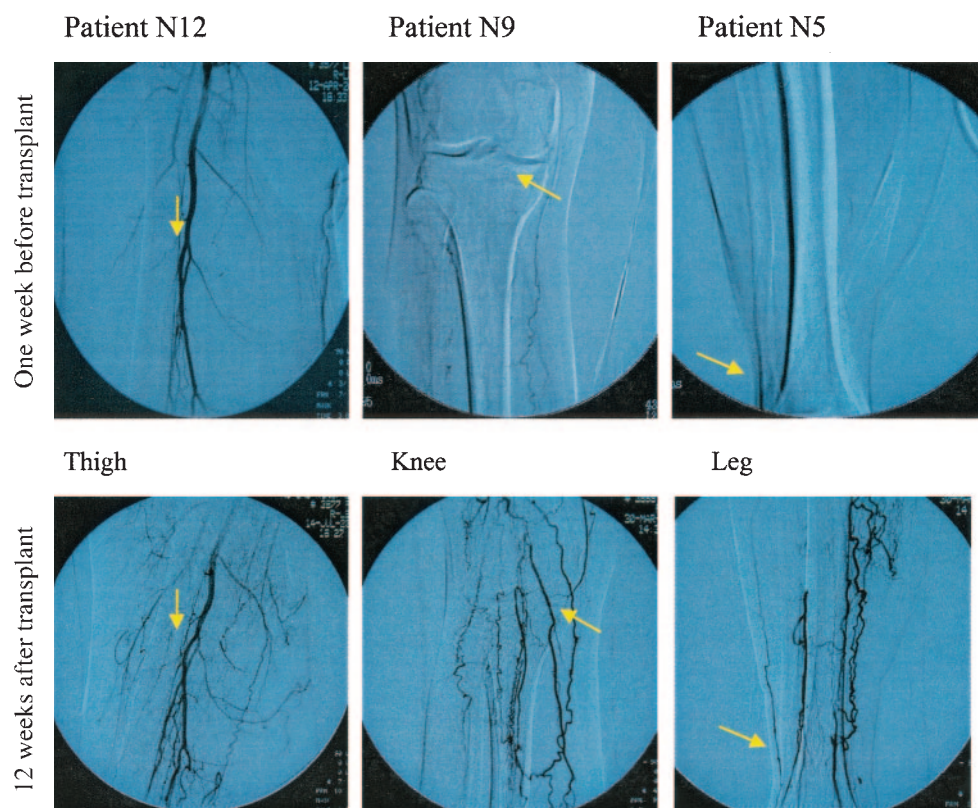


Figure 2—Change in digital subtraction angiography of lower limbs in three patients (N5, N9, and N12) from the transplant group before and after cell transplantation, showing a significantly increased formation of new collateral vessels. Arrows direct to the same place of vessel or bone before and after transplantation.

and wound healing in ischemic diseases because of its ability to mobilize EPCs into peripheral blood (23). G-CSF augments the differentiation of marrow cells into endothelial cells of blood vessels, resulting in early recovery of blood flow in the ischemic tissues (24). However, some investigations reported that treatment by G-CSF improves symptoms but not signs of myocardial ischemia in patients with severe ischemic heart disease (25). The adjunctive treatment with G-CSF for 3 weeks was well tolerated but could not significantly affect the clinical and biological parameters of the healing process in diabetic patients with severe limb-threatening infection (26). We have also treated two patients with diabetic CLI G-CSF alone and have not observed obvious improvement of the main clinical manifestations at 12 weeks (data not shown). There are at least two possibilities to explain why G-CSF mobilization plus the transplantation of PBMNCs into ischemic local muscles can result in an excellent therapeutic effectiveness. The first is that the intramuscular injections of G-CSF-mobilized PBMNCs into ischemic thighs and legs directly bring a number of EPCs into ischemic foci where the EPCs can initiate angiogenesis. The second is that a

large number of transplanted PBMNCs can secrete in vivo in the injected sites several angiogenic factors to activate the EPCs nearby ischemic tissues to form new vessels and repair impaired vessels (7). The two possibilities may coexist in this therapeutic approach.

