High-Density Lipoprotein-Induced Tube Formation Requires the Activation of Ras/Raf/Mitogen-Activated Protein Kinase in Human Coronary Artery Endothelial Cells

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Objectives: High density lipoprotein (HDL) levels have been shown to be inversely correlated with coronary artery disease, but the mechanisms of the direct protective effect of HDL on endothelial cells (ECs) are not fully understood. In this study, we investigated the role of the HDL-mediated promotion of angiogenesis in human coronary artery ECs (HCECs).

Methods and Results: We developed an in vitro model of HCEC tube formation on a matrix gel. HDL induced tube formation, in which the dose-response showed that the maximum effective dose of HDL was 100 µg/mL. We also examined the effect of sphingosine-1-phosphate (SIP), which is a carrier of bioactive lipids of HDL, to analyze tube formation. Since HDL contains SIP (about 180 pmol/mg protein), 100 µg/mL of HDL contains about 0.018 µM of SIP. Although we observed 0.02 µM of SIP significantly induced tube formation, it was only 20% of 100 µg/mL-induced tube formation. HDL-induced tube formation was not affected by SB203580, an inhibitor of p38 MAPK activity, suppressed HDL-induced tube formation. Dominant-negative Ras N17 inhibited HDL-induced tube formation. Activated Ras by Ras pull-down assay and its effect was inhibited by PD98059, an inhibitor of MEK1/2 (p42/44 MAPK) pathway. Although HDL activated MEK1/2 (p42/44 MAPK), while Ras N17 blocked HDL-induced pdp42/44 MAPK. Conclusions: These results indicate that HDL induced a potent signal through a Ras MAPK pathway mediated by PTX-sensitive G-protein coupled receptor to the angiogenic phenotype in HCECs.

Monocytes, but Not Neutrophils or Lymphocytes Are Essential Mediators of Arteriogenesis

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Background: Blood vessel growth following arterial occlusion is mediated by infiltrating leukocytes. While all three leukocyte subpopulations (neutrophils, lymphocytes, monocytes) can play an important role in arteriogenesis, only monocytes have been shown to influence the growth of collateral arteries (arteriogenesis). In this study, we examined the importance of neutrophils and lymphocytes in a rabbit model of arteriogenesis.

Methods: 36 Rabbits received either Interleukin-8 (IL-8), Neutrophil-activating-protein-2 (NAP-2) or Lyphotacticin (Ltn) via osmotic minipumps directly into the collateral circulation after femoral artery ligation. NAP-2 is a relatively selective activator of neutrophils. Ltn chemoattracts lymphocytes and has an effect on both cell types. PB6 and MCP-1 treated groups served as controls. After one week, leukocytes and growing collateral arteries were quantified via immunohistology and effects on integrin markers of leukocytes activation (Mac-1, LFA-1) were assessed by flow-cytometry. Collateral conductions were measured using fluorescent microspheres.

Results: A significant increase in neutrophil accumulation after IL-8 and NAP-2 treatment was detected in vivo (cells/mm³: PBS:8.33±2.87, MCP-1:2,25±3.03,1.81±0.83, IL-8:36.6±12.65, NAP-2:2.20±6.7, 4.71±4.0, Ltn:2.27±5.5, 4.4±4.0, p<0.05 vs controls). No significant increase in collateral formation was observed. Ltn treatment resulted in leukocyte accumulation (cells/mm³: PBS:2.48±1.24, MCP-1:1.44±0.9, 1.6±4.5, 8.65±2.5, 2.85±0.6, 2.20±0.5, 2.27±0.4, 2.67±0.3, 2.27±0.3, 2.70±0.2, 2.70±0.1, 2.70±0.5, but not in collateral artery growth. Collateral conductance: [m²/min/100mmHg]: PBS-50.70±5.15, MCP-1-1339±60.19, IL-8-1358±91.56, NAP-2:2.66±83.8±72, Ltn:52.8±50.7 ±37. Conclusion: While neutrophils and lymphocytes are known to participate in arteriogenesis, their importance for arteriogenesis seems to be negligible.

Granulocyte-Colony Stimulating Factor Mobilizes and Activates Endothelial Progenitor Cells in Patients With Coronary Artery Disease

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Background: Circulating endothelial progenitor cells may function to repair cardiovascular injury, but are reduced in patients with coronary artery disease. Granulocyte-colony stimulating factor (G-CSF) mobilizes hematopoietic stem cells (CD34+) in healthy subjects, but whether this cytokine mobilizes endothelial progenitor cells capable of endotelial maturation in coronary artery disease patients is unknown.

