Modulation of vascular remodeling : a role for the immune system, growth factors, and transcriptional regulation

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Bone marrow derived cells in arteriogenesis: a crucial role for leukocytes

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Abstract

In recent years cell therapeutic approaches for induction of arteriogenesis, including injection of autologous bone marrow, have gained popularity for experimental treatment of patients with coronary or peripheral arterial ischemic disease. However, beneficial results of these cell therapies in patients remain inconsistent to date. Evidence points towards a supportive paracrine role of bone marrow derived cells by secretion of various pro-arteriogenic cytokines and growth factors. Moreover, inflammatory cells, originating from the bone marrow, are recently pointed out as the key regulators of arteriogenesis. This chapter focuses on the role of leukocytes in arteriogenesis. In particular, the role of monocytes, natural killer cells and T-cells, including various subpopulations of T-cells, are addressed. Activation (or inhibition) of selective leukocyte subpopulations may ameliorate pro-arteriogenic cell therapy in the future.

Introduction

In the past ten years, therapeutic arteriogenesis using bone marrow derived cells has become a promising treatment for patients with either coronary or peripheral ischemic disease [1-6]. Although initial reports of autologous bone marrow transplantation for patients with limb ischemia were very promising [7], beneficial results of cell therapy in patients with peripheral arterial disease (PAD) are not very consistent to date [8].

Although the concept of the development of adult collateral arteries from a pre-existing arteriole network is indisputable for therapeutic intervention, the exact cellular mechanism underlying arteriogenesis remains unclear. There are different hypotheses on the role of bone marrow derived cells in arteriogenesis. Nowadays, evidence points towards a more supportive paracrine role of bone marrow derived cells by secretion of various factors instead of the initially proposed role in promoting arteriogenesis by incorporating into the vessel walls [9]. Also the role of different inflammatory cells, originating from the bone marrow, has extensively been studied in order to evolve a more efficient cell therapeutic approach than infusion of the total bone marrow derived cell fraction. Previously, numerous animal studies have established that ischemia induced angiogenesis and shear stress induced arteriogenesis can be enhanced with different types of leukocytes, cytokines and growth factors. The molecular and cellular mechanisms that play a role in collateral artery formation start to become unravelled more recently. Increased shear stress induces arteriogenesis by stimulating the attraction, adhesion and invasion of circulating inflammatory cells [10] such as monocytes, T-lymphocytes and NK-cells. Growth factors that are already present in the ischemic tissue, as well as those that are produced by the invading cells play an important role too. Recently, also subpopulations of T-lymphocytes, such as CD4+ T cells, CD8+ T cells and CD4+CD25+FoxP3+ T cells and their role in arteriogenesis have been studied in more detail. This chapter will focus on the role of leukocytes in collateral artery formation.

Monocytes in arteriogenesis

Monocytes play a crucial role in mediating neovascularization in response to increased shear stress and ischemia. Already in the 1970s monocytes were found to invade the vessel wall of developing coronary collateral arteries [11,12]. Later on, functional proof for the participation of monocytes in arteriogenesis has been reported by the results that after increasing blood monocyte concentration in a hind limb ischemia mouse model, arteriogenesis is enhanced [11-13]. In contrast, after depletion of monocytes, collateral artery formation is much attenuated [12]. Increased shear stress, due to the arterial occlusion, activates the vascular endothelium. The activated endothelial cells upregulate the monocyte chemoattractant MCP-1 and adhesion molecules (eg. ICAM-1 and VCAM-

1) to which the receptor of monocytes binds [14,15]. This leads to monocyte adhesion and infiltration with the subsequent production of growth factors and proteases. In the next paragraph we will discuss these aspects of monocytes in arteriogenesis into more detail. The local production of cytokines and chemokines within the first 3 days after ischemia mediates the recruitment of monocytes to the ischemic area [16-19]. To stimulate arteriogenesis, monocytes must home and retain around the arterial occlusion. There is evidence that MCP-1, but also VEGF and SDF-1α contribute to the recruitment and retention of monocytes [19-23]. For example, local infusion of MCP-1 [24] or MCP-1 gene transfer [25] in a hind limb ischemia rabbit model increase the local number of monocytes in the ischemic area and enhance revascularization. Voskuil et al. [26] reported impaired monocyte recruitment after hind limb ischemia in MCP-1-/- mice. This effect was reversed with treatment of the MCP-1 protein. Furthermore, Heil et al. [27] showed that disruption of the MCP-1 receptor (CCR2) in CCR2-/- mice leads to an impaired blood flow restoration after femoral artery ligation. This impairment was based on a reduced migration capacity of these monocytes.