We were surprised to observe that transplantation of G-CSF-mobilized PBMNCs may also improve blood glucose metabolism in addition to an effective vascular and skin wound repair. The level of plasma glucose after cell transplantation was decreased in the transplant group of patients compared with control patients. In diabetic animal models (8,17,27), evidence has been provided that in the animals with induced β -cell injury, transplanted bone marrow EPCs were detectable throughout the pancreas after cell transplantation, suggesting that EPCs are recruited to the pancreas in response to islet injury and that EPC-mediated neovascularization of the pancreas could in principle facilitate the recovery of nonterminally injured β -cells (27). Recently, reports of the potential existence of pancreatic stem cells and their utility in rescuing the diabetic state further raise the same possibilities of generating insulin-producing β -cells as well as other cell

types of the pancreatic islet from a stem cell (28).

In conclusion, we have developed a simple, safe, and effective therapy for diabetic CLI. Future studies are needed to evaluate precisely the efficacy of this therapy in a large number of patients and to determine the mechanism of action in provoking therapeutic angiogenesis, blood glucose metabolism improvement, and repopulation of β -cells.

Acknowledgments— This work is supported by grants of 863 (2001AA215311, 2002AA223354) and 973 (001CB5101) projects from the Ministry of Science & Technology of China and a grant from the China Medical Board of New York (01-748 to Z.C.H.).

We thank Dr. David T. Scadden of the Harvard Stem Cell Institute for his critical review of this manuscript and suggestions and the colleagues in the Department of Internal Medicine, Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College for their support and assistance.

