Peripheral artery disease is caused by obstructing athero-
sclerotic 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
mortality rates exceed 50%. Therefore, new therapeutic
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Promotion of arteriogenesis, which refers to the enlarge-
ment and functionalization of preformed collateral arteri-
oles, represents a promising therapeutic approach in critical
limb ischemia patients. Several clinical studies have used
the administration of growth factors (mostly basic fibroblast
growth factor or vascular endothelial growth factor, either
as protein or gene therapy) or stem and progenitor cells. In
addition, exercise rehabilitation programs have been shown
to improve symptoms of claudication. Mechanistic under-
standing of how physical exercise increases collateral artery
formation is inadequate.

The new study from Schirmer et al. 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 geneti-
cally deleted or macrophages and monocytes were pharmaco-
logically 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 for-
formation has been previously established. Furthermore, exer-
cise training has been shown to improve regional perfusion in
ischemic syndromes by facilitating the release of progenitor
cells. Interestingly, symptomatic tissue ischemia seems to
be a prerequisite for induction of proangiogenic cell-mediated
reparative mechanisms. 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 NO family. They are either
constitutively expressed in neurons and endothelial cells or
induced—as in case of iNOS—by endotoxin or proinflamma-
tory 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 par-
ticipation of iNOS in cellular mechanisms of postischemic
arteriogenesis represents a novel concept meritoriously intro-
duced 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 pro-
motes 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 tran-
scription. In addition, iNOS promoter coactivators have been
recently reported, showing the binding of p300 to the iNOS
promoter region and demonstrating that p300 overexpres-
sion 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 isch-
emia. It would be relevant to investigate if a similar inductive
mechanism occurs in proangiogenic progenitor cells previ-
ously associated with exercise training 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. KLK1 is
crucial for cell migration/invasion and is highly abundant not
only on proangiogenic CD34 cells but also on CD16 nonclas-
csical monocytes. Inhibition of iNOS or scavenging of NO

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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,11 and CD16 monocytes.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.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.

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Training Monocytes by Physical Exercise: Good Practice for Improving Collateral Artery Development and Postischemic Outcomes

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