Peripheral vascular disease (PVD) is a major cause of morbidity and significant health care cost in the United States. It is estimated that >8 million Americans have PVD, and its prevalence is expected to increase concurrently with the increase of the aging population. The high rate of morbidity associated with this disease has created a need for identification of novel therapies for adjunctive treatment of patients with PVD.

Collateral vessels are preexisting arteriolar connections that may not be used to provide perfusion under normal conditions but can be recruited to bypass the site of acute or chronic vessel occlusion. Collateral vessel growth (arteriogenesis) provides a natural adaptive mechanism to lessen tissue injury caused by PVD. However, this process is insufficient in many patients, and therapies to augment it are needed. Several investigators have shown that intramuscular injection of ex vivo–processed autologous mononuclear cells leads to short-term symptomatic improvement in patients with PVD. Others have reported a correlation between patient outcomes and the number of intramuscularly injected CD34+ cells within the patient’s mononuclear cell population. Although these studies have demonstrated favorable safety and feasibility of cell-based therapy for the treatment of patients with PVD, the need for ex vivo processing of mononuclear cells before delivery is a major impediment to the performance of a large-scale study in these patient populations. This creates a need for a novel method of rapid in vivo mobilization of mononuclear cells for the treatment of patients with PVD.

Human blood outgrowth cells (HBOCs) are cultured CD34+/vascular endothelial growth factor receptor (VEGFR) 2+ mononuclear cells from healthy human subjects. These late-outgrowth cells have a slow initial rate of growth, and they exhibit tremendous proliferative capacity after several weeks in culture, thus serving as useful tools for screening the biological features of vasculoprotective mononuclear cells in vitro assays. It was recently reported that a 12-mer peptide ligand that binds with high affinity to HBOCs has...
sequence homology to human interleukin-11 (IL-11). This finding, coupled with reports of high expression of IL-11 receptor α (IL-11Rα) in highly vascular tissues, suggests a potential role for IL-11 as a putative ligand for in vivo mobilization of mononuclear cells and vascular remodeling.

IL-11 is a member of the IL-6 cytokine family. IL-11 is produced by a variety of tissues and has pleiotropic effects on multiple tissues, including promotion of human and murine megakaryocytopoiesis and thrombopoiesis, protection of endothelial cells from alloxinjury by upregulation of survivin, and protection against endothelial cell death. Fortuitously, recombinant human IL-11 (rhIL-11) has already been used extensively in patients. Because the clinical efficacy and safety parameters of rhIL-11 have been well characterized in humans, we performed a preclinical study to investigate the role of rhIL-11 as a potential pharmacological agent for in vivo mobilization of CD34+/VEGFR2+ mononuclear cells and collateral vessel remodeling using a mouse model of hindlimb ischemia. We chose CD34+/VEGFR2+ cells because these markers are present in humans and mice, have been used to identify mouse progenitor cells that enhance neovascularization during hindlimb ischemia, and will allow for correlation of CD34+/VEGFR2+ cells in mice and future human studies.

Methods

The supplemental material (available online at http://atvb.ahajournals.org) provides methods not described herein.

Unilateral Hindlimb Ischemia

Unilateral femoral artery ligation was performed using 10-week-old Sv129 mice, as previously described. Briefly, mice were anesthetized with 1.25% isoflurane/O2 during hindlimb depilation (1 day before surgery) and during hindlimb ischemia surgery. The right femoral artery was exposed through a 2-mm incision and ligated with two 7-0 sutures placed proximal to the origin of the lateral caudal femoral artery. The artery was transected and separated. The wound was irrigated with sterile saline and closed; and ceftazolin (50 mg/kg IM), topical furazolidone, and pentazocine (10 mg/kg IM) were administered. The University of North Carolina (Chapel Hill) Institutional Animal Care and Use Committee approved all of our animal procedures.