References

1. Kugler CF, Rudofsky G: The challenges of treating peripheral arterial disease. *Vasc*

- Med 8:109–114, 2003
2. Eckardt A, Kraus O, Kustner E, Neufang A, Schmiedt W, Meurer A, Schollner C, Schadmand-Fischer S: Interdisciplinary treatment of diabetic foot syndrome. *Orthopade* 32:190–198, 2003
 3. Loomans CJM, de Koning EJP, Staal FJT, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld A-J: Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 53:195–199, 2004
 4. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC: Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106:2781–2786, 2002
 5. Lechner A, Habener JF: Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. *Am J Physiol Endocrinol Metab* 284:E259–E266, 2003
 6. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA: Regeneration of cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 107:1395–1402, 2001
 7. Iba O, Matsubara H, Nozawa Y, Fujiyama S, Amano K, Mori Y, Kojima H, Iwasaka T: Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. *Circulation* 106:2019–2025, 2002
 8. Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA: Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. *J Clin Invest* 106:571–578, 2000
 9. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T: Ischemia and cytokine-induced mobilization of bone marrow-derived endothelial cells for neovascularization. *Nat Med* 5:434–438, 1999
 10. Al-Khaldi A, Al-Sabti H, Galipeau J, Lachapelle K: Therapeutic angiogenesis using autologous bone marrow stromal cells: improved blood flow in a chronic limb ischemia model. *Ann Thorac Surg* 75:204–209, 2003
 11. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T; Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators: Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 360:427–435, 2002
 12. Yang C, Zhang ZH, Li ZJ, Yang RC, Qian GQ, Han ZC: Enhancement of neovascularization with cord blood CD133+ cell-derived endothelial progenitor cell transplantation. *Thromb Haemost* 91:1202–1212, 2004
 13. Dorros G, Jaff MR, Dorros AM, Mathiak LM, He T: Tibioperoneal (outflow lesion) angioplasty can be used as primary treatment in 235 patients with critical limb ischemia: five-year follow-up. *Circulation* 104:2057–2062, 2001
 14. Fukumoto Y, Miyamoto T, Okamura T, Gondo H, Iwasaki H, Horiuchi T, Yoshizawa S, Inaba S, Harada M, Niho Y: Angina pectoris occurring during granulocyte colony-stimulating factor-combined preparatory regimen for autologous peripheral blood stem cell transplantation in a patient with acute myelogenous leukaemia. *Br J Haematol* 97:666–668, 1997
 15. Kawachi Y, Watanabe A, Uchida T, Yoshizawa K, Kurooka N, Setsu K: Acute arterial thrombosis due to platelet aggregation in a patient receiving granulocyte colony-stimulating factor. *Br J Haematol* 94:413–416, 1996
 16. Oyibo SO, Jude EB, Tarawneh I, Nguyen HC, Harkless LB, Boulton AJM: A comparison of two diabetic foot ulcer classification systems. *Diabetes Care* 24:84–88, 2001
 17. Sivan-Loukianova E, Awad OA, Stepanovic V, Bickenbach J, Schatteman GC: CD34+ blood cells accelerate neovascularization and healing of diabetic mouse skin wounds. *J Vasc Res* 40:368–377, 2003
 18. Szmítko PE, Fedak PW, Weisel RD, Stewart DJ, Kutryk MJ, Verma S: Endothelial progenitor cells: new hope for a broken heart. *Circulation* 107:3093–3100, 2003
 19. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP: Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 105:71–77, 2000
 20. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S: Expression of VEGFR-2 and AC133 by circulating human CD34 (+) cells identifies a population of functional endothelial precursors. *Blood* 95:952–958, 2000
 21. Gordon PR, Leimig T, Babarin-Dorner A, Houston J, Holladay M, Mueller I, Geiger T, Handgretinger R: Large-scale isolation of CD133+ progenitor cells from G-CSF mobilized peripheral blood stem cells. *Bone Marrow Transplant* 31:17–22, 2003
 22. Huang PP, Li SZ, Han MZ, Xiao ZJ, Yang RC, Han ZC: Autologous transplantation of peripheral blood stem cells as treatment for arteriosclerosis obliterans of lower extremities. *Thromb Haemost*, 91:606–609, 2004
 23. Seiler C, Pohl T, Wustmann K, Hutter D, Nicolet PA, Windecker S, Eberli FR, Meier B: Promotion of collateral growth by granulocyte-macrophage colony-stimulating factor in patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. *Circulation* 104:2012–2017, 2001
 24. Minamino K, Adachi Y, Okigaki M, Ito H, Togawa Y, Fujita K, Tomita M, Suzuki Y, Zhang Y, Iwasaki M, Nakano K, Koike Y, Matsubara H, Iwasaka T, Matsumura M, Ikehara S: Macrophage colony-stimulating factor (M-CSF), as well as granulocyte colony-stimulating factor (G-CSF), accelerates neovascularization. *Stem Cells* 23:347–354, 2005
 25. Wang Y, Tagil K, Ripa RS, Nilsson JC, Carstensen S, Jorgensen E, Sondergaard L, Hesse B, Johnsen HE, Kastrup J: Effect of mobilization of bone marrow stem cells by granulocyte colony stimulating factor on clinical symptoms, left ventricular perfusion and function in patients with severe chronic ischemic heart disease. *Int J Cardiol* 100:477–483, 2005
 26. de Lalla F, Pellizzer G, Strazzabosco M, Martini Z, Du Jardin G, Lora L, Fabris P, Benedetti P, Erle G: Randomized prospective controlled trial of recombinant granulocyte colony-stimulating factor as adjunctive therapy for limb-threatening diabetic foot infection. *Antimicrob Agents Chemother* 45:1094–1098, 2001
 27. Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA: Recruitment of bone marrow-derived endothelial cells to sites of pancreatic β -cell injury. *Diabetes* 53:91–98, 2004
 28. Peshavaria M, Pang K: Manipulation of pancreatic stem cells for cell replacement therapy. *Diabetes Technol Ther* 2:453–460, 2000