Short Communication

Adenosine A_{2A} Receptor Activation Promotes Wound Neovascularization by Stimulating Angiogenesis and Vasculogenesis

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Recent reports indicate that circulating endothelial progenitor cells (EPCs) may be recruited to sites of neovascularization where they differentiate into endothelial cells (EC). As we have previously demonstrated that adenosine A_{2A} agonists promote neovascularization in wounds, we sought to determine whether adenosine A_{2A} receptor agonist-augmented wound healing involves vessel sprouting (angiogenesis) or EPC recruitment (vasculogenesis) or both. Four weeks after bone marrow reconstitution from donor FVB/N Tie2GFP transgenic mice, two full-thickness excisional wounds were performed on the dorsum of FVB/N wild-type mice and treated with either an A_{2A} receptor agonist (CGS-21680) or vehicle alone. Vessel density, as measured by CD31 staining, and density of EPC-derived vessels, as measured by GFP expression, were quantified in a blinded fashion using two-color fluorescence microscopy. We observed nearly a three-fold increase in CD31-positive vessels and a more than 10-fold increase in GFP-positive cells in A_{2A} agonist-treated 3-day old wounds, but by 6 days after wounding the differences between A_{2A} agonist-treated and vehicle-treated wounds were no longer statistically significant. In conclusion, this is the first evidence that an exogenous agent such as an adenosine A_{2A} receptor agonist increases neovascularization in the early stages of wound repair by increasing both EPC recruitment (vasculogenesis) and local vessel sprouting (angiogenesis). (*Am J Pathol* 2004, 164:1887–1892)

In wound healing revascularization of the wound bed is essential to supply oxygen, nutrients, and inflammatory cells to the newly growing tissue. Angiogenesis, the formation of new vessels from pre-existing ones, consists of an orderly sequence of events triggered by growth factors secreted from the surrounding hypoxic tissues. This complex process of angiogenesis occurs through the sprouting or partitioning of the already existing vessels and encompasses activation, migration and proliferation of pre-existing, fully differentiated endothelial cells resident within the parent vessels. For many years, it has been assumed that neovascularization in wounds occurred exclusively as a result of angiogenesis. In contrast, vasculogenesis, the initial series of events in vascular growth in which endothelial cell precursors (angioblasts) differentiate *in situ* and assemble into solid endothelial cords, had been thought to play a role in neovascularization only during embryonic development.¹ However, endothelial progenitor cells (EPCs) have been isolated from peripheral blood and subsequently incorporated into sites of active neovascularization, suggesting that both angiogenesis and vasculogenesis contribute to the development of new vessels in the adult.²

Adenosine is a metabolic messenger that may be generated intracellularly or extracellularly as a result of ATP catabolism in ischemic or inflamed tissues. Adenosine mediates a wide variety of physiological effects through its interaction with cell-surface receptors, of which there are four subtypes, A₁, A_{2A}, A_{2B} and A₃. Most of these receptors are expressed on all of the cellular components of the healing wound including neutrophils, macro-

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phages, fibroblasts and endothelial cells. The participation of the A_{2A} receptor in promoting wound healing and angiogenesis has previously been demonstrated in two ways: 1) selective A_{2A} receptor antagonists reverse the effect of CGS-21680 on wound healing³; and 2) CGS-21680 has no effect on wound healing in mice lacking A_{2A} receptors.⁴ Moreover, A_{2A} receptor-deficient mice suffer from disordered wound healing with poor matrix formulation and diminished blood vessel formation in the granulation tissue of excisional wounds and mechanically injured skin.

The aim of the present study was to determine whether topical application of an adenosine A_{2A} receptor agonist promotes new blood vessel formation in wounds by stimulating angiogenesis, sprouting of new vessels from pre-existing vessels, by increasing recruitment of endothelial precursors with *de novo* vessel formation, vasculogenesis, or both processes. We report here that topical application of an adenosine A_{2A} receptor agonist stimulates both angiogenesis and vasculogenesis.

