A substantial proportion of cardiovascular mortality is caused by atherosclerosis of large and midsized arteries that supply the brain. Stroke is the main complication of cerebral atherosclerosis and ranks second as cause of death worldwide. Recruitment of some myeloid cell subpopulations to atherosclerotic lesions can drive plaque inflammation and predispose to stroke. After a stroke, myeloid cells may also be harmful by directly aggravating the reperfusion injury to the brain. The latter possibility is of significant clinical relevance because epidemiological data have suggested that increased numbers of circulating leukocytes, particularly monocytes and neutrophils, after stroke correlate with poor outcomes. However, the functional mechanistic links between monocyto-sis and neutrophilia and stroke were unknown.

In the current issue of Circulation Research, Courties, Nahrendorf et al addressed this intriguing question and elucidate how stroke speeds up the turnover of hematopoietic stem cells (HSCs) to specifically generate myeloid cell populations and release them into the blood circulation. In their study, the authors have chosen an established mouse stroke model, in which the middle cerebral artery was transiently occluded. After this procedure, the numbers of monocytes and neutrophils resident in the bone marrow increased within 3 days, an effect caused by enhanced proliferation of myeloid progenitors, such as granulocyte-macrophage progenitors and monocyte/dendritic cell progenitors. Interestingly, this effect seems to be specific for myeloid cells because neither noninflammatory Ly6C<sup>+</sup> monocytes, nor B cells or lymphoid progenitors showed increased turnover. In fact, the numbers of nonmyeloid cells, as well as expression of their lineage-characterizing factors, such as IL-7 receptor, decreased, whereas the myeloid transcription factor PU-1 increased, suggesting a specific effect on the myeloid lineage. In seeking the most upstream cause of this myeloid bias—a state defined by an increased myeloid-to-lymphoid cell ratio in the bone marrow—the authors found that HSC proliferation and cell cycle turnover was significantly promoted after stroke. So what activated these HSCs to cycle? The authors hypothesized that the tone of the autonomous nervous system could provide this link. Indeed, sympathetic nerves branching out into the bone marrow can directly activate the bone marrow niche, a part of the bone marrow microenvironment comprising all nonhematopoietic cells involved in HSC maintenance, quiescence, proliferation, and mobilization. Sympathetic nerve terminals secrete noradrenaline (also known as norepinephrine), which binds and activates adrenergic receptors expressed on cells of the bone marrow niche. In the current study, the authors show that mice deficient for the β3-adrenergic receptor do not show enhanced HSC cycling after transient middle cerebral artery occlusion, concluding that sympathetic nerve signaling contributes to the HSC-activating effect of acute cerebral ischemia (Figure).

The current study completes a compelling series of studies by this same group demonstrating the disease-specific consequences of adrenergic signaling on HSC homeostasis—a fundamental principle discovered by Frenette et al. This landmark study demonstrated that the chemokine stromal cell-derived factor 1/CXC-motif chemokine 12 (CXCL12), a factor required to retain HSCs in the bone marrow niche, is controlled by β3-, but not β2-, adrenergic signaling. Upon activation of β3-adrenergic receptor, the nuclear transcription factor Sp1 is dephosphorylated and degraded, most likely by cooperation of a serine protease and the S26 proteasome, resulting in downregulation of CXCL12 and impaired HSC retention. As an effect of this upstream activation by catecholamines, it was demonstrated that the appearance of HSC-enriched, lineage-negative, Sca-1<sup>+</sup> c-Kit<sup>+</sup> (LSK) cells in the blood circulation depends on circadian oscillations that correlate with sympathetic nerve outflow. Interestingly, HSC circulation in the blood stream was inversely correlated to CXCL12 expression in the bone marrow, and circadian variation was absent in animals deficient in genes of the molecular clock, such as genes encoding the transcription factor Aryl hydrocarbon receptor nuclear translo-cator-like, also known as Arntl or Bmal1. It is noteworthy that the particular cellular compartment in the bone marrow niche targeted by the nervous system remains to be fully identified. It has been demonstrated that cells of the endosteal bone marrow niche, such as osteoblasts, highly express CXCL12 to retain HSCs and progenitor cells by interaction with the chemokine receptor CXCR4 expressed on these cells. However, β3-adrenergic receptor is not expressed by osteoblasts, raising the possibility that other cells could be involved in mediating HSC mobilization after sympathetic activation. Interestingly, it has been proposed earlier that
mesenchymal stem cells are regulated by β3-adrenergic signaling and thus represent a possible candidate cell type.5,11 The authors of the current report expanded the concept that adrenergic signaling controls the bone marrow. Before the current report, they showed that this mechanism causes monocytosis during myocardial infarction4 and chronic stress.5 In addition to the mechanism presented in this report, they demonstrated earlier that bone marrow–derived monocytes infiltrate the atherosclerotic wall, aggravate local and systemic inflammation, and eventually enhance the probability of subsequent myocardial infarction. Notably, monocytes emanating from the bone marrow engrafted secondary niches, such as the spleen, to fill up the myeloid reservoir for later inflammatory events.4,12 In summary of all these studies, the authors provide evidence for the existence of a psycho-cellular vicious cycle, in which pain, anxiety, stress (and presumably circadian events) are potent drivers of bone marrow–derived monocytosis and HSC release with a myeloid bias, which in turn are likely to aggravate atherosclerosis and its complications like myocardial infarction and stroke. In the current work, the different imaging techniques used are of particular appeal. Several in vitro and in vivo imaging approaches directly visualize the inside of the bone marrow niche. Most illustratively, the authors induced experimental stroke in transgenic mice expressing the firefly enzyme luciferase under control of the cell cycle gene nuclear factor-Y–dependent cyclin B2 promoter. This whole body imaging approach allows monitoring and localizing proliferating, bioluminescent cells in living animals, including HSCs in the bone marrow. The authors also directly monitored stem cell proliferation by intravital microscopy in the skull bone at 2 different time points in the same animal. In using Nestin-GFP transgenic mice, many mesenchymal stem cells within the bone marrow niche are fluorescently marked and can be used as

