Tissue Resident Cells Play a Dominant Role in Arteriogenesis and Concomitant Macrophage Accumulation

Eugen Khmelewski, Aileen Becker, Thomas Meinertz, Wulf D. Ito

Ap—Collateral growth is characterized by macrophage accumulation, suggesting an important role of circulating cells. To study origin and function of macrophages during arteriogenesis, we related the extent of macrophage accumulation to vascular proliferation and investigated the fate of fluorescently (CMFDA) labeled blood cells that were injected at the time of femoral artery occlusion. The effect of bone marrow depletion via cyclophosphamide before femoral artery occlusion on collateral proliferation and macrophage accumulation was studied, and we looked for the presence of bone marrow–derived stem cells in the vicinity of growing collateral vessels. Finally, we investigated the arteriogenic effect of macrophage activation via MCP-1 in bone marrow–depleted animals. Maximal macrophage accumulation occurred during the first 3 days after femoral artery occlusion and paralleled the extent of vascular proliferation. Fluorescently labeled leukocytes homed to spleen and wound but they were absent in proliferating collateral arteries during maximal macrophage accumulation. Depletion of circulating cells did neither affect macrophage accumulation nor collateral growth. Staining of monocyte-depleted animals for BrdUrd and ED2, αSMA, or VE-Cadherin demonstrated local proliferation of macrophages and vascular cells, whereas C-Kit, SSEA1, or Thy1-positive bone marrow–derived stem cells were not detectable. Enhancement of macrophage accumulation via MCP-1 was independent of circulating monocytes and promoted arteriogenesis in the absence of direct effects on vascular cells. We propose that the initial phase of vascular growth is characterized by local proliferation of tissue resident precursors rather than by migration of blood born cells. The full text of this article is available online at http://circres.ahajournals.org.

Key Words: arteriogenesis ■ collateral growth ■ macrophages ■ circulating bone marrow–derived cells ■ resident progenitor cells

Tissue resident precursor cells play an important role in the regeneration of several adult tissues, particularly brain, muscle tissue, and liver.1,2 Skeletal muscle tissue is replenished by resident satellite cells throughout adult life. In the adult liver, resident cells differentiate into hepatocytes and blood cells.2,3 Multipotent progenitor cells, capable of giving rise to both neurons and glia, line the cerebral ventricles of all adult organisms.1,4 Resident immunoreactive microglial cells appear to direct migration and differentiation of neural precursor cells.1 As far as vascular tissue is concerned, circulating cells have primarily been implicated to play a major role.5,6 Asahara et al5 were able to show that circulating CD34+ cells are capable of assuming an endothelial phenotype and incorporate into sites of active angiogenesis. Lyden et al6 demonstrated that vascular cells and macrophages, accumulating around growing vessel, originate from donor bone marrow after lethal irradiation and transplantation. The study design, however, did not allow the distinction between tissue resident cells originating from transplanted precursors and transplanted cells recirculating from bone marrow because it has been shown that transplanted cells do not only incorporate into bone marrow but also into other tissues.7 The same caveats apply to a recently published investigation in which the authors were not able to detect incorporation of transplanted bone marrow–derived cells into the vascular wall but demonstrated accumulation of these transplanted cells as macrophages around growing collateral vessels.8 The authors of this study used a similar lethal irradiation and transplantation model as Lyden et al and likewise were not able to distinguish between cells originating from transplanted cells primarily incorporating as tissue resident cells around the vessel and cells recirculating from the repopulated bone marrow.

Our own group previously revealed that the initial proliferative phase of collateral growth was associated with macrophage accumulation around growing vessels.9 Macrophages were the main source of vascular growth factors like bFGF and infusion of the chemokine MCP-1 increased collateral conductance more than 2-fold during the first week after femoral artery occlusion in the rabbit.10 Furthermore, we
identified preexisting arteriolar anastomoses as substrate for growing collateral vessels.\textsuperscript{11,12} Based on these findings, we hypothesized that increased shear forces in preexisting arteriolar anastomoses lead to upregulation of cell adhesion molecules and chemokines and thus to adhesion and migration of monocytes.\textsuperscript{12} Migrated monocytes become macrophages and enhance arteriogenesis indirectly via secretion of vascular growth factors and metalloproteinases.\textsuperscript{12} According to this hypothesis, MCP-1 leads to further recruitment of monocytes to the growing collateral artery. Most studies, however, demonstrate a downregulation of cell adhesion molecules and chemokines with increasing shear forces and monocyte migration itself was primarily observed under low shear force conditions.\textsuperscript{13–15} According to current homing concepts, locally infused MCP-1 would not suffice to increase monocyte homing to collateral vessels if cell adhesion molecules and selectins were not upregulated simultaneously.\textsuperscript{16}