Materials and Methods

Bone Marrow Transplantation

FVB/N-TgN(Tie2/GFP)287Sato (Tie2/GFP) 10-week-old male mice (Jackson Laboratory, Bar Harbor, ME), which express the marker gene green fluorescent protein (GFP) under the endothelial specific Tie2 promoter, served as bone marrow donors.⁵ Following cervical dislocation the long bones of mice were flushed with PBS, filtered through sterile 33- μ m Nytex mesh (Sefar America, Kansas City, MO) resuspended in cold PBS and counted. 15 FVB/N (wild-type) 10-week-old female mice (Jackson Laboratory) recipient mice were lethally irradiated with a total of 12 Gy administered in two doses, 3 hours apart. Three hours later, the irradiated recipient mice were randomly repopulated via intravenous tail vein injection with whole non-fractionated bone marrow (BM) (2×10^6 cells) of male Tie2/GFP donors. All mice were maintained under veterinary supervision at the New York University Medical Center Animal Facility in accordance with the guidelines established by the NIH for the care of laboratory animals and all procedures were approved by the Institutional Animal Care and Use Committee.

Excisional Wound Formation

Four weeks after the bone marrow transplantation, two sterile, full-thickness excisional wounds (10 mm in diameter) were created on the dorsum of anesthetized mice using a template and scissors. Wounds were treated daily with topical application of 20 μ l of either the adenosine agonist CGS-21680 (2-*p*-phenethyl-amino-5'-*N*-ethylcarboxamido-adenosine) (Sigma Chemical Co., St. Louis, MO) (250 μ g/ml) or vehicle (1.5% w/v carboxymethylcellulose in PBS). This dose was selected based on previous experiments in which the dose-response curve for CGS-21680 on wound healing revealed that the agonist concentration used here (5 μ g/wound) was optimal

for this effect.⁶ Mice were kept individually caged to minimize licking of wounds. Mice were anesthetized on the stated day and perfused with PBS followed by 4% paraformaldehyde. Wounds were then excised and fixed in 4% paraformaldehyde overnight, followed by dehydration in an 18% sucrose solution.

Immunofluorescence Staining

Frozen tissues sections (10 μ m thick) were stained with monoclonal rat antibody to CD31 (PharMingen, San Diego, CA) followed by Texas red-conjugated rabbit antibody to rat IgG (Molecular Probes). The nuclei were counterstained with DAPI. Tissue sections were reviewed at low magnification ($\times 20$) using a Nikon fluorescence microscope. Counting of GFP-positive/CD31-positive endothelial cells (from bone marrow origin) and GFP-negative/CD31-positive stained endothelial cells was performed blindly on five random fields at a higher magnification ($\times 400$) using two-color fluorescence microscopy. The results are expressed as the mean ($\pm$ SEM) of the values obtained from the areas studied. Differences between groups were analyzed by means of one-way analysis of variances (analysis of variance) using SigmaStat (SPSS, Inc., Chicago, IL).

Fluorescence in Situ Hybridization

Frozen tissues sections (10 μ m thick) were hybridized with a digoxigenin (DIG) -labeled probe specific for the mouse Y chromosome (Cambio, Cambridge, UK). After hybridization, the probe was detected with a Fab fragment of anti-DIG coupled to rhodamine. The nuclei were counterstained with DAPI and the slides examined with a Nikon fluorescence microscope.

Results

We studied neovascularization in healing excisional wounds in mice that had undergone lethal irradiation followed by bone marrow transplantation from donor congenic mice that express the marker gene green fluorescent protein (GFP) under the endothelia-specific Tie2 promoter. Tie2 is a specific receptor predominantly found in vascular endothelial cells, therefore the recipient mice will express GFP in those vascular endothelial cells that were originated in the bone marrow. The evaluation of tissue sections from untreated mice revealed that 3 days after wounds were inflicted there were very few GFP-positive cells in the incipient granulation tissue at the very base of the wound, near the muscle layer. The number of GFP-positive cells in the granulation tissue of the wounds increased dramatically from day 3 to day 6 postwound. Immunofluorescence staining for CD31 was performed to detect all endothelial cells in the wounds and there was a marked increase in the number of CD31-positive cells in the wounds from day 3 to day 6 postwound (Figure 1).