The interaction of the monocyte with the activated endothelium is a complex multistep process. Auffray et al. [28] reported recently the importance of the role of monocytes in the initial recruitment of blood monocytes. They observed a subset of monocytes that could crawl on endothelial cells. These resident monocytes patrol healthy blood vessels and allow rapid tissue invasion by monocytes in case of ischemic injury or tissue damage [28]. Two integrins are responsible for the interaction of monocytes with endothelial cells: Mac-1 and LFA. These intergrins interact with adhesion molecules on the endothelial surface, like selectins, intercellular adhesion molecules (ICAM-1 and ICAM-2) and vascular adhesion molecules (VCAM-1) [14]. It was demonstrated that factors like MCP-1 and VEGF, released by the activated endothelial cells, can increase the expression of integrins on monocytes and enhance monocyte adhesion [29-31]. After monocyte adhesion, integrins also mediate transmigration of monocytes into the perivascular tissue. Hoefer et al. showed that *in vivo* treatment with antibodies against ICAM-1 blocked collateral artery growth [32].

For invading into deeper vessel wall layers, monocytes can use their capacity to produce proteases such as matrix-metalloproteinases and u-PA. Their proteolytic activity can create gaps by which monocytes can migrate further into the vessel wall [33-35]. After transmigration into the perivascular tissue, monocytes differentiate into macrophages and secrete growth factors and cytokines that attract other inflammatory cells and stimulate smooth muscle cell proliferation and endothelial cell mitosis, both necessary for collateral growth [36]. One of the pro-inflammatory factors secreted by monocytes and macrophages is TNF-α. In a rabbit hind limb ischemia model, it is demonstrated that arteriogenesis is attenuated after treatment with TNF-α inhibitors, most likely due

to the inhibition of leukocyte infiltration around collateral arteries and lower vascular smooth muscle cell proliferation [37]. Also fibroblast growth factors (FGF), provided by monocytes in a paracrine way, do have a stimulating effect on arteriogenesis [13].

The potential of transplantation of monocytes to stimulate collateral artery formation in the clinical situation has now been studied by using the hind limb ischemia animal model. Herold et al. [15] showed that although autologous monocytes only demonstrated a marginal increase in arteriogenesis, their transduction with GM-CSF before transfusion into the hind limb ischemia model resulted in a robust stimulation of arteriogenesis. Also Urbich et al. [38] provided evidence that transplantation of engineered monocytes represents a highly effective therapeutic approach to stimulate collateral artery formation. In contrast, monocytes injected directly after isolation, without any stimulation, did not show any stimulating effect on arteriogenesis. Isolation of monocytes can easily be done in humans by leukapheresis and these cells have been safely administered for patients with malignancies. The strategy of transplantation of pro-arteriogenic monocytes seems feasible, but further research towards the side effects (e.g. pro-atherogenic effects) is warranted.

T cells in arteriogenesis

More recently, it was found that other leukocytes than monocytes also contribute to collateral artery formation. T lymphocytes also play a role in many vascular diseases ranging from a pro-atherogenic role in atherosclerosis towards a beneficial role in arteriogenesis. Couffinhal [39] showed that nude mice, which are deficient for all types of mature T lymphocytes, did have a hampered capacity to form collateral arteries after induction of hind limb ischemia. The role of T lymphocytes in arteriogenesis has recently been studied more extensively, with focus on arteriogenic function of specific subpopulations of T lymphocytes. Although the exact role is not completely understood yet, we will discuss the contribution of CD4+ T cells (Helper T cells), CD8+ T cells (Cytotoxic T cells), CD3+CD31+CXCR4+ T cells (angiogenic T cells) and CD4+CD25+FoxP3+ T cells (regulatory T cells). See Figure **1** for a schematic representation.