We here resolve this discrepancy and present evidence that collateral growth and concomitant macrophage accumulation occur via proliferation of tissue resident precursors, independently of circulating cells, and thus demonstrate remarkable similarities between reparative processes in the brain and vascular tissue. Identification of the exact nature of macrophages accumulating around growing vessels and demonstration of their arteriogenic effect is a prerequisite for further studies on the precise mechanisms via which these cells contribute to collateral growth.

Materials and Methods

Animal Model

This study was performed according to section 8 of the German Law for the Protection of Animals. After femoral artery occlusion, MCP-1 (450 ng/mL) in phosphate-buffered saline (PBS) was infused via osmotic minipumps (Alzet model 2 mL, 10 μL per hour for 7 days, Durect Corporation), as described previously.\textsuperscript{11} For proliferation studies, animals (Charles River Deutschland, Kißlegg, Germany) were supplied with subcutaneous osmotic minipumps (Alzet Model 2 mL, 10 μL per hour for 7 days, Durect Corporation) filled with 5′-bromo-2′-deoxyuridine (BrdUrd, Sigma, Germany; 62 mg of BrdUrd in 3 mL 0.5 mol NaHCO\textsubscript{3}-Buffer, pH 9.8), as described previously.\textsuperscript{11}

In Vivo Homing Experiments With Fluorescently Labeled Blood Cells

For leukocyte homing studies, blood from syngenic rats was labeled with CellTracker Green CMFDA (Molecular Probes, Inc) according to the protocol provided by the manufacturer. Heparinized blood (4 mL) was centrifuged and the cell pellet incubated for 1 hour at 37°C and 5% CO\textsubscript{2} in 10 μmol/L CMFDA diluted in PBS as loading buffer. Labeled blood cells were washed 3 times with PBS before they were injected directly into the collateral circulation after femoral artery occlusion via a catheter placed into the femoral artery. Three days after femoral artery occlusion, the animals were killed and blood smears as well as biopsies from the operation wound, kidney, liver, muscle tissue, and collateral vessel were obtained.

Bone Marrow Depletion

Bone marrow depletion was achieved via intraperitoneal injections of cyclophosphamide (80 mg/kg body weight 5 days before occlusion followed by an additional injection of 40 mg/kg body weight 3 days before occlusion). Depleted animals were only investigated 3 days after femoral artery occlusion.

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Assessment of Leukocyte and Differential Counts

Total leukocyte count was determined in an automatic counter (Beckman/Coulter GmbH) from blood samples obtained from experimental animals 5 days before occlusion, the day of occlusion, and 3 days after occlusion. The differential blood count was performed manually by a blinded observer.

Determination of Proliferative Index and Proliferation Kinetics

Proliferation index was assessed using the 5-Bromo-2′-deoxyuridine Labeling and Detection Kit 2 (Roche Diagnostics GmbH), a Cy2-conjugated goat-anti-mouse IgG (Lot Nr.11110, Dianova) antibody, and a 0.001% propidium iodide solution as nuclear staining (P-4170, Sigma-Aldrich Chemie GmbH), as described previously.\textsuperscript{11} For counting, pictures were taken from 4 to 5 sections of 3 collateral midzone segments. The proliferative index was calculated as number of BrdUrd positive nuclei (green fluorescence) to the total number of nuclei (red propidium iodide fluorescence). Proliferation was investigated in 9 animals per group 7 days after occlusion and in 5 animals per group 3 days after femoral artery occlusion. One animal in the MCP-1 with cyclophosphamide group was excluded because the minipump had failed to deliver BrdUrd.