Laser Doppler Perfusion Imaging

Laser Doppler perfusion imaging was performed as previously described. Briefly, mice were anesthetized with 1.25% isoflurane/O2 and their body temperature was strictly maintained at 37°C ±0.5°C, during the entire procedure. Laser Doppler perfusion imaging of plantar foot or adductor thigh of both legs was performed before, immediately after, and at 2, 4, 6, 8, and 14 days after femoral ligation. Images were analyzed using computer software (MoorLDIV5.0). Regions of interest were drawn with respect to anatomic landmarks, and flow rate was calculated. Perfusion in the ligated leg was normalized to the unligated leg. Foot appearance was scored as an index of ischemia: 0, normal; 1 to 5, cyanosis or loss of nail(s) (the score is dependent on the number of nails affected); 6 to 10, partial or complete atrophy of digit(s) (the score reflects number of digits affected); and 11, partial atrophy of the forefoot. Hindlimb use was scored as an index of muscle function: 0, normal; 1, no toe flexion; 2, no plantar flexion; and 3, dragging foot.

All data are reported as mean±SE. Differences were subjected to unpaired t tests (2-tailed). P<0.05 was considered significant.

Results

HBOCs Express IL-11Rα and Administration of rhIL-11 Activates Downstream STAT-3

We recently identified peptide ligands that bind specifically to HBOCs by screening phage display libraries to identify novel surface markers of vasculoprotective mononuclear cells (Figure 1A, cartoon). One such 12-mer peptide ligand has sequence homology with human IL-11. This observation, coupled with reports of high expression of IL-11Rα in highly
vascular tissues,7 prompted us to investigate the potential significance of the IL-11/HBOC interaction on vascular remodeling. As a first step, we confirmed the phenotype of HBOCs by multiparametric investigation consisting of identification of their characteristic cobblestone morphological features,5 acetylated low-density lipoprotein uptake,18 and expression of cell surface markers (eg, VEGFR2, CD34, and CD31). These characteristics, along with the lack of expression of CD45 (data not shown),19 suggest that these HBOCs have properties of dedifferentiated mononuclear cells.20 To determine whether HBOCs express IL-11R, we incubated HBOCs with anti–IL-11R antibody and found that HBOCs robustly express IL-11Rα on their surface (Figure 1B). To determine whether signaling via the IL-11Rα/rhIL-11 (receptor/ligand) axis was intact, we stimulated HBOCs with rhIL-11 and found that rhIL-11–treated cells displayed time-dependent phosphorylation of signal transducer and activator of transcription-3 (STAT-3) (Figure 1C), a downstream effector molecule that supports cell survival by upregulation of antiapoptotic proteins (eg, survivin)10 and upregulation of proangiogenic factors (eg, VEGF).21 These data demonstrate that HBOCs express a functional IL-11Rα and suggest a link between IL-11/HBOC interaction and IL-11Rα signaling.

rhIL-11 Stimulates Directed Migration and Tubule Formation in HBOCs

To study the physiological effects of rhIL-11 on HBOCs, we administered rhIL-11 to HBOCs in a Boyden chamber. rhIL-11 administration at 25 ng/mL led to optimal cell migration of HBOCs toward a concentration gradient of rhIL-11 when compared with control (Figure 2A). Similarly, treatment of HBOCs with 25-ng/mL rhIL-11 resulted in a 3-fold increase in HBOC proliferation (Figure 2B). Because cell migration and cell proliferation are cellular events that support blood vessel assembly, we performed a spheroid assay using HBOCs that were stimulated with rhIL-11 (Figure 2C) and counted the cumulative sprout length and total number of sprouts for each spheroid. As shown in Figure 2D and 2E, rhIL-11–treated HBOCs showed an 11-fold increase in cumulative sprout length (Figure 2D) and an 8-fold increase in sprouts/spheroids (Figure 2E) when compared with HBOCs treated with PBS control. These observations indicate that rhIL-11 enhances in vitro migration and proliferation of HBOCs and suggest a potential role of rhIL-11 for in vivo mobilization of CD34+/VEGFR2+ cells to sites of vascular remodeling.

rhIL-11 Treatment Leads to In Vivo Mobilization of CD34+/VEGFR2+ Mononuclear Cells

After demonstrating that HBOCs express CD34+/VEGFR2+ markers in vitro, we investigated the potential role of rhIL-11 on in vivo mobilization of CD34+/VEGFR2+ mononuclear cells by implanting Sv129 mice with osmotic pumps that continuously deliver 200-μg/kg rhIL-11 or PBS daily. This dose resulted in maximum in vivo mobilization of CD34+/VEGFR2+ mononuclear cells (data not shown). Blood was drawn 1, 3, and 7 days after minipump implantation for characterization of mononuclear cells by flow cytometry. Figure 3A illustrates mononuclear cells profiled according to
rhIL-11 Improves Functional Recovery and Collateral Vessel Growth