The importance of CD4+ T cells in collateral artery formation has been demonstrated recently. CD4-/- mice, which do have a normal development of CD8+ T cells and myeloid cells showed a markedly reduction in ischemia-induced collateral artery formation. Compared to controls, there was a 25% decrease in blood flow restoration after femoral artery ligation during 28 days in these CD4-/- mice [40]. In a rescue experiment, CD4-/- mice received spleen-derived purified CD4+ T cells and restored blood flow recovery after ischemia to the levels of wild type mice [40]. Additionally, CD4 depleted mice, show significantly impaired blood flow restoration after femoral artery ligation [41]. Furthermore, MHC class II deficient mice, which are characterized by the selective lack of the maturation of CD4+ T cells, display impaired arteriogenesis too [41].
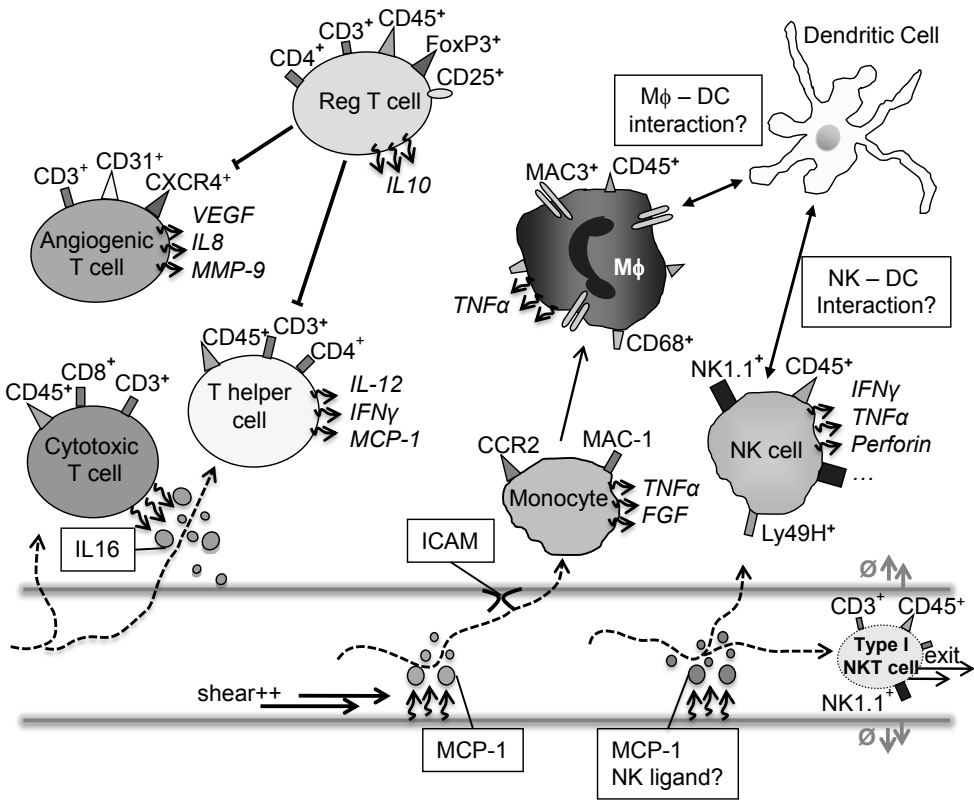
Figure 1. Involved leukocyte subsets in outward remodeling of collateral arteries. Cytotoxic CD8+ T cells invade the vessel wall early and secrete IL-16, which in turn attracts CD4+ T helper lymphocytes. These CD4+ T helper lymphocytes secrete a variety of pro-arteriogenic cytokines IL-12, IFNγ and the chemoattractant MCP-1. The latter induces of perivascular monocyte recruitment. Monocytes adhere to activated endothelium, which expresses increased levels of ICAM-1 and VCAM-1 molecules. Next, monocytes invade in the vessel wall, excrete growth factors such as Fibroblast Growth Factor (FGF) and cytokines such as TNFα. Furthermore, monocytes can differentiate towards macrophages. NK cells are also attracted by MCP-1, and probably via NK ligands that are produced upon endothelial cell stress. In the vessel wall NK cells are supposed to act as cytokine factories by secreting an array of pro-arteriogenic cytokines; IFNγ, TNFα and the cytolytic perforin. Furthermore, they might have relevant interactions with dendritic cells. Recent experiments did not demonstrate involvement of type I NK-T cells in arteriogenesis. Regulating T lymphocytes inhibit the pro-arteriogenic action of CD4+ T helper lymphocytes and of the angiogenic CD3+, CD31+, CXCR4+ T lymphocytes. The latter are attracted by hypoxia derived factors such as SDF-1 and have the ability to secrete VEGF, IL8 and MMP-9. The subtle balance of this inflammatory cascade should optimally lead to enlargement of the collateral diameter. *See color figure on page 213.*