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**Figure. Proposed mechanism of monocyte release from the bone marrow following stroke.**3 Sympathetic nerve terminals, which end in the bone marrow, secrete noradrenaline after stroke. Noradrenaline binds to β3-adrenergic receptors (ADRB3) expressed on bone marrow stromal cells. In the resting bone marrow, vascular cell adhesion molecule 1 (VCAM-1) binds to the integrin α4β1 (VLA-4) to retain hematopoietic stem cells (HSCs) in the stem cell niche. Additionally, high concentrations of the bone marrow stromal C-X-C motif chemokine 12 (CXCL12) with high affinity to the receptor CXCR4 expressed on HSCs contribute to their retention. After stroke, expression of VCAM-1 and CXCL12 decreases by an yet undefined mechanism. As a result, HSCs, myeloid progenitor cells, such as granulocyte-macrophage progenitors (GMPs) and monocyte/dendritic cell progenitors (MDPs), and monocytes enter the blood circulation. Moreover, ADRB3 signaling enhances HSC proliferation, increases the myeloid transcription factor PU-1, and generates a myeloid bias with higher numbers of proinflammatory Ly6Chigh monocytes. Whether stroke-associated monocytosis stroke is protective by improving host defense or detrimental by worsening reperfusion injury and neuronal survival is currently unknown (both possibilities indicated).
guide posts for identifying the same area in a second imaging session. The authors show fascinating pictures, in which proliferating, previously transferred HSCs, build clusters of stem cells only after stroke, but not after a sham procedure. In addition, the current work shows confocal imaging of increased expression of tyrosine hydroxylase—a rate limiting enzyme in norepinephrine synthesis—and its precise lining of bone marrow arterioles.

Beyond the basic science appeal, the current study may contribute to a better understanding of and treatment for post-stroke patients. One might propose that myeloid bias has been shaped by evolution because it provides a benefit to the host. It is not known whether this mechanism is protective or detrimental. One can speculate that an increased rate of infection after myocardial infarction, or stroke, such as pneumonia, urinary tract infection, and sepsis, would warrant an increased frequency and surveillance activity of innate immune cells. This hypothesis is supported by a study of Nguyen et al, who demonstrate that Bmal1 (Arntl) suppresses monocyte mobilization by repressing chemokine expression, such as CCL2. Notably, releasing monocytes from the bone marrow by inactivation of Bmal1 might directly contribute to host defense by conferring protection from 

leukotrienes. On the other hand, stroke-associated monocytosis may represent a less-functional, myeloid-biased and malignancy-predisposing phenotype of the bone marrow as previously suggested. Monocyte released from the bone marrow are expected to aggravate reperfusion injury to the ischemic brain and worsen neuronal survival. The authors of the current study are well aware of this possible dichotomy and propose further studies to fully understand the consequences of the myeloid deployment after stroke. It will also be challenging to decipher the exact mechanism by which adrenergic signaling fuels HSC proliferation. Functional loss of CXCL12 can explain why HSCs are not retained in the niche any more—but how exactly does adrenergic signaling cause increased myeloid cell proliferation as observed by the authors in their model? This may indeed be explained by previous studies showing that adrenergic receptors are expressed on myeloid cells throughout different stages of myeloid differentiation to regulate activation, motility, and proliferation, particularly of HSCs. Notably, β3-receptors are restricted to bone marrow stromal cells, whereas β2-receptors are highly expressed within the hematopoietic and the stromal compartment. Consistent with this, the authors of the current study observed that genetic deletion of β3-adrenergic receptor abolishes the turnover of HSCs, whereas downstream progenitor cells remained responsive to norepinephrine. Whether this activation was dependent on β2-receptor signaling and confined to monocyte progenitors (and not lymphoid cells) was not tested, but the existence of β2-receptors on both HSCs and monocyte precursors may provide a plausible explanation for the effects observed. Also, synergistic activation of both β2- and β3 receptors may be required for the activation and proliferation of some, but not of all subpopulations of bone marrow stem cells as previously suggested. Conversely, it is also possible that the myeloid bias observed in the current study is the result of a failure of lymphoid progenitors to respond to the inflammatory stimuli, resulting in overwhelming production of myeloid cells.

After stroke, the clinical outcome inversely correlates with leukocytosis in some studies, proposing that preventing monocytosis may be beneficial for patients. In the current study, the authors suggest that at least some of the remote catecholamine effects on the bone marrow are mediated by β3-adrenergic signaling—a target accessible by existing pharmacological strategies. However, whether modulation of stress-associated bone marrow hyperactivity can be prevented by β-blockers will have to be investigated in further studies. In clinical practice, β-receptor blocking drugs with specificity for cardiac expressed β1-receptors have superseded earlier strategies using nonselective β1/β2-blockers, which also target β2-receptors in the GI-tract, bronchi, blood vessels, and muscles. In particular, specific β1-blockers have proven effective in the treatment of hypertension and after myocardial infarction. In this regard, blockade of cardiac β-receptors can mitigate the effects of toxic levels of catecholamines in the acute and chronic scenario and prolong life by preventing heart failure. The new perspective now provided by Courties et al may initiate a search for drugs that specifically target the unwanted catecholamine signaling in HSCs and myeloid precursors. Such drugs would need to target the bone marrow or the myeloid limb of differentiating leukocyte precursors because β3-receptors are widely expressed in other tissues, including adipose tissue and the myocardium, where their inhibition could precipitate unwanted side effects.

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Disclosures

None.

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