To determine the functional significance of the observed increase in reperfusion previously described, we used a hindlimb use score (index of hindlimb muscle function) and appearance score (index of hindlimb ischemia) and discovered that rhIL-11–treated mice exhibited better hindlimb use and appearance scores than their PBS-treated counterparts (supplemental Figure VA and VB). To measure preexisting collateral vessel remodeling directly, we performed histomorphometry of the single collateral present in each of the anterior and posterior gracilis muscles at 8 and 21 days after femoral artery ligation. Mice treated with rhIL-11 showed a 1.5-fold increase in collateral vessel luminal diameter on day 8 (data not shown) and a 3-fold increase in collateral vessel luminal diameter on day 21 after femoral artery ligation (Figure 5A and 5B), compared with PBS-treated control mice, indicating that rhIL-11 plays a significant role in time-dependent collateral vessel growth. By comparison, we did not observe a difference in angiogenesis measured by isolecitin B4+ cell analysis 21 days after femoral artery ligation (supplemental Figure VI).

rhIL-11 Enhances Influx of CD34+/VEGFR2+ Mononuclear Cells Into Perivascular Tissues

Because we observed a 20-fold increase in circulating CD34+/VEGFR2+ cells associated with rhIL-11 treatment (Figure 3), we hypothesized that rhIL-11–mediated collateral vessel growth is functionally linked to mobilized CD34+/VEGFR2+ mononuclear cells. To examine this further, we performed immunohistochemical analysis of the anterior and posterior gracilis muscles to identify CD34+/VEGFR2+ mononuclear cells in the perivascular tissues 8 days after femoral artery ligation. As shown in Figure 5C and supplemental Figure VIIA, mice treated with rhIL-11 displayed a 4.4-fold increase in perivascular CD34+/VEGFR2+ mononuclear cells (Figure 3D), compared with PBS-treated control mice. Seventy-two hours after rhIL-11 administration, there was a 20-fold increase in the number of circulating CD34+/VEGFR2+ cells (Figure 3D), compared with PBS-treated mice. By comparison, we observed a 2.8-fold increase in circulating platelets and a 2.5-fold increase in monocytes; we did not observe a significant change in red blood cells, total white blood cells, lymphocytes, or granulocytes (supplemental Figure III). These data provided the basis for further physiological characterization of rhIL-11.

rhIL-11 Increases Recovery of Perfusion After Femoral Artery Ligation

We used a model of hindlimb ischemia in which the right femoral artery is ligated proximal to the origin of the lateral caudal femoral artery. High-resolution infrared laser Doppler perfusion imaging with a 2-mm sampling depth was then used to measure perfusion in the plantar foot (which is dependent on collateral vessels in the thigh and is a good index of overall leg perfusion) and in the adductor region. Blood flow decreased to low values in both PBS- and rhIL-11–treated mice immediately after ligation and continued to be decreased in both groups of animals 2 days later (Figure 4A through 4C), confirming successful femoral artery ligation in both animal groups. In addition, the lack of significant difference in blood flow between PBS- and rhIL-11–treated animals at these points suggests that rhIL-11 does not have an immediate effect on postocclusive reperfusion. However, when examined at 4, 6, and 8 days after ligation, a greater recovery of plantar perfusion was seen in rhIL-11–treated mice when compared with syngeneic mice treated with PBS (Figure 4A and 4B and supplemental Figure IVA). In addition, rhIL-11–treated mice exhibited a greater increased measure of perfusion in the center of the adductor region, which contains collaterals interconnecting the saphenous and popliteal artery trees with medial trees branching from the femoral and iliac arteries proximal to the lateral circumflex femoral artery (Figure 4C and 4D and supplemental Figure IVB). Together, these observations suggest that rhIL-11 augments preexisting collateral vessel remodeling.
clear cells when compared with control mice. By comparison, we only observed a 2.6-fold increase in perivascular CD11b cells after rhIL-11 treatment (supplemental Figure VIIB and VIIC). Taken together, these data suggest that rhIL-11 enhances collateral vessel growth by augmentation of the influx of CD34+/H11001/VEGFR2+/H11001 and CD11b+/H11001 mononuclear cells into perivascular tissues.