The most likely mechanisms contributing to the arteriogenic-enhancing effects of CD4+ T cells appears to be that CD4+ T cells play an important role in the classic immune response in the ischemic area. In wildtype C57BL/6 mice, CD4+ T cells accumulate around collateral arteries within 3 to 7 days after ligation of the femoral artery [41]. It is believed that perivascular presence of T cells is necessary to destroy cells to create space for collateral growth [42]. Also exogenous CD4+ T cells home to the inflammatory cell infiltration in the ischemic hind limb within 24 hours after transfusion. In contrast, CD4 negative cells do not have selective homing to the ischemic hind limb [40]. CD4+ T cells secrete various cytokines for example to induce monocytes/macrophage accumulation in the ischemic muscle [43]. These monocytes and macrophages secrete large arrays of cytokines and growth factors which further facilitate arteriogenesis [43]. Recently, van Beem et al. [44] described the role of CD4+ T cells in the stimulation of CD14+ monocytes to differentiate into a pro-angiogenic cell type, namely endothelial progenitor cell colonies (CFU-ECs). Paracrine factors produced by activated CD4+ T cells present in the ischemic tissue facilitate CFU-EC formation. The exact cocktail of soluble factors stimulating CD14+ monocytes and the role of these CD4+ T cell - stimulated monocytes need to be further investigated.

The role of CD8+ T cells in arteriogenesis is demonstrated by an impaired blood flow restoration in CD8-/- mice after hind limb ischemia. An attenuated blood flow persisted from day 3 till day 28 after femoral artery ligation compared to controls. After transfusion of exogenous CD8+ T cells into CD8-/- mice, the impaired blood flow recovery after ligation was rescued [45]. CD8+ T cells are one of the first cells involved in collateral artery formation [45]. When CD8+ T cells infiltrate into the ischemic muscle, they express IL-16, which is an important chemo-attractant for several immune cells such as monocytes, eosinophils and dendritic cells [46-48]. Similarly, IL-16 participate in the recruitment of CD4+ T cells since it has been shown that IL-16 is a natural ligand for CD4 [48].

More recently, the role of a specific subset of CD4+ T cells, the regulatory T cells, has been studied. CD4+CD25+FoxP3+ regulatory T cells constitute 10% of CD4+ T cells in the peripheral blood. This T cell population is specialized to suppress immune responses and contributes to the maintenance of immunological self-tolerance and immune homeostasis. In addition, these regulatory T cells do have an important function in the T cell homeostasis and suppress T cell responses against self-antigens or foreign antigens. Interestingly, it has been shown that regulatory T cells participate in the control of the development of atherosclerotic lesions. The role of regulatory T cells in ischemia-induced collateral artery formation has been recently studied by Zouggari et al. [49]. They modulated regulatory T cell levels by intervening in the B7/CD28 interaction, which is required for regulatory T cell generation and homeostasis. In this regard, hind limb ischemia was induced in CD28-/- and B7-1/2-/- mice, which are deficient for

regulatory T cells. In these mice, a 1.2 to 2.0 fold increase in neovascularization after hind limb ischemia induction was found. In line with this, collateral artery formation was also increased in anti-CD25-treated mice compared to controls [49]. Taken together, regulatory T cell reduction with B7- or CD28 deficiency or anti-CD25 treatment, increases post-ischemic neovascularization. So, regulatory T cells play an important role in arteriogenesis, most probably by controlling the effector immune cell response [49]. Modulation of the regulatory component of post-ischemic inflammatory cell response, could provide a novel level of therapeutic intervention for pro-arteriogenic strategies for the treatment of PAD.

Another subpopulation of T cells, CD3+CD31+CXCR4+ T cells, has been suggested as a potential target for ischemic cardiovascular diseases. This cell is also referred as angiogenic T cell in literature. Hur et al. [50] report that the central cluster of endothelial progenitor cells (EPCs), which play an important role in neovascularization [51,52], is composed of CD3+CD31+CXCR4+ T cells. These angiogenic T cells are required for colony formation and early EPC differentiation as was investigated by depletion and adding these T cells during EPC-culture.

Furthermore, CD3+CD31+CXCR4+ T cells secrete various pro-angiogenic cytokines such as VEGF, IL-8, IL-17 and granulocyte colony-stimulating factor. Moreover, these cells also secrete MMP-9 which is known to play an important role in angiogenesis related extracellular matrix degradation. *In vitro* experiments as tube formation assays, as well as *in vivo* experiments further illustrate the pro-angiogenic capacity of CD3+CD31+CXCR4+ T cells. Infusion of CD3+CD31+CXCR4+ T cells in a hind limb ischemia mouse model improved blood flow recovery significantly. Clinical studies showed that the level of angiogenic T cells in peripheral blood MNCs is decreased when risk scores for cardiovascular disease are increased. This stresses the clinical relevance of this subpopulation T cells and further research towards this cell as target for cell therapy or as biomarker of cardiovascular disease is necessary [50].