Methods: G-CSF (10 mcg/kg) was administered daily for 5 days to 12 coronary artery disease patients, and circulating CD34+ cells, endothelial progenitor cells (CD133/VEGFR-2+), and mature endothelial cells [CD144 (VE-cadherin), CD31 (PECAM), CD141 (alpha beta 3 integrin)] were measured by flow cytometry. Results: G-CSF increased CD34+ cells from <1 cell/µl at baseline to 60±18 cells/µl within 24 hours of the last dose (p<0.05 vs baseline). One week following completion of treatment, CD34+ cells and endothelial progenitor cells had returned to baseline, but levels of mature endothelial cells remained increased over baseline (p<0.05). Conclusion: These findings establish that G-CSF administration to coronary artery disease patients mobilizes CD34+ cells and activates the endothelial progenitor cell subset, into the circulation. Mobilization is associated with increased cells expressing mature endothelial markers, which persist even at one week following the last dose of G-CSF. This suggests sustained G-CSF-stimulated differentiation into mature endothelial cell lineage, with potential therapeutic implications for revascularization of ischemic myocardium.

Impaired Arteriogenic Response to Acute Hindlimb Ischemia in CD8 Knockout Mice

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Background: CD8+ cytotoxic T lymphocytes regulate cellular responses of the immune system, which play a pivotal role in modulating collateral vessel development. The aim of our study was to investigate if the absence of circulating CD8+ T-cells impairs collateral development after femoral artery ligation in CD8-/- mice.

Methods and Results: After surgical excision of the femoral artery, Laser Doppler Perfusion Imaging demonstrated reduced collateral flow induction in CD8-/- mice compared to control mice (CS7BL/6J) at day 3 (0.21±0.01 vs 0.29±0.03, p<0.01) which persisted to day 28 (0.69±0.04 vs 0.93±0.04, p<0.01). In CD8+/- mice, when compared to controls, the reduced collateral flow was evidenced by hindlimb ischemia (ambulatory impairment score: 1.73 ± 0.18 vs 0.86 ± 0.19, p<0.01), greater calf muscle atrophy (mean area 76 ± 678 vs 1067 ± 69, μm², p<0.01), and increased fibrotic tissue content (14 ± 1% vs 7 ± 1%, p<0.01). Exogenous CD8+ T-cells, when infused into CD8-/- mice immediately after ischemia, selectively homed at the site of collateral formation and, over time, significantly increased collateral flow, improved hindlimb functional recovery, and reduced muscle atrophy/fibrosis. Conclusions: These results demonstrate that CD8+ T-cells are a critical component of the immune system in regulating the essential phase of normal collateral development. CD8+ T-cells demonstrated both delayed and impaired blood flow recovery after femoral artery ligation, and infusion of CD8+ T-cells immediately after surgery rescued the phenotype. Our study provides further evidence that the immune system is critical in modulating collateral development in response to peripheral ischemia.

Monocyte Chemoattractant Protein-1 Activates Vascular Endothelial Growth Factor and Tumor Necrosis Factor-Alpha-Mediated Angiogenesis in Ischemic Hindlimbs of Mice

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Recently, we and others have suggested that macrophage accumulation plays a role in angiogenesis in hindlimb ischemia model. Macrophage chemoattractant protein (MCP)-1 is a key molecule to trigger inflammatory changes in various diseases. Thus, we sought to determine the role of the endogenous MCP-1 in ischemia-induced angiogenesis. At day 0, unilateral hindlimb ischemia was induced by excising surgically entire left femoral artery and vein in mice. Immediately after operation, plasmid DNA encoding 7ND, a dominant-negative mutant of MCP-1, or the empty plasmid (as control) was injected into the ipsilateral thigh adductor muscle. Serial laser Doppler blood flow analysis showed an abrupt decrease in blood flow, followed by a remarkable recovery, in ischemic hindlimbs of controls. Control mice showed well-developed collateral vessels and capillary formation as assessed by postmortem angiography and immunohistostaining for CD31, respectively, at day 21 after induction of ischemia. In 7ND-treated mice, although the extent of the early decrease in laser Doppler blood flow was similar to that in controls, the recovery was impaired. At day 3, macrophage infiltration and inductions of vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF)-alpha, known angiogenic factors, were prominent in the adductor muscle of ischemic hindlimbs in controls. 7ND treatment significantly reduced the infiltrates macrophages and repressed VEGF and TNF-alpha inductions in response to ischemia at day 3. Moreover, the number of angiographically visible collateral vessels and the capillary density were significantly decreased in ischemic hindlimbs of 7ND-treated mice at day 21. In conclusion, MCP-1-mediated macrophage accumulation may play an important role in ischemia-induced angiogenesis at least in part by activating angiogenic factors such as VEGF and TNF-alpha in ischemic hindlimbs.

Fiber Type-Specific Angiogenic Dysregulation in a Genetic Mouse Model of Heart Failure

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Introduction. Chronic heart failure (CHF) leads to intrinsic skeletal muscle abnormalities including slow-to-fast oxidative fiber type switching, decreased capillary density, and reduced mitochondrial function. These skeletal muscle abnormalities contribute to clinical exercise intolerance. Methods. A genetic mouse model of heart failure induced through cardiac-targeted overexpression of the sarcoplasmic reticulum Ca** storage protein calsequestrin (Csq) has been recently characterized. Skeletal muscle (plantaris) from Csq/CHF mice and wild type (WT) mice was analyzed with triple color immunofluorescence using antibodies specific for myosin heavy chain I, IIa, lb, and endothelial cells. Results. A decrease in oxidative myofibers (I + IIa), a concurrent increase in glyco-