**Discussion**

Although native collateral vessels are functional after vessel occlusion, they are often not well developed in short- or long-term vessel occlusion, resulting in significantly de-

![Figure 4](image)

**Figure 4.** rhIL-11–treated mice show faster recovery of perfusion. Sv129 mice were pretreated with rhIL-11 or PBS for 72 hours before femoral artery ligation. A, Laser Doppler perfusion imaging of the plantar foot of rhIL-11–treated mice showed increased plantar perfusion and faster blood flow recovery compared with PBS control mice. B, Graph showing the ratio of perfusion rate in the ligated/nonligated plantar foot. rhIL-11–treated mice have significantly increased perfusion rates from day 4 to day 8 after femoral artery ligation. Each point denotes the mean±SEM of 9 mice (unpaired Student t test, 2-tailed *P<0.05 and **P<0.01). The typical region of interest used to calculate the perfusion rate is marked in white (arrow).

![Figure 5](image)

**Figure 5.** Mice treated with rhIL-11 have increased collateral vessel luminal diameter and an influx of perivascular CD34+/VEGFR2+ cells. A, Cyano-Masson-elastin staining of anterior and posterior gracilis muscles showing increased collateral vessel luminal diameter in rhIL-11–treated mice 21 days after femoral artery ligation. B, Graph showing that rhIL-11–treated mice have a 3-fold increase in luminal diameter of the adductor collateral vessel compared with PBS control. Each bar depicts the mean±SEM of 9 mice (unpaired Student t test, ***P<0.0001; ×10 magnification). C, Anterior and posterior gracilis muscles were stained for influx of perivascular CD34+/VEGFR2+ cells. Mice treated with rhIL-11 have a 4.4-fold increase in perivascular CD34+/VEGFR2+ cells 8 days after femoral artery ligation. Each bar depicts the mean±SEM of 8 mice (unpaired Student t test, 2-tailed **P<0.01).
creased chances of clinical improvement in symptoms. The clinical utility of mononuclear cells for adjunctive treatment of patients with PVD is based on the ability of mononuclear cells to revascularize occluded vessels by growth of preexisting collaterals. Current methods of delivering mononuclear progenitor cells to patients with PVD include intramuscular injection of ex vivo–processed bone marrow–derived or apheresed peripheral blood–derived mononuclear cells into the ischemic limb. These methods of mononuclear cell delivery are cumbersome, invasive, and are also associated with clinically significant procedural complications. Therefore, the identification of a simple and less invasive strategy of revascularization by in vivo mobilization of progenitor cells is crucial to successful treatment of patients with PVD.

Herein, we describe a novel role for rhIL-11 on in vivo mobilization of CD34+/VEGFR2+ mononuclear cells and augmentation of collateral vessel growth after femoral artery ligation. We observed that rhIL-11 treatment led to a 20-fold increase in circulating CD34+/VEGFR2+ mononuclear cells and that mobilized CD34+/VEGFR2+ cells home into sites of collateral vessel remodeling, resulting in collateral vessel growth and faster recovery of perfusion to ischemic limb in a manner that closely correlated with mobilization of CD34+/VEGFR2+ cells. These observations suggest that rhIL-11–mobilized CD34+/VEGFR2+ mononuclear cells, in part, participate in collateral vessel growth and are consistent with other reports showing a correlation between circulating CD34+ cells and the extent of collateral vessel remodeling during cerebral26 or hindlimb ischemia in humans and augmentation of regional blood flow and neovascularization28 during cerebral or hindlimb ischemia in mice.29,30

Our histological analysis of anterior and posterior gracilis muscles shows an increase in the luminal diameter of collateral vessels that was significant (1.5-fold) 8 days after femoral artery ligation and more pronounced (3-fold) 21 days after femoral artery ligation (Figure 5A and 5B). This finding complements our laser Doppler perfusion imaging data showing faster recovery of plantar and adductor perfusion in rhIL-11–treated mice as early as 4 days after femoral artery ligation (Figure 4 and supplemental Figure IV).