Natural killer cells in arteriogenesis

Natural Killer (NK) cells were recently proven to contribute to arteriogenesis as well [41]. As explained previously, collateral growth is initiated by shear stress induced release of chemo-attractants, such as MCP-1. Not only is MCP-1 a potent chemo-attractant for monocytes [24,53] but also for NK cells [54].

The role of NK cells in processes of vascular remodeling, e.g. atherosclerosis [55] but also remodeling of spiral arteries in the placenta [56] was already described. Mice deficient for functional NK cells have reduced atherosclerotic lesions and mice deficient for uterine NK (uNK) fail to modulate spiral arteries feeding the placenta.

The role for NK cells in arteriogenesis was established when these cells were found to accumulate around collateral arteries and by the observation of impaired arteriogenesis following femoral artery ligation in mice depleted or deficient for NK cells [41]. Unlike a well established role for NK-T cells in atherosclerosis [57], this could not be proven for type 1 NK-T cells in arteriogenesis. Especially since J-alpha281-knockout mice, that selectively lack V-alpha14 NK-T cells, did not show impaired arteriogenesis after femoral artery ligation [41]. However, the role of type 2 NKT cells is unclear.

NK cells are cytolytic effector cells of the innate immune system primarily involved in the defense against infectious pathogens, especially viruses. Their state of action is determined by dual signaling via inhibitory and activating surface receptors [58]. Many of these receptors, e.g. NKG2D, NKrp1c (NK1.1), CD94/NKG2, and the highly polymorphic Ly49 receptor family, code for in the Natural Killer gene complex (NKC) [58,59]. Ly49 and CD94/NKG2 receptors are expressed in a stochastic and independent fashion, which results in NK cell subsets expressing distinct combinations of these receptors. NK cell function is, in part, based on the interaction of their inhibitory receptors with Major Histocompatibility Complex class I (MHC I) molecules, expressed on the target cell surface in a complex with β2-microglobulin (β2m) [60]. Often, activating NK cell receptors recognize inducible molecules on the target cell, which can be induced upon cellular stress such as hypoxia or infection [61].

Upon activation NK cells produce a variety of cytokines, e.g. perforin, TNF-α, TNF-β, IL10 and most dominantly IFN-γ and sometimes even vascular growth factors [62]. By their IFN-γ production, NK cells are an early source important in T-helper 1(Th1) polarization [63] which is associated with profound vascular remodeling, unlike Th2 that leads to less profound vascular remodeling [64].

The Th1 response leads to rapid arteriogenesis in C57BL/6 mice, whereas BALB/c mice respond poorly due to a Th2 response. Introduction of C57BL/6 genes into BALB/c mice by the creation of a C57BL/6 x BALB/c F1 led to significant improved arteriogenesis when compared to the parent BALB/c strain [41]. This indicates that BALB/c mice are lacking a crucial factor for proper arteriogenesis.

The genetic differences between C57BL/6 and BALB/c mice also include a relevant difference in the content of the above-mentioned Natural Killer gene complex (NKC), as BALB/c mice lack a 200 kb region. This region codes for members of the Ly49 receptor family of which the C57BL/6 NKC region possesses 12 Ly49 genes, whereas the BALB/c strain only has 7 Ly49 genes. Importantly, BALB/c mice lack the NK receptor Ly49H [65] which is crucially involved in murine cytomegalovirus (mCMV) resistance, and is therefore specifically targeted against the viral mCMV glycoprotein m157 [66]. There are strong indications that the key NK cell receptor for arteriogenesis lies within this gene locus, which is normally lacking in BALB/c.

Current research now focuses on the identification of this key NK cell receptor in arteriogenesis. This will also give insight in possible therapeutic ligands to specifically stimulate NK cells for therapeutic arteriogenesis. Next, more insight in NK cell dynamics during arteriogenesis is required, including insight in local effector function. The local effector function could include perivascular cytolysis to create space for outward remodeling, but also cytokine production for modulating the local inflammatory response.