Determination of Macrophage Subpopulations

To determine numbers of immature macrophages, we used ED3 (S erotec) and TRP-M-3 (RD) as marker. Mature tissue macrophages were stained by ED2 (S erotec) and KiM2R (Bachem/Peninsula). Cryostat acetone-fixed sections (7 μm) were incubated with the primary antibody followed by incubation with PO-coupled goat-anti-mouse (Dianova). Revelation was performed using amino-ethyl carbamyl in sodium acetate (0.1 mol/L, pH 4.8). Negative controls were obtained for every staining procedure by omitting the first antibody or by using an isotype control. They were negative for all immunohistochemical stainings presented in this study.

Double Staining for Macrophages, α-Smooth Muscle Actin, von Wilbrand Factor, and Proliferating Cells

For identification of proliferating cell types we combined the BrdUrd staining protocol either with a PE-labeled anti-ED2 (S erotec) antibody or with an anti-VE-cadherin, or anti-α-smooth muscle actin antibody linked to NHS-rhodamine (Pierce) using protocols provided by the manufacturers.\textsuperscript{11}

Staining of Bone Marrow and Collateral Vessels for Stem Cell Markers c-Kit, SSEA1, and Thy1

The presence of cells in the collateral region that carried stem cell markers was tested via staining of collateral sections with monoclonal anti-CD90 (S erotec), monoclonal anti-SSEA-1 (Chemicon Technology), and monoclonal anti-c-Kit antibody (Santa Cruz Biotechnol ogy). Revelation was performed as described earlier. Rat bone marrow served as positive control. Negative controls were obtained as described.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) and human coronary artery smooth muscle cells (CASMCs) were obtained from PromoCell (Heidelberg, Germany). HUVECs were cultured in Endothelial Cell Growth Medium (PromoCell), and CASMCs were cultured in Smooth Muscle Cell Medium (PromoCell). Both cell types were grown on fibronectin (Gibco)-coated cell culture dishes at 37°C and 5% CO\textsubscript{2}.

Migration Studies of Endothelial and Smooth Muscle Cells via Time-Lapse Videomicroscopy

Cell migration assays using time lapse videomicroscopy was performed as described previously.\textsuperscript{17} Analysis of migration course and
speed was done using the Imagoquant Multi-Track-Software Version 2.01 (Mediquant). Experiments were repeated 4 times.

**Cell Proliferation Assay**

Cell proliferation was assessed as described previously using the BrdUrd Flow Kit (PharMingen GmbH). MCP-1 was added together with BrdUrd at concentrations of 1 and 10 ng/mL representing concentrations well above the ED 50 of MCP-1 in vitro and the effective concentration in vivo. The number of cells staining positive for BrdUrd were counted in a flow cytometer (FACS Calibur, Becton Dickinson) and were related to the total amount of cells counted. Cells from 6 wells were analyzed in each group. Experiments were repeated 4 times.

**Statistical Analysis**

Data are presented as mean ± SD. Statistical comparisons between groups were performed via regression analysis (ANOVA). Bonferroni tests were used for intergroup comparisons. Differences among means were considered significant if \( P < 0.05 \).

**Results**

**Local Macrophage Accumulation Around Collateral Vessels Paralleled the Time Course of Collateral Proliferation After Femoral Artery Occlusion**

Macrophage accumulation was maximal at day 3 after femoral artery occlusion increasing 3- to 6-fold in comparison to control vessels (Figure 1b). The increase involved both immature macrophages (ED3/TRPM3 positive) and mature tissue macrophages (ED2/KiM2R positive). Between day 3 and 7, the number of macrophages accumulating around collateral vessels decreased again significantly by more than a third. Proliferative index was maximal during the first 3 days after occlusion and decreased toward day 7, paralleling the decline in the number of macrophages (Figure 1a).