Figure 1. Fluorescence microscopy of representative sections of untreated and CGS-21680-treated excisional wounds in Tie2GFP-transplanted FVB/N mice. Lethally irradiated FVB/N wild-type mice received bone marrow (BM) reconstitution from donor FVB/N Tie2GFP transgenic mice, which express the marker gene green fluorescent protein (GFP) under the endothelial specific Tie2 promoter. Tie2 is a specific receptor predominantly found in vascular endothelial cells, therefore the recipient mice will express GFP in those vascular endothelial cells that were originated in the bone marrow. Four weeks later, two excisional wounds were formed on the backs of transplanted mice and these were treated daily with either a topical A_{2A} receptor agonist (CGS-21680) ($5 \mu\text{g}/\text{wound}$) or vehicle alone (control). On the stated day, mice were killed and the wounds dissected out. Tissue sections were stained with an antibody to CD31 as described in Materials and Methods and counterstained with DAPI to provide nuclear staining. Shown are representative sections of treated and untreated 3-day-old and 6-day-old wounds depicting the granulation tissue at the base of the wound near the underlying muscle. Each section was examined using three-color fluorescence microscopy: blue fluorescence shows DAPI staining of the nuclei; red fluorescence shows CD31 staining of all vessels and green fluorescence shows GFP-positive endothelial cells indicating their bone marrow origin (original magnification, $\times 400$). The panels at the bottom show superimposition of red and green fluorescence, in which CD31 and GFP-positive cells are yellow.

As previously described,⁴ topical treatment with the selective adenosine A_{2A} agonist CGS-21680 significantly increased the number of factor VIII/von Willebrand factor-positive and CD34-positive blood vessels present in the granulation tissue of 3-day-old wounds. We analyzed, in a blinded fashion, the superimposed images of the red and green fluorescence and counted the GFP $^-$ /CD31 $^+$ cells as an index of angiogenesis and the GFP $^+$ /CD31 $^+$ cells as an index of vasculogenesis. There was nearly a threefold increase in GFP $^-$ /CD31 $^+$ vessels in adenosine A_{2A} agonist- versus vehicle-treated wounds (64.4 ± 8.7 versus 26.1 ± 5.0 cells/ mm^2 , $n = 8$ and 6, respectively, $P < 0.001$). The CGS-21680-induced increase in angiogenesis persisted in 6-day wounds although it did not achieve statistical significance (125.8 ± 21.9 versus 108.3 ± 13.1 cells/ mm^2 , $n = 8$ and 6, respectively, $P = \text{NS}$) (Figures 1 and 2A). These changes in CD31-positive cells were nearly identical to the changes in angiogenesis observed when endothelial cells were labeled with

factor VIII/vWF and CD34. Interestingly, the selective adenosine A_{2A} agonist CGS-21680 induced a more than 10-fold increase in GFP $^+$ /CD31 $^+$ cells in 3-day-old wounds (3.58 ± 0.92 versus 0.33 ± 0.33 cells/ mm^2 , $n = 6$ and 8, respectively, $P < 0.01$) but, as with the increase in GFP $^-$ /CD31 $^+$ cells, the CGS-21680-mediated increase did not differ significantly by 6 days after wounding (5.26 ± 1.52 versus 3.34 ± 0.63 cells/ mm^2 , $n = 6$ and 8, respectively).

Since recipient mice were females and donor mice were males, we stained the wound tissue sections with a probe for Y chromosome to confirm the validity and efficiency of the bone marrow transplant. All cells that originated in the bone marrow, such as inflammatory cells and endothelial cells derived from EPCs, possessed a Y chromosome, as detected by fluorescence microscopy. Superimposition of red and green fluorescence showed that all of the GFP-positive endothelial cells were of bone marrow origin (Figure 3).

