Peripheral artery disease is caused by obstructing atherosclerotic plaques that critically reduce blood flow during exercise. The disease affects ≈4% of people >40 years and 15% to 20% of subjects above 65 years of age. Critical limb ischemia, the most severe manifestation of peripheral artery disease, describes patients with chronic ischemic rest pain, or patients with ischemic skin lesions, either ulcers or gangrene. It requires foot amputation in 25% of cases within 1 year from the diagnosis. Revascularization therapies are indicated in critical limb ischemia patients, but they are often ineffective or unfeasible; and in the latter case, the reported amputation and mortality rates exceed 50%. Therefore, new therapeutic approaches are urgently needed.

The new study from Schirmer et al⁴ shows that voluntary training confers mice with an improved capacity to recover from operatively induced limb ischemia when compared with sedentary controls. The positive outcome is associated with homing of inducible nitric oxide synthase (iNOS)-expressing mononuclear cells. The importance of this cellular mechanism is highlighted by ablative studies in which iNOS was genetically deleted or macrophages and monocytes were pharmacologically depleted. Likewise, bone marrow reconstitution of irradiated mice with iNOS knockout cells inhibits collateral formation and blood flow recovery after ischemia. The authors conclude that iNOS-expressing mononuclear cells might be therapeutically useful for therapeutic collateralization. This could be achieved by stimulation of mononuclear cells release and homing or direct cell transplantation into the ischemic limb.

The important role of monocytes in collateral artery formation has been previously established.¹ Furthermore, exercise training has been shown to improve regional perfusion in ischemic syndromes by facilitating the release of progenitor cells.²,³ Interestingly, symptomatic tissue ischemia seems to be a prerequisite for induction of proangiogenic cell-mediated reparative mechanisms.⁷ A novel concept of Schirmer’s study consists of the documentation that iNOS plays a key role in postischemic collateralization. The generation of NO from oxidation of l-arginine (to give citrulline and NO) is catalyzed by 3 distinct members of the NOS family. They are either constitutively expressed in neurons and endothelial cells or induced—as in case of iNOS—by endotoxin or proinflammatory cytokines, such as interleukin-1, tumor necrosis factor-α, and interferon-γ mainly in macrophages. The role of iNOS in cancer angiogenesis is well acknowledged, whereas participation of iNOS in cellular mechanisms of postischemic arteriogenesis represents a novel concept meritiously introduced by Schirmer et al. However, it remains to be elucidated whether physical training induces the expression of iNOS in monocytes (and if so by which mechanism) or rather promotes the homing of a specific population of iNOS-expressing monocytes.

With regard to transcriptional regulation, the sequences of cloned iNOS promoters of all species investigated to date exhibit homologies to binding sites for transcription factors known to be involved in cytokine-mediated induction of transcription. In addition, iNOS promoter coactivators have been recently reported,⁸ showing the binding of p300 to the iNOS promoter region and demonstrating that p300 overexpression increases interferon-γ-induced iNOS promoter activity. It is therefore possible, yet to be demonstrated, that exercise training exerts a preconditioning action on iNOS transcription through a cytokine-mediated mechanism amplified by ischemia. It would be relevant to investigate if a similar inductive mechanism occurs in proangiogenic progenitor cells previously associated with exercise training⁶ or instead limited to a specific subpopulation of monocytes/macrophages (Figure). In this respect, we have demonstrated that iNOS expression is induced in cultured circulating proangiogenic progenitor cells by forced expression of human tissue kallikrein (KLK1), a serine protease of the kallikrein–kinin system.⁹ KLK1 is crucial for cell migration/invasion and is highly abundant not only on proangiogenic CD34 cells but also on CD16 nonclassical monocytes.¹⁰ Inhibition of iNOS or scavenging of NO
blocks the invasive effect of KLK transduction, supporting the idea that potentiating iNOS can result in better homing of proangiogenic cells. This effect may be dependent on the concomitant presence of the kinin receptor B2R, highly abundant in CD34 progenitor cells,\textsuperscript{11} and CD16 monocytes.\textsuperscript{12}

Exercise programs improve pain free and maximum walking distance in peripheral artery disease patients, but may be impracticable in patients with critical limb ischemia. The direct use of iNOS-expressing monocytes may be considered as a surrogate of physical training in a global therapeutic strategy to alleviate ischemic symptoms. However, caution is necessary when translating promising preclinical studies to the clinical field. For instance, diabetic patients represent a special high cardiovascular risk group in which strategies to ameliorate collateralization of ischemic limbs is particularly desirable. Unfortunately, proangiogenic cells are impaired both at the bone marrow and peripheral blood level with an imbalance in the monocytes subtype proportion toward the M1 proinflammatory counterpart.\textsuperscript{13,14} Strategies increasing M1 monocytes abundance at the ischemic site could be detrimental in the evolution of peripheral artery disease.

In conclusion, iNOS-expressing monocytes seem to play an important role in the collateralization of ischemic limbs of mice. Further studies are necessary to determine their pathogenic role and therapeutic activity in chronic atherosclerotic vascular disease.

After a femoral artery ligation, mononuclear cells and progenitor cells are mobilized from the bone marrow and released into the peripheral blood, to be homed into the site of injury. In mice, a regular exercise would favor the increase of a particular class of bone marrow–derived iNOS-overexpressing monocytes, which finally engraft into the ischemic muscle and accumulate around collateral arteries at 7 days post ligation. iNOS-monocytes release NO that, together with the exercise-induced enhancement of shear stress, promotes the growth of collateral arteries (observed as increased number and size of collateral arteries), thus improving the perfusion of the ischemic limb.

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Disclosures

None.

References


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