**Reinjected Labeled Leukocytes Home to Spleen and Wound Tissue But Not to Collateral Vessels During Maximal Proliferation and Macrophage Accumulation**

Fluorescently labeled blood cells injected directly after femoral artery occlusion into the bed of the collateral circulation were clearly detectable 3 days after occlusion in the circulating blood (Figure 2a and 2b). In particular, large numbers of cells resembling monocytes and lymphocytes as well as thrombocytes were labeled. Fluorescent lymphocytes accumulated as a rim of cells around the pulpa of the spleen (Figure 2d). Within the operation wound, we detected cylindrical fluorescent blood clots as well as fluorescent leukocytes (Figure 2c). In contrast to spleen and wound tissue, not a single fluorescent cell was visible in tissue surrounding collateral vessels or in the collateral vessel itself indicating that homing of blood cells to collateral vessels does not occur during maximal collateral proliferation and macrophage accumulation (Figure 2e and 2f).
Monocyte Depletion Does Not Affect Collateral Proliferation and Concomitant Macrophage Accumulation

Cyclophosphamide treatment led to a pronounced pancytopenia (pretreatment, 10.5 ± 1.4 × 10⁹/L; time of occlusion, 0.6 ± 0.4 × 10⁹/L; 3 days after occlusion, 0.1 ± 0.07 × 10⁹/L) (Figure 3a). Differential counts revealed that the reduction of leukocytes involved all subpopulations (data only shown for monocytes in Figure 3a). Monocytes were reduced to 1% of their original population (pretreatment, 10.4 ± 1.1%, respectively, 0.991 ± 0.047 × 10⁹/L; time of occlusion, 10.6 ± 2.7%, respectively, 0.067 ± 1.06 × 10⁹/L; 3 days after occlusion, 7 ± 2.8%, respectively, 0.012 ± 0.012 × 10⁹/L) (Figure 3a). Despite the pronounced pancytopenia, we observed the same increase in proliferative index in depleted as in nondepleted animals after femoral artery occlusion (Figure 3b). Furthermore, we observed the same increase in all macrophage subpopulations in monocyte depleted animals as in nondepleted animals (Figure 3c and 3d).

Bone Marrow–Derived Stem Cells Positive for c-Kit, SSEA1, and Thy1 Do Not Migrate to Growing Collateral Vessels

After staining of collateral sections and bone marrow with antibodies against the stem cell markers c-Kit, SSEA1, and Thy1, we detected not a single positively stained cell around the collateral vessel whereas numerous cells were positive for these stem cell markers in the bone marrow (Figure 4a through 4f).

All Macrophages Accumulating Around Growing Collateral Vessels as well as Endothelial and Smooth Muscle Cells Are Subject to Cell Division During the First 3 Days After Femoral Artery Occlusion

Double staining with ED2 and a BrdUrd antibody revealed that all macrophages accumulating around collateral vessels had divided within the first 3 days after femoral artery occlusion (Figure 5i and 5j). Proliferation also involved endothelial and smooth muscle cell staining for VE-Cadherin and α-smooth muscle actin (Figure 5a through 5h). This proliferative response of macrophages and vascular cells was observed in depleted as well as in nondepleted animals.

Enhancement of Macrophage Accumulation via MCP-1 Occurs in Absence of a Significant Number of Circulating Monocytes and Promotes Collateral Growth

MCP-1 infusion increased both parameters maximally during the first three days after femoral artery occlusion (Figure 1c...
and 1d) and lead to the same rise in proliferative index and enhancement of macrophage accumulation in monocyte-depleted animals as in monocyte-competent animals (Figure 3b through 3d). Thus, the increase of mature as well as immature macrophages is not dependent on monocyte homing. In vitro, we were able to exclude a direct effect of MCP-1 on vascular cells (Figure 6). In contrast to monocytes, CCR-2 was only slightly expressed on endothelial and smooth muscle cells (data not shown). We were not able to detect any effect of MCP-1 on endothelial cell or smooth muscle cell migration, nor did concentrations in a range from 1 to 10 ng/mL enhance endothelial cell or smooth muscle cell migration (Figure 6a and 6b). MCP-1 also did not increase the number of HUVECs or CASMCs incorporating BrdUrd after 7 hours of incubation (Figure 6c and 6d). However, we observed an increase in variation of the number of endothelial cells incorporating BrdUrd with increasing MCP-1 concentrations (Figure 6c and 6d).