Figure 2. Number of GFP-negative/CD31-positive and GFP-positive/CD31-positive endothelial cells in the granulation tissue of untreated and CGS-21680-treated excisional wounds in Tie2GFP-transplanted FVB/N mice. Excisional wounds were created on the dorsum of Tie2GFP-transplanted mice on day 0 and treated daily with topical application of vehicle or CGS-21680 (5 μ g/wound), as described in Materials and Methods. Vessel density, as measured by CD31 staining, and density of EPC-derived vessels, as measured by GFP expression, were quantified in a blinded fashion using two-color fluorescence microscopy. **A:** Number of GFP- / CD31+ cells (means $\pm$ SEM), as an index of angiogenesis, counted in five different fields of 3-day-old wounds from six control (vehicle-treated) and eight CGS-21680-treated animals. **B:** Number of GFP+ / CD31+ (means $\pm$ SEM), as an index of vasculogenesis, counted in five different fields of 6-day-old wounds from six control (vehicle-treated) and eight CGS-21680-treated animals. Significance of the difference observed was tested by means of Student's *t*-test.

Discussion

In addition to the classic mechanisms of angiogenesis, it is generally accepted that circulating endothelial cell precursor cells participate in the process of regenerative neovascularization.¹ Early reports showed a greater number of circulating endothelial cells in smokers and patients with vascular pathologies and in experimentally injured animals (reviewed in ref. 7). Moreover, culture of both human and chicken peripheral blood leukocytes under proper conditions leads to formation of capillary-like structures filled with blood cells (reviewed in ref. 7). Thus, endothelial progenitor cells (EPCs) are a unique subtype of circulating, bone marrow-derived cells which have the potential to proliferate and differentiate into mature endothelial cells. EPCs can be isolated from the

Figure 3. Fluorescence *in situ* hybridization microscopy of representative sections of untreated and CGS-21680-treated excisional wounds in Tie2GFP-transplanted FVB/N mice. The bone marrow of lethally irradiated FVB/N wild-type female mice was reconstituted with bone marrow (BM) from donor FVB/N Tie2GFP transgenic male mice. Four weeks later, two excisional wounds were formed on the backs of transplanted mice and these were treated daily with topical application of vehicle (control) or CGS-21680 (5 μ g/wound). On the stated day, mice were killed and the wounds dissected out. Tissue sections were stained with a probe for the Y chromosome as described in Materials and Methods and counterstained with DAPI to provide nuclear staining. Each section was examined using three-color fluorescence microscopy: blue fluorescence for DAPI staining of the nuclei; red fluorescence for Y chromosome staining of all cells originated from the bone marrow and green fluorescence for GFP-positive cells indicating their endothelial cell lineage. Shown are representative sections of treated and untreated 3-day-old and 6-day-old wounds with red and green fluorescence superimposed (original magnification, $\times 400$).

peripheral blood of adult humans by magnetic bead selection on the basis of cell surface antigen expression and have the ability to incorporate into sites of active neovascularization, both in animal models of ischemia^{2,8} and humans.⁹

Endothelial precursors with phenotypic and functional characteristics of embryonic hemangioblasts, a common ancestor of EPCs and hematopoietic stem cells (HSCs), are present in human adult bone marrow. Conditions such as ischemia, trauma, and wound repair and cytokines such as granulocyte macrophage-colony stimulating factor (GM-CSF) stimulate mobilization of EPCs and HSCs from the bone marrow into the circulation^{8,10} and further evidence for incorporation of bone marrow-derived circulating EPCs into sites of postnatal neovascularization has been garnered from murine models of bone marrow transplantation.^{10–14} Our results further confirm that the recruitment of EPCs is involved in the process of wound neovascularization. Using a similar bone marrow transplantation model, Asahara and colleagues¹¹ showed that there was recruitment of bone marrow-derived EPCs into the neovasculature and stroma of granulation tissue at 4 and 7 days after wounding. We found very few (approximately 1%) bone marrow-derived endothelial cells in the newly formed vasculature of untreated 3-day-old wounds. The proportion of endothelial cells originating in the marrow increased to nearly 3% by 6 days after wounding.