The mechanism of rhIL-11–mediated collateral vessel growth is an important topic of future study. Our data suggest that it is likely related to rhIL-11–induced circulating CD34+/VEGFR2+ mononuclear cells that are recruited to the perivascular ischemic region of the hindlimb, where they may release cytokines/growth factors (paracrine effect),31 activate STAT–3–dependent pathways (supplemental Figure VIII), and result in a reduction of apoptosis-mediated cell death (supplemental Figure IX), as shown by other researchers.32 Because monocytes and platelets are involved in collateral vessel growth,32,33 our data suggest that CD34+/VEGFR2+ cells are candidate cells that also play a role in collateral vessel growth. We did not characterize the relative contribution of each of the previously described cells to the observed collateral vessel growth, and we cannot exclude the possibility that the observed effects are direct effects of rhIL-11. Because of these limitations, additional studies using chimeric mice with tissue and/or cellular depletion of IL-11 will be necessary for further characterization of this mechanism.

In summary, this report shows that rhIL-11 treatment leads to in vivo mobilization of CD34+/VEGFR2+ mononuclear cells in mice. Pretreatment of Sv129 mice with rhIL-11 before femoral artery ligation leads to an increase in collateral vessel growth and faster recovery of perfusion, which correlates with functional recovery of hindlimb use. Because rhIL-11 has been used for the treatment of other human diseases, this report suggests the possibility that rhIL-11 could be used as an adjunctive treatment for patients with PVD.

Acknowledgments

We thank Kirk McNaughton, PhD, Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill Histology Research Core Facility, for assistance in preparing tissue specimens; and Robert Bagnell, Department of Pathology and Laboratory Medicine, Microscopy Services Laboratory of the University of North Carolina at Chapel Hill for assistance with microscopy.

Sources of Funding

This study was supported by grants K08HL085293 (Dr Aitsebaomo), 090655 and 062584 (Dr Faber), and R01HL065619, PO1 AG 024282, and CDC H75DP001750 (Dr Patterson) from the National Institutes of Health; and grant 09BGI2260549 from the American Heart Association (Dr Aitsebaomo).

Disclosures

None.

References


Recombinant Human Interleukin-11 Treatment Enhances Collateral Vessel Growth After Femoral Artery Ligation

Julius Aitsebaomo, Siddharth Srivastava, Hua Zhang, Sushmita Jha, Zhongjing Wang, Stephan Winnik, Anka N. Veleva, Xinchun Pi, Pamela Lockyer, James E. Faber and Cam Patterson

Arterioscler Thromb Vasc Biol. 2011;31:306-312; originally published online November 11, 2010;
doi: 10.1161/ATVBAHA.110.216986
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/31/2/306

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2010/11/11/ATVBAHA.110.216986.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Methods:

Isolation and characterization of HBOCs: The Institutional Review Board of the University of North Carolina, Chapel Hill, approved the use of human blood samples. Blood was drawn from normal healthy individuals and added to buffered sodium citrate and subsequently diluted 1:1 with HBSS (Sigma-Aldrich, Milwaukee, WI) containing 1mM EDTA and 0.5% BSA. Mononuclear cells were obtained by density gradient centrifugation as previously described and cultured in EGM-2 complete medium (Cambrex Bioscience, Walkerville, MD) prior to plating in a single well of a 6-well culture dish (Costar, Lowell, MA) coated with type 1 Collagen (BD Biosciences, Bedford, MA). Non-adherent cells were removed 72 hrs after plating and every second day thereafter. HBOC colonies with typical cobblestone morphology appeared within 3 to 4 weeks, and HBOCs were expanded by standard cell culture techniques.

Flow cytometry of HBOCs and peripheral blood: HBOCs or mouse peripheral blood-derived mononuclear cells were analyzed by flow cytometry as previously described. Briefly, HBOCs or mouse mononuclear cells (separated from peripheral blood by Histopaque 1077 density-gradient) were incubated with CD45-PerCP, CD34-APC, CD31-FITC (BD Bioscience, San Jose, CA), CD133-PE (Miltenyi Biotech, Auburn, CA), VEGFR2 (eBioscience, San Diego, CA) or anti IL-11 receptor (K-20) antibody (Santa Cruz Biotechnology, Santa Cruz CA) conjugated with Alexa 488 (Invitrogen, Carlsbad, CA). Control cells were incubated with appropriate isotype control antibodies. HBOCs or mononuclear cells were analyzed on a Beckman Coulter (Dako) Cyan ADP flow cytometer. Flow cytometry data was analyzed by SummitV4.3 software.