Discussion

In this study, we present three lines of evidence that circulating cells do not contribute to the early phase of collateral proliferation. First, fluorescently labeled blood cells do not home to collateral tissue; second, bone marrow depletion does not affect collateral proliferation and concomitant macrophage accumulation; and third, cells staining for markers of bone marrow-derived stem cells are not detectable in the vicinity of growing collateral vessels. Macrophage accumulation and collateral growth rather appear to rely on proliferation and differentiation of tissue resident precursors. Furthermore, evidence is presented for a significant role of tissue macrophages during collateral growth.

We concentrated on the proliferative phase of collateral growth because macrophage accumulation was confined to this stage and refrained from hemodynamic measurements because maximal proliferation precedes maximal improvement of collateral hemodynamics, and we had previously demonstrated the pronounced effect of MCP-1 on collateral conductance one week after femoral artery occlusion.10,11 To test whether homing of circulating cells was responsible for the pronounced macrophage accumulation at day 3 after femoral artery occlusion, we reinjected labeled blood cells into the collateral circulation on femoral artery occlusion. These fluorescent cells accounted for 30% to 50% of all blood cells and were clearly visible three days after femoral artery occlusion. The fact that we detected these cells as a rim of lymphocytes around spleen pulpa and in wound tissue, indicated that homing mechanisms of these cells were intact. Homing of labeled circulating blood cells into collateral tissue, however, did not occur at the time of maximal collateral proliferation and concomitant macrophage accumulation.

Next, we investigated whether depletion of bone marrow-derived circulating cells has an impact on collateral proliferation and macrophage accumulation. Bone marrow depletion was accomplished via treatment with the antiinflammatory, antiproliferative, and antiangiogenic agent cyclophosphamide.18,19 Total numbers of leukocytes were determined via automated blood cell counters, an established method for obtaining absolute numbers of leukocytes, which is superior to flow cytometric analysis, that necessitates the use of microspheres as internal standard.20 Subpopulation analysis was performed via manual differential counts, which are not
inferior to flow cytometric subpopulation analysis because monocytes markers like CD11b are also present on granulo-
cytes. Most importantly, we were not able to obtain enough cells from the small volume of blood detained from rats to achieve sufficient sensitivity and specificity in flow cytom-
eytology analysis. For the assessment of macrophage accumu-
lation, we used 4 different antibodies against rat macrophages that have been validated in numerous studies. These antibodies do not stain any other interstitial cells like fibro-
blasts and have been used to distinguish between tissue (ED2/KiM2R) and exsudative macrophages (ED3/TRPM3). Our finding that the same amount of exsudative (ED3/TRPM3 positive) and tissue (ED2/KiM2R positive) macrophages were accumulating after monocyte reduction to less than 1% of the original population excluded that circu-
lating cells play a dominant role in macrophage accumulation

Figure 4. Bone marrow–derived stem cells are not detectable in collateral tis-

Figure 5. Local proliferation of endotheli-
al cells, smooth muscle cells, and mac-
rophages in collateral tissue. a through d, Double staining of BrdUrd and VE-Cadherin (a, green fluorescence, BrdUrd; b, red fluorescence, VE-Cadherin; c, both fluorences combined; d, nuclear staining: small arrows, proliferating endothelium). e through h, Double staining of BrdUrd and α-smooth muscle actin (e, green fluorescence, BrdUrd; f, red fluorescence, α smooth muscle actin; g, both fluorences combined; h, nuclear staining: arrowheads, proliferating smooth muscle cells). i through l, Double staining of ED2-positive macrophages and BrdUrd after cyclophosphamide depletion (i, green fluorescence, BrdUrd; j, red fluorescence, ED2; k, both fluorences combined; l, nuclear staining: small arrow, proliferating vascular cell; large arrows, proliferating macrophages; scale bars=40 μm).
during collateral growth in the rat. These results also questioned the purely exudative character of ED3- and TRPM3-positive cells that rather appear to represent immature macrophages independent of their origin.