Adenosine is released as result of ATP catabolism; ATP is one of the most abundant molecules in cells and adenine nucleotides may be released into the extracellular milieu following stress or trauma. Extracellular adenine nucleotides are dephosphorylated to AMP by NTP

dephosphorylase (CD39) and further dephosphorylated to adenosine by the plasma membrane-associated enzyme 5'-ectonucleotidase (CD73).¹⁵ Adenosine is a potent regulator of physiological functions at the cellular and organ level via occupancy of specific receptors on the cell surface. There are four known receptors for adenosine, A₁, A_{2A}, A_{2B} and A₃, all of which belong to the superfamily of seven transmembrane-spanning G-protein-coupled receptors. Marked species differences have been observed for agonists and antagonists of adenosine receptors with respect to potency and selectivity. In particular, binding affinity studies at rat adenosine subtypes showed that the adenosine derivative CGS-21680 is an adenosine A_{2A} receptor-selective agonist; however, CGS-21680 showed similar affinity for human A_{2A} and A₃ receptors.^{16,17} Nonetheless, in A_{2A} receptor-deficient mice CGS-21680 does not affect wound closure or angiogenesis in healing wounds but markedly increases VEGF production by macrophages in A₃ receptor-deficient but not A_{2A} receptor-deficient mice.^{4,18} Thus, the effects of CGS-21680 on wound healing and angiogenesis are most consistent with an A_{2A} receptor-mediated phenomenon and there is little evidence for involvement of any other adenosine receptors.

We have previously demonstrated that exogenous agonists for adenosine A_{2A} receptors promote wound healing and angiogenesis in healing wounds.^{3,4} To our knowledge, the results shown here are the first evidence that topical application of an exogenous agent can promote recruitment of EPCs from the bone marrow to wound neovasculature. Activation of the A_{2A} receptor induces the expression of the angiogenic growth factor VEGF by endothelial cells, smooth muscle cells^{19–22} and macrophages.¹⁸ The regulatory role of VEGF in angiogenesis and vasculogenesis in fetal development and postnatal neovascularization has been previously established.¹ Moreover, VEGF is an initial determinant of hemangioblast differentiation into endothelial progenitor cells and hematopoietic stem cells. VEGF administration *in vivo* produced an increase in circulating EPCs, as consequence of induced mobilization of bone marrow-derived EPCs, which resulted in increased differentiation of EPCs *in vitro* and augmented corneal neovascularization *in vivo*.²³ Thus, it is likely that topical application of adenosine A_{2A} receptor agonists might promote angiogenesis in wounds by stimulating local VEGF production and by increasing systemic levels of this growth factor as well. It has also been observed that adenosine A_{2A} receptor agonists directly stimulate endothelial proliferation as well. Whatever its mechanism, adenosine A_{2A} receptor activation increases neovascularization in healing wounds by increasing both local vessel sprouting and recruitment of endothelial progenitor cells from the bone marrow, a process most marked in the early stages of wound repair.