Western blot analysis: Western blotting was performed as previously described. Briefly, HBOC were incubated with 25ng/ml rhIL-11 for indicated time. Cells were subsequently lysed with RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO). Western blotting was performed by incubating with antibodies against phosphorylated (Cell Signaling, Danvers, MA) and un-phosphorylated (Cell Signaling, Danvers, MA) STAT-3. Blots were developed with ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Spheroid angiogenesis assay: HBOC spheroids were generated as previously described. Spheroids were added to a freshly prepared gel of neutralized collagen and
carboxymethylcellulose at a ratio of 1:1. The spheroid-containing gel was aliquoted into 24-well culture plates and incubated at 37°C until polymerization occurred. Spheroids hung in the polymerized gel and produced sprouts in response to pro-angiogenic stimuli such as rhIL-11 or VEGF. Images of the sprouts were taken at 10 x magnification and imported into image J (NIH, Bethesda, MD) to measure number and cumulative length of sprouts per spheroid. For each experimental condition, at least 10 spheroids were analyzed.

**Cell migration and proliferation assay:** Cell migration was performed using 48-well chamber apparatus (NeuroProbe, Cabin John, MD) as previously described 5. Briefly, HBOC were suspended in EGM-2 minimal medium in the upper chamber while various concentrations of rhIL-11 or VEGF were added to the lower chambers prior to incubation for 4hr and 30min at 37°C. Cells on the side of the filter facing lower chamber were stained with hematoxylin and counted using a 10 x objective on a Nikon Eclipse TS100 inverted microscope (Nikon, Melville, NY). Cell proliferation was performed using 96 well plates coated with collagen solution. Wells were neutralized with HBSS and 100µl of cell suspension containing 10^5 HBOC cells were added to the well and cultured until they retained 75% confluence. Subsequently, the indicated concentrations of rhIL-11 or VEGF were added, and cells were incubated for 48 hours in CO₂ incubator. Cells were washed once in PBS and manually counted under microscope at 10 x. Four replicates of each well were used for each assay and 4 microscope observation fields were used from each well for calculations. Data are representative of three independent experiments.

**Histologic analysis:** Histology was performed as previously described 6. Briefly, 8 or 21 days after femoral artery ligation, mice were cannulated through the descending aorta and perfused with PBS and 4% paraformaldehyde. The adductor tissue was dissected, paraffin embedded and sectioned to 5 µm sections prior to Cyano-Masson-elastin staining. Adductor sections were deparaffinized and rehydrated by incubation with alcohol prior to staining for VEGFR2 (rabbit anti mouse VEGFR2 receptor Ab, 2479, Cell Signaling, Danvers, MA) and CD34 (rat anti mouse CD34 Ab, 553731, BD Pharmingen, San Jose, CA).
Recombinant human interleukin-11 administration: rhIL-11 was obtained from R&D Systems and systemically administered at a dose of 200µg/kg/day by subcutaneously implanted mini-pumps (ALZET Osmotic Pumps) for the indicated duration of the experiment.

Reference:


Supplemental Figure Legend:

Supplemental Figure I: Representative flow data of mobilized CD34+/VEGFR2+ cells after rhIL-11 treatment. Peripheral blood mononuclear cells were stained with antibody against cell surface proteins, CD34 (Abcam, MA) and VEGFR2 (eBioscience, CA). Peripheral blood mononuclear cells were sorted based on forward and side scatter, and cells were further analyzed according to staining with antibody against CD34 and VEGFR2. As shown in the figure, rhIL-11 treated mice consistently have several fold increase in circulating CD34+VEGFR2+ cells compared with PBS-treated mice. The two representative sets of figures show homogeneity in our data.

Supplemental Figure II: rhIL-11 treatment increased circulating CD34+ and VEGFR2+ cells. Peripheral blood mononuclear cells were stained with antibody against cell surface proteins, CD34 (Abcam, MA) and VEGFR2 (eBioscience, CA). Figure shows a representative
increase of CD34+ or VEGFR2+ cells identified with their individual cell markers after rhIL-11 treatment. Unpaired student T-test, two tailed *= P< 0.05.