Cyclophosphamide-induced monocyte reduction should have had a measurable effect on macrophage content and collateral proliferation if they were to play a major role. After cyclophosphamide treatment, the whole rat contains \( \approx 270 \times 10^3 \) monocytes at the time of occlusion and \( 45 \times 10^3 \) monocytes at day 3 after femoral artery occlusion, assuming a total blood volume of 5 mL in the rat. We observed an increase of \( \approx 100 \) cells staining positive for ED3 and TRPM3 around one collateral section. Midzone length is \( \approx 4 \) cm. With a macrophage size of 20 \( \mu \)m, the total increase of cells carrying the markers ED 3 and TRPM 3 is \( \approx 200 \times 10^3 \) cells in one collateral vessel. This means that nearly all of the remaining monocytes need to be attracted to this one specific collateral vessel if the observed increase of ED3-positive cells were attributable to monocyte homing. This is extremely unlikely considering that there are several collateral vessels. De novo production of monocytes during the first 3 days after occlusion was also ruled out. A direct effect of cyclophosphamide is improbable. One would need to propose that cyclophosphamide is a proinflammatory and proarteriogenic agent able to compensate the loss of circulating bone marrow–derived cells, if one were to assume that circulating cells play a role during collateral proliferation. This is definitely not the case.\(^{18,19}\)

Our results appear to contradict previous reports by Heil et al\(^ {20}\) but the reported perfusion and oxygenation deficits in 5FU-treated mice, that persisted despite a more than 10-fold increase in monocytes in the rebound phase, likely were attributable to other factors than monocyte depletion.

Next, we investigated the possibility that small amounts of mobilized bone marrow–derived stem cells acutely migrate into the collateral tissue and give rise to vascular cells and macrophages. Transplantation experiments were not suitable because, as Kennedy et al\(^ {7}\) have shown, these studies do not allow to distinguish between donor cells that primarily migrated into peripheral tissues and remained there as tissue resident precursors and cells that originate from repopulated bone marrow. We therefore used markers specific for rat bone marrow–derived stem cells and indeed were able to demonstrate that certain subpopulations of bone marrow cells stained positive. We, however, did not detect any cells staining positive for these markers in collateral tissue. This does not rule out that to date unknown bone marrow–derived precursor cells home to growing vessels or that the amount of cells carrying markers of bone marrow–derived cells are too small to be detected by our methods. In case one assumes the presence of putative circulating progenitor cells, the number of these cells has to be extremely small in the light of our findings. They would be too small to be detected as reinjected blood cells or cells carrying markers of bone marrow–derived stem cells and assuming a cyclophosphamide independent or enhanced recruitment of these stem cells too small to lead to a measurable rise of any leukocyte population. Given the large number of proliferating vascular cells and macrophages as seen in this study, such a putative progenitor cell had to proliferate far more than 100-fold within the first 3 days after occlusion. This appears rather unlikely and contradicts every finding from prior in vitro studies on isolated vascular progenitor cells.\(^ {25}\) Our data therefore underlines the notion that homing of circulating bone marrow–derived cells does not play a major role during the initial phase of collateral growth.

In this study, we explicitly did not study a therapeutical effect of enriched and thus inevitable activated circulating progenitor cells or monocytes but rather concentrated on their role in the natural process of arteriogenesis. Our findings therefore do not contradict the results of prior studies investigating the effect of a massive enhancement of circulating cells or the injection of enriched and activated subpopulations.\(^ {20}\)

Our observation that homing of circulating cells is not responsible for the pronounced macrophage accumulation during collateral growth inevitably raised the question about the origin of increased amounts of vascular cells and more importantly about the origin of accumulating macrophages. Using double staining procedures, we were able to demonstrate that virtually all tissue macrophages originated from locally dividing cells during the first three days after occlusion. In keeping with previous reports, we were able to demonstrate that a large portion of vascular cells were proliferating as well.\(^ {26}\) In line with our previous reports, BrdUrd-positive cells were not detected in control legs or control vessels of the same leg excluding that BrdUrd incorporation was attributable to reasons other than proliferation, eg, in the context of cell repair.\(^ {9,11}\) In our experimental setting, BrdUrd appeared to be more suitable than other markers of proliferation like Ki67 because it allowed to determine proliferation within a time period, whereas other markers, eg, Ki67, only reveal proliferating cells at a specific point in time.\(^ {9}\) Our findings strongly suggest that vascular...
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cells as well as macrophages accumulating during the acute phase of collateral growth originate from local precursors.