References

1. Conway EM, Collen D, Carmeliet P: Molecular mechanisms of blood vessel growth. *Cardiovasc Res* 2001, 49:507–521

2. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM: Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997, 275:964–967
3. Montesinos MC, Gadangi P, Longaker M, Sung J, Levine J, Nilsen D, Reibman J, Li M, Jiang CK, Hirschhorn R, Recht PA, Ostad E, Levin RI, Cronstein BN: Wound healing is accelerated by agonists of adenosine A₂ (G_αs-linked) receptors. *J Exp Med* 1997, 186:1615–1620
4. Montesinos MC, Desai A, Chen JF, Yee H, Schwarzschild MA, Fink JS, Cronstein BN: Adenosine promotes wound healing and mediates angiogenesis in response to tissue injury via occupancy of A(2A) receptors. *Am J Pathol* 2002, 160:2009–2018
5. Motoike T, Loughna S, Perens E, Roman BL, Liao W, Chau TC, Richardson CD, Kawate T, Kuno J, Weinstein BM, Stainier DY, Sato TN: Universal GFP reporter for the study of vascular development. *Genesis* 2000, 28:75–81
6. Victor-Vega C, Desai A, Montesinos MC, Cronstein BN: Adenosine A_{2A} receptor agonists promote more rapid wound healing than recombinant human platelet-derived growth factor (Becaplermin gel). *Inflammation* 2002, 26:19–24
7. Tomanek RJ, Schatteman GC: Angiogenesis: new insights and therapeutic potential. *Anat Rec* 2000, 261:126–135
8. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhardt D, Wang J, Homma S, Edwards NM, Itescu S: Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001, 7:430–436
9. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM: Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002, 106:3009–3017
10. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Wagner M, Isner JM, Asahara T: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999, 5:434–438
11. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearney M, Wagner M, Isner JM: Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999, 85:221–228
12. Davidoff AM, Ng CY, Brown P, Leary MA, Spurbeck WW, Zhou J, Horwitz E, Vanin EF, Nienhuis AW: Bone marrow-derived cells contribute to tumor neovascularization and, when modified to express an angiogenesis inhibitor, can restrict tumor growth in mice. *Clin Cancer Res* 2001, 7:2870–2879
13. Zhang ZG, Zhang L, Jiang Q, Chopp M: Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. *Circ Res* 2002, 90:284–288
14. Grant MB, May WS, Caballero S, Brown GA, Guthrie SM, Mames RN, Byrne BJ, Vaught T, Spoerri PE, Peck AB, Scott EW: Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. *Nat Med* 2002, 8:607–612
15. Morabito L, Montesinos MC, Schreibman DM, Balter L, Thompson LF, Resta R, Carlin G, Huie MA, Cronstein BN: Methotrexate and sulfasalazine promote adenosine release by a mechanism that requires ecto-5'-nucleotidase-mediated conversion of adenine nucleotides. *J Clin Invest* 1998, 101:295–300
16. Fredholm BB, AP IJ, Jacobson KA, Klotz KN, Linden J: International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors *Pharmacol Rev* 2001, 53:527–552
17. Klotz KN, Hessling J, Hegler J, Owmán C, Kull B, Fredholm BB, Lohse MJ: Comparative pharmacology of human adenosine receptor subtypes: characterization of stably transfected receptors in CHO cells. *Naunyn Schmiedeberg's Arch Pharmacol* 1998, 357:1–9
18. Leibovich SJ, Chen JF, Pinhal-Enfield G, Belem PC, Elson G, Rosania A, Ramanathan M, Montesinos C, Jacobson M, Schwarzschild MA, Fink JS, Cronstein B: Synergistic up-regulation of vascular endothelial growth factor expression in murine macrophages by adenosine A(2A) receptor agonists and endotoxin. *Am J Pathol* 2002, 160:2231–2244
19. Fischer S, Sharma HS, Karliczek GF, Schaper W: Expression of

- vascular permeability factor/vascular endothelial growth factor in pig cerebral microvascular endothelial cells and its upregulation by adenosine. *Mol Brain Res* 1995, 28:141–148
20. Takagi H, King GL, Ferrara N, Aiello LP: Hypoxia regulates vascular endothelial growth factor receptor KDR/Fik gene expression through adenosine A2 receptors in retinal capillary endothelial cells. *Invest Ophth Vis Sci* 1996, 37:1311–1321
21. Takagi H, King GL, Robinson GS, Ferrara N, Aiello LP: Adenosine mediates hypoxic induction of vascular endothelial growth factor in retinal pericytes and endothelial cells. *Invest Ophth Vis Sci* 1996, 37:2165–2176
22. Gu JW, Brady AL, Anand V, Moore MC, Kelly WC, Adair TH: Adenosine upregulates VEGF expression in cultured myocardial vascular smooth muscle cells. *Am J Physiol* 1999, 277:H595–602
23. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM: VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J* 1999, 18:3964–3972