Summary

The role of leukocytes in arteriogenesis is evident, but highly complex. To design new arteriogenic treatment strategies using leukocytes, it is necessary to refine our knowledge on which subsets of leukocytes are involved in collateral formation and how. Ex-vivo activation (or inhibition) of specific inflammatory cell subpopulations may prove beneficial for stimulation of arteriogenesis by cell therapy in the future. A major challenge for the development of these therapies remains that pro-arteriogenic leukocytes and growth factors may contribute to adverse effects such as plaque progression and neointima formation [67,68].

References

1. Isner JM, Pieczek A, Schainfeld R, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet* 1996 Aug 10;348(9024):370-4.

2. Baumgartner I, Pieczek A, Manor O, et al. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation* 1998 Mar 31;97(12):1114-23.

3. Isner JM, Baumgartner I, Rauh G, et al. Treatment of thromboangiitis obliterans (Buerger's disease) by intramuscular gene transfer of vascular endothelial growth factor: preliminary clinical results. *J Vasc Surg* 1998 Dec;28(6):964-73.

4. van Weel V, van Tongeren RB, van Hinsbergh VW, van Bockel JH, Quax PH. Vascular growth in ischemic limbs: a review of mechanisms and possible therapeutic stimulation. *Ann Vasc Surg* 2008 Jul;22(4):582-97.

5. van Oostrom MC, van OO, Quax PH, Verhaar MC, Hoefer IE. Insights into mechanisms behind arteriogenesis: what does the future hold? *J Leukoc Biol* 2008 Dec;84(6):1379-91.

6. Simons M, Ware JA. Therapeutic angiogenesis in cardiovascular disease. *Nat Rev Drug Discov* 2003 Nov;2(11):863-71.

7. Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002 Aug 10;360(9331):427-35.

8. Gupta R, Tongers J, Losordo DW. Human studies of angiogenic gene therapy. *Circ Res* 2009 Oct 9;105(8):724-36.

9. Ziegelhoeffer T, Fernandez B, Kostin S, et al. Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res* 2004 Feb 6;94(2):230-8.

10. Heil M, Schaper W. Pathophysiology of collateral development. *Coron Artery Dis* 2004 Nov;15(7):373-8.

11. Schaper J, Konig R, Franz D, Schaper W. The endothelial surface of growing coronary collateral arteries. Intimal margination and diapedesis of monocytes. A combined SEM and TEM study. *Virchows Arch A Pathol Anat Histol* 1976 Jun 22;370(3):193-205.

12. Schaper W. Pathophysiology of coronary circulation. *Prog Cardiovasc Dis* 1971 Nov;14(3):275-96.

13. Deindl E, Hoefer IE, Fernandez B, et al. Involvement of the fibroblast growth factor system in adaptive and chemokine-induced arteriogenesis. *Circ Res* 2003 Mar 21;92(5):561-8.

14. Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res* 2004 Sep 3;95(5):449-58.

15. Herold J, Pipp F, Fernandez B, et al. Transplantation of monocytes: a novel strategy for in vivo augmentation of collateral vessel growth. *Hum Gene Ther* 2004 Jan;15(1):1-12.

16. Paoni NF, Peale F, Wang F, et al. Time course of skeletal muscle repair and gene expression following acute hind limb ischemia in mice. *Physiol Genomics* 2002 Dec 3;11(3):263-72.

17. Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. *J Leukoc Biol* 1994 Mar;55(3):410-22.

18. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002 Nov;23(11):549-55.

19.	Capoccia BJ, Gregory AD, Link DC. Recruitment of the inflammatory subset of monocytes to sites of ischemia induces angiogenesis in a monocyte chemoattractant protein-1-dependent fashion. *J Leukoc Biol* 2008 Sep;84(3):760-8.

20.	Lu B, Rutledge BJ, Gu L, et al. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J Exp Med* 1998 Feb 16;187(4):601-8.

21.	Cursiefen C, Chen L, Borges LP, et al. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest* 2004 Apr;113(7):1040-50.

22.	Zittermann SI, Issekutz AC. Endothelial growth factors VEGF and bFGF differentially enhance monocyte and neutrophil recruitment to inflammation. *J Leukoc Biol* 2006 Aug;80(2):247-57.

23.	De Falco E, Porcelli D, Torella AR, et al. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. *Blood* 2004 Dec 1;104(12):3472-82.

24.	Ito WD, Arras M, Winkler B, Scholz D, Schaper J, Schaper W. Monocyte chemotactic protein-1 increases collateral and peripheral conductance after femoral artery occlusion. *Circ Res* 1997 Jun;80(6):829-37.