**Supplemental Figure III:** Mice treated with rhIL-11 showed increase in circulating platelets and monocytes. Sv129 mice were implanted with osmotic pumps loaded with either PBS or rhIL-11 for 3 days, and whole mouse blood was collected by cardiac puncture. One hundred microliters of whole mouse blood was aliquoted in BD microtainer tubes with EDTA (Becton Dickinson, NJ) and sent to the University of North Carolina Animal Clinical Core Laboratory for differential cell count using automated counter. Mice treated with rhIL-11 have a 2.8-fold increase in platelets (A) and 2.5-fold increase in monocytes (B). However, there were no significant changes in the number of red blood cells, white blood cells, lymphocytes or granulocytes (C, D, E & F). Each time point denotes mean ± SEM of 6 mice. Unpaired student T-test, two tailed *= P< 0.05, **= P< 0.01.

**Supplemental Figure IV:** Mice treated with rhIL-11 have faster recovery of perfusion after femoral artery ligation. “Raw data” of laser Doppler perfusion imaging of the plantar (A) and adductor (B) region of rhIL-11- or PBS-treated mice showing faster recovery of perfusion after rhIL-11 treatment. A modified version of this is shown as manuscript figure 4.

**Supplemental Figure V:** Treatment with rhIL-11 improves recovery of hindlimb function. After femoral artery ligation, animals were individually inspected for hindlimb use and appearance score. Recombinant human IL-11-treated mice have better functional recovery assessed by both hindlimb use (A) and hindlimb appearance (B) score when compared to PBS control mice (higher numbers indicate worse hindlimb functional recovery). Each time point denotes mean ± SEM of 9 mice. Unpaired student T-test, two tailed *= P< 0.05, **= P< 0.01.

**Supplemental Figure VI:** Treatment with rhIL-11 has no significant effect on angiogenesis in gastrocnemius vessels 21 days after femoral artery ligation. Gastrocnemius muscles were stained with FITC-labeled isoelectin B4, and numbers of capillaries stained with isoelectin B4 were counted/field at 20-x magnification. There was no significant difference in capillary density 21 days after femoral artery ligation in rhIL-11- or PBS-treated mice.
Supplemental Figure VII: Mice treated with rhIL-11 have influx of CD34+/VEGFR2+ cells and monocytes in ligated limb. Adductor muscle sections of PBS- and rhIL-11-treated mice were stained with the indicated antibodies to determine influx of cells in perivascular tissues distal to the site of femoral artery ligation. There was a 4.4-fold increase in CD34+/VEGFR2+ cells as shown in Figure A and manuscript Figure 5 C and a 2.6-fold increase in monocytes (B and C) 8 days after femoral artery ligation (20x magnification). Unpaired student T-test, two tailed *= P< 0.05.

Supplemental Figure VIII: Treatment with rhIL-11 induces STAT-3 phosphorylation in adductor muscle. Adductor muscle sections in ligated limb were stained with anti p-STAT-3 antibodies (Cell signaling, MA). Mice treated with rhIL-11 showed increase in phosphorylated STAT-3 in adductor muscle when compared with control mice.

Supplemental Figure IX: Mice treated with rhIL-11 have reduced apoptosis in perivascular cells. Adductor muscle sections of PBS- and rhIL-11-treated mice were stained with FITC-labeled antibodies to detect double stranded DNA break (A) using in situ cell death detection (Roche, NJ). A 1.8-fold more apoptotic cells are observed in PBS-treated mice compared to their rhIL-11-treated counterpart (B). Unpaired student T-test, two tailed *= P< 0.05.
Supplemental Figure III

A

Platelet count (×10^3/µl)

B

Monocyte count (×10^3/µl)

C

Red blood cell count (×10^6/µl)

D

White blood cell count (×10^3/µl)

E

Lymphocyte count (×10^3/µl)

F

Granulocyte count (×10^3/µl)
Supplemental Figure V

A

Hindlimb use score

PBS  rhIL-11

Time after ligation (day)

B

Hindlimb appearance score

PBS  rhIL-11

Time after ligation (day)
Supplemental Figure IX

A

PBS

rhIL-11

B

TUNEL^+ cells in perivascular region of remodeled collaterals

*
Supplemental Figure VII

A

PBS

rhIL-11

α-CD34- FITC  α-VEGFR2- Alexa594  Overlay

B

α-CD11b Ab- FITC

α-CD11b Ab- FITC

PBS  rhIL-11

C

No. of CD11b+ cells/ Muscle fibers

PBS  rhIL-11
Supplemental Figure VIII

Phosho-STAT-3

PBS

rhIL-11