Next, we investigated whether local macrophage accumulation had a direct impact on the proliferation of vascular cells. We were able to demonstrate that MCP-1 lead to an increase of local macrophage accumulation in the absence of circulating monocytes. The observed (statistically nonsignificant) increase of macrophages in cyclophosphamide-treated animals was likely attributable to an early rebound effect in preparation of the massive release of circulating cells observed 7 days after occlusion.18–20 This notion is supported by the fact that MCP-1 only has a significant impact on the number of immature macrophages in cyclophosphamide-treated animals in contrast to untreated animals in which MCP-1 leads to a significant increase in mature tissue macrophages. We were able to exclude that the arteriogenic effect of MCP-1 was attributable to a direct effect on vascular cells, although we also were able to detect low CCR-2 expression on endothelial and smooth muscle cells, as reported previously.27 MCP-1, however, had no significant effect on endothelial and smooth muscle cell proliferation and migration. We observed an increase in the variation of the number of proliferating HUVECs with increasing MCP-1 concentrations indicating that a certain subset of endothelial cells might be sensitive to MCP-1 treatment. This would explain the discrepancy between the findings of Salcedo et al27 and the data publish in this article, which agree with previous reports by Goede et al.28

Our results substantiate that activation and proliferation of tissue macrophages play an important role during the proliferative phase of arteriogenesis and that augmentation of their local accumulation via MCP-1 enhances collateral growth. The angiogenic potential of macrophages has been well known since the early experiments of Polverini et al29 and was confirmed in numerous studies.10,30 The mechanisms that are responsible for their contribution to vascular growth still remain to be determined independently of their origin from tissue or circulation. Production of growth factors and metalloproteinases certainly contribute to the angiogenic potential of macrophages as demonstrated previously by Arras et al.9 Previous studies also demonstrated that certain macrophage subsets have the potential to transform into other cell types including vascular cells.31,32 However, it remains unknown whether these phenotypical changes relate to a functional integration of these cells into growing vessels.

Our findings reconcile observations of an inverse correlation between monocyte homing and shear force and the accumulation of macrophages around growing collateral vessels under high shear force conditions.9,13,14,33 The presence of tissue resident precursors has been demonstrated in central nervous system, liver, and skeletal muscle.1–4 The similarities encountered during reparative processes throughout the organism and vascular remodeling suggest a common acute response that involves tissue resident precursors. Biologically this would cohere with the necessity of a locally confined, rapid response. Mobilization of resident precursor conceivably requires less time and is regionally more limited than mobilization of bone marrow–derived stem cells. The nature of local precursor cells during vascular growth however remains to be determined. Our study does not rule out a continuous low turnover of bone marrow–derived cells in peripheral tissues. This is, in fact, suggested by previous reports.8 Such a general turnover conceivably can be augmented via mobilization of bone marrow–derived cells or reinsertion of activated cells as demonstrated previously.20 Our results indeed suggest that there are signals from the peripheral tissue that augment the inflammatory burden throughout the organism because we observed an increase in the macrophage counts not only in collateral vessels but also in control vessels albeit much lower.

Thus, we propose that the acute phase of collateral growth is characterized by local proliferative responses of tissue bound precursors. At the same time, signals are released from the ischemic tissue that lead to augmentation of a basal turnover of circulating bone marrow–derived cells that might have an impact on later stages of vascular remodeling. In the light of therapeutical approaches using circulating cells for revascularization and ambiguous effects of mononuclear cells in the context of atherosclerotic disease, this has important clinical implications. Our findings suggest that a targeted support of local processes, ie, local overexpression or activation of survival or differentiation factors, might be more rewarding than injection of circulating cells.

Acknowledgments

This study was supported by the German Research foundation (Deutsche Forschungsgemeinschaft [DFG]: IT 13/2-1, DFG: IT 13/2-2, and DFG: IT 13/2-3) and by the Bundesministerium für Bildung und Forschung (BMBF) 01GN0127. We thank Juliane Bergmann and Petra Tiede for technical assistance.

References


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_Circ Res._ 2004;95:e56-e64; originally published online August 26, 2004;
doi: 10.1161/01.RES.0000143013.04985.E7

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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