25.	Muhs A, Lenter MC, Seidler RW, Zweigerdt R, Kirchengast M, Weser R, et al. Nonviral monocyte chemoattractant protein-1 gene transfer improves arteriogenesis after femoral artery occlusion. *Gene Ther* 2004 Dec;11(23):1685-93.

26.	Voskuil M, Hoefer IE, van Royen N, et al. Abnormal monocyte recruitment and collateral artery formation in monocyte chemoattractant protein-1 deficient mice. *Vasc Med* 2004 Nov;9(4):287-92.

27.	Heil M, Ziegelhoeffer T, Wagner S, et al. Collateral artery growth (arteriogenesis) after experimental arterial occlusion is impaired in mice lacking CC-chemokine receptor-2. *Circ Res* 2004 Mar 19;94(5):671-7.

28.	Auffray C, Fogg D, Garfa M, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 2007 Aug 3;317(5838):666-70.

29.	Heil M, Clauss M, Suzuki K, et al. Vascular endothelial growth factor (VEGF) stimulates monocyte migration through endothelial monolayers via increased integrin expression. *Eur J Cell Biol* 2000 Nov;79(11):850-7.

30.	Takagi J, Springer TA. Integrin activation and structural rearrangement. *Immunol Rev* 2002 Aug;186:141-63.

31.	Hogg N, Henderson R, Leitinger B, McDowall A, Porter J, Stanley P. Mechanisms contributing to the activity of integrins on leukocytes. *Immunol Rev* 2002 Aug;186:164-71.

32.	Hoefer IE, van RN, Rectenwald JE, et al. Arteriogenesis proceeds via ICAM-1/Mac-1-mediated mechanisms. *Circ Res* 2004 May 14;94(9):1179-85.

33.	Kusch A, Tkachuk S, Haller H, et al. Urokinase stimulates human vascular smooth muscle cell migration via a phosphatidylinositol 3-kinase-Tyk2 interaction. *J Biol Chem* 2000 Dec 15;275(50):39466-73.

34.	Menshikov M, Elizarova E, Plakida K, et al. Urokinase upregulates matrix metalloproteinase-9 expression in THP-1 monocytes via gene transcription and protein synthesis. *Biochem J* 2002 Nov 1;367(Pt 3):833-9.

35.   Khan KM, Falcone DJ. Role of laminin in matrix induction of macrophage urokinase-type plasminogen activator and 92-kDa metalloproteinase expression. *J Biol Chem* 1997 Mar 28;272(13):8270-5.

36.   Schirmer SH, van Nooijen FC, Piek JJ, van Royen N. Stimulation of collateral artery growth: travelling further down the road to clinical application. *Heart* 2009 Mar;95(3):191-7.

37.   Grundmann S, Hoefer I, Ulusans S, et al. Anti-tumor necrosis factor-{alpha} therapies attenuate adaptive arteriogenesis in the rabbit. *Am J Physiol Heart Circ Physiol* 2005 Oct;289(4):H1497-H1505.

38.   Urbich C, Heeschen C, Aicher A, Dernbach E, Zeiher AM, Dimmeler S. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation* 2003 Nov 18;108(20):2511-6.

39.   Couffinhal T, Silver M, Kearney M, et al. Impaired collateral vessel development associated with reduced expression of vascular endothelial growth factor in ApoE-/- mice. *Circulation* 1999 Jun 22;99(24):3188-98.

40.   Stabile E, Burnett MS, Watkins C, et al. Impaired arteriogenic response to acute hindlimb ischemia in CD4-knockout mice. *Circulation* 2003 Jul 15;108(2):205-10.

41.   van Weel V, Toes RE, Seghers L, et al. Natural killer cells and CD4+ T-cells modulate collateral artery development. *Arterioscler Thromb Vasc Biol* 2007 Nov;27(11):2310-8.

42.   Schaper W, Scholz D. Factors regulating arteriogenesis. *Arterioscler Thromb Vasc Biol* 2003 Jul 1;23(7):1143-51.

43.   Delves PJ, Roitt IM. The immune system. First of two parts. *N Engl J Med* 2000 Jul 6;343(1):37-49.

44.   van Beem RT, Noort WA, Voermans C, et al. The presence of activated CD4(+) T cells is essential for the formation of colony-forming unit-endothelial cells by CD14(+) cells. *J Immunol* 2008 Apr 1;180(7):5141-8.

45.   Stabile E, Kinnaird T, la Sala A, et al. CD8+ T lymphocytes regulate the arteriogenic response to ischemia by infiltrating the site of collateral vessel development and recruiting CD4+ mononuclear cells through the expression of interleukin-16. *Circulation* 2006 Jan 3;113(1):118-24.

46.   Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. *J Leukoc Biol* 2000 Jun;67(6):757-66.

47.   Wilson KC, Center DM, Cruikshank WW. The effect of interleukin-16 and its precursor on T lymphocyte activation and growth. *Growth Factors* 2004 Jun;22(2):97-104.

48.   Girgis L, Davis MM, Fazekas de St Groth B. The avidity spectrum of T cell receptor interactions accounts for T cell anergy in a double transgenic model. *J Exp Med* 1999 Jan 18;189(2):265-78.

49.   Zouggari Y, Ait-Oufella H, Waeckel L, et al. Regulatory T cells modulate postischemic neovascularization. *Circulation* 2009 Oct 6;120(14):1415-25.

50.   Hur J, Yang HM, Yoon CH, et al. Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. *Circulation* 2007 Oct 9;116(15):1671-82.

51.   Kalka C, Masuda H, Takahashi T, et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 2000 Mar 28;97(7):3422-7.

52. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 2002 Dec 10;106(24):3009-17.

53. Hoefer IE, Grundmann S, van Royen N, et al. Leukocyte subpopulations and arteriogenesis: specific role of monocytes, lymphocytes and granulocytes. *Atherosclerosis* 2005 Aug;181(2):285-93.

54. Allavena P, Bianchi G, Zhou D, et al. Induction of natural killer cell migration by monocyte chemotactic protein-1, -2 and -3. *Eur J Immunol* 1994 Dec;24(12):3233-6.

55. Whitman SC, Rateri DL, Szilvassy SJ, Yokoyama W, Daugherty A. Depletion of natural killer cell function decreases atherosclerosis in low-density lipoprotein receptor null mice. *Arterioscler Thromb Vasc Biol* 2004 Jun;24(6):1049-54.

56. Croy BA, Esadeg S, Chantakru S, et al. Update on pathways regulating the activation of uterine Natural Killer cells, their interactions with decidual spiral arteries and homing of their precursors to the uterus. *J Reprod Immunol* 2003 Aug;59(2):175-91.

57. van Puijvelde GH, van Wanrooij EJ, Hauer AD, de Vos P, van Berkel TJ, Kuiper J. Effect of natural killer T cell activation on the initiation of atherosclerosis. *Thromb Haemost* 2009 Aug;102(2):223-30.

58. Ryan JC, Naper C, Hayashi S, Daws MR. Physiologic functions of activating natural killer (NK) complex-encoded receptors on NK cells. *Immunol Rev* 2001 Jun;181:126-37.:126-37.

59. Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol* 2003 Apr;3(4):304-16.

60. Pamer E, Cresswell P. Mechanisms of MHC class I--restricted antigen processing. *Annu Rev Immunol* 1998;16:323-58.:323-58.

61. Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci U S A* 1996 Oct 29;93(22):12445-50.

62. Trinchieri G. Biology of natural killer cells. *Adv Immunol* 1989;47:187-376.:187-376.

63. Martin-Fontecha A, Thomsen LL, Brett S, et al. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat Immunol* 2004 Dec;5(12):1260-5.

64. Schulte S, Sukhova GK, Libby P. Genetically programmed biases in Th1 and Th2 immune responses modulate atherogenesis. *Am J Pathol* 2008 Jun;172(6):1500-8.

65. Anderson SK, Dewar K, Goulet ML, Leveque G, Makrigiannis AP. Complete elucidation of a minimal class I MHC natural killer cell receptor haplotype. *Genes Immun* 2005 Sep;6(6):481-92.

66. Smith HR, Heusel JW, Mehta IK, et al. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci U S A* 2002 Jun 25;99(13):8826-31.

67. van Royen N., Hoefer I, Bottinger M, et al. Local monocyte chemoattractant protein-1 therapy increases collateral artery formation in apolipoprotein E-deficient mice but induces systemic monocytic CD11b expression, neointimal formation, and plaque progression. *Circ Res* 2003 Feb 7;92(2):218-25.

68. Epstein SE, Stabile E, Kinnaird T, Lee CW, Clavijo L, Burnett MS. Janus phenomenon: the interrelated tradeoffs inherent in therapies designed to enhance collateral formation and those designed to inhibit atherogenesis. *Circulation* 2004 Jun 15;109(23):2826-31