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Lifestyle effects on hematopoiesis and atherosclerosis

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Abstract

Diet, exercise, stress and sleep are receiving attention as environmental modifiers of chronic inflammatory diseases, including atherosclerosis, the culprit condition of myocardial infarction and stroke. Accumulating data indicate that psychosocial stress and a high-fat, high-cholesterol diet aggravate cardiovascular disease, whereas regular physical activity and healthy sleeping habits help prevent it. Here we raise the possibility that inflammation-associated leukocyte production plays a causal role in lifestyle effects on atherosclerosis progression. Specifically, we explore whether and how potent real-life disease modifiers influence hematopoiesis' molecular and cellular machinery. Lifestyle, we hypothesize, may rearrange hematopoietic topography, diverting production from the bone marrow to the periphery, thus propagating a quantitative and qualitative drift of the macrophage supply chain. These changes may involve progenitor-extrinsic and intrinsic communication nodes that connect organ systems along neuro-immune and immuno-metabolic axes, ultimately leading to an altered number and phenotype of lesional macrophages. We propose that, in conjunction with improved public health policy, future therapeutics could aim to modulate the quantitative and qualitative output, as well as the location, of the hematopoietic tree to decrease the risk of atherosclerosis complications.

Keywords

atherosclerosis; hematopoiesis; monocyte; macrophage; bone marrow; spleen; proliferation; stress; sleep; diet

Introduction

In recent decades, perceptions of atherosclerosis' pathophysiology morphed considerably. We once imagined atherosclerosis as a lipid storage disease in need of mechanical artery reopening but now recognize it to be a multifactorial condition largely driven by an overactive and dysfunctional immune system^{1–4}. Some therapeutic goals have been met, leading, for instance, to reduced acute myocardial infarction incidence and mortality⁵. Another major success was the identification and partial elimination of smoking as a risk factor. However, atherosclerosis still leads the world's mortality statistics⁶. The life expectancy of atherosclerosis patients may have changed, but their numbers are legion

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despite medical progress⁶. Here we take a fresh look at four lifestyle-associated risk factors by asking how they may be linked to inflammation in the vascular wall. More specifically, we ask how psychosocial stress, diet, physical activity and sleeping patterns influence the supply and phenotype of innate immune cells in atherosclerotic plaque. Epidemiological data confirm these four lifestyle-related disease modifiers as highly potent risk factors for atherosclerotic disease⁷. For instance, daily fruit and vegetable consumption combined with regular physical activity associate with an odds ratio of 0.6 for myocardial infarction⁷. Motivated by emerging connections between stress, diet, physical activity, sleep and the immune system, we review data (or lack thereof) connecting lifestyle modifiers with immunity and hematopoiesis and identify areas that require further study. Discussing these links, we propose testing several hypotheses to deepen our understanding of how lifestyle influences crosstalk between hematopoiesis, immune cells and the cardiovascular system. Lifestyle is associated with potent preventive and anti-inflammatory effects: we argue that uncovering the involved molecular and cellular pathways could guide us towards new therapeutic targets.

Atherosclerosis risk factors

Epidemiological studies identify a number of risk factors for atherosclerosis, including age, gender, hypertension, LDL and HDL cholesterol, triglycerides, smoking, family history, obesity, diabetes and preexisting chronic inflammatory conditions such as rheumatoid arthritis^{7, 8}. These risk factors vary in prevalence and potency and are often combined in patients with severe atherosclerosis, which supports the concept that atherosclerosis is a multifactorial disease⁷. Identifying risk factors' putative mechanisms of action has already led to some successful therapeutic strategies, particularly statins and antihypertensive drugs that inhibit atherosclerotic progression and reduce its complications by lowering LDL and reducing blood pressure, respectively. These examples may serve as motivating blueprints for scrutinizing stress, diet, physical activity and sleep habits as potential modifiers of immunity, hematopoiesis and ultimately cardiovascular disease.

Role of macrophages in atherosclerotic plaque

Macrophages are innate immune cells that reside in large numbers in all major healthy and diseased tissues. Named for one of their functions — phagocytosis of pathogens and other foreign bodies — macrophages' numerous specific tasks depend on their lineage, host tissue and phenotype. Macrophages' tissue specific functions are diverse, spanning bone resorption, iron recycling, antigen presentation, temperature regulation, instruction of hematopoiesis and tissue-regenerating progenitors, modulation of neural synaptic activity, among others. Recent work highlights the abundance and importance of tissue-resident macrophages, which also inhabit the healthy vascular wall and myocardium (reviewed in^{9, 10}). While resident macrophages in the steady state vascular wall and plaque macrophages belong to the same class of innate immune cells, they may be functionally distinct (reviewed in¹⁻⁴). In the diseased vessel wall, macrophages accumulate progressively and fail to remove intimal cholesterol deposits. Instead, macrophages assume tissue destructive inflammatory phenotypes, resembling what has been termed M1 polarization *in vitro*^{11, 12}. Macrophages may give rise to foam cells and die locally, forming the necrotic

cores frequently found in ruptured plaques. While in plaque, inflammatory macrophages interact with other immune and stromal cells and secrete pro-inflammatory molecules that lead to tissue destruction. The cells are important sources for proteases that destabilize the arterial wall by digesting extracellular matrix, contributing to thinning of the fibrous cap and rendering lesions more vulnerable to rupture. It is quite clear that macrophages are central to atherogenesis and its ischemic complications; however, we are still limited in our understanding, and far from therapeutically harnessing, the triggers that induce the drastic atherosclerosis-associated changes in arterial macrophage number, phenotype and function linked to myocardial infarction and stroke.

The macrophage lineage

While recent reports suggest that macrophage-like cells in plaque may also derive from smooth muscle cells¹³, the bulk of data support the idea that lesional macrophages derive from genuine hematopoietic progenitors residing in the bone marrow and spleen⁴. The life span of plaque macrophages is rather short: BrdU tracking experiments in 4-month old *Apoe*^{-/-} mice illustrate that the entire plaque macrophage population renews within one month¹⁴ and consequently relies on newly produced cells. Depending on disease stage, macrophage production is a function of monocyte recruitment from circulation and local plaque macrophage proliferation¹⁴, whereas reduced recruitment¹⁵, along with macrophage death and exit from plaque likely contributes to lower macrophage numbers in the arterial wall^{2, 16}. Below we discuss current knowledge on factors that regulate monocyte and macrophage supply in the inflamed vessel wall.

Bone marrow hematopoiesis

After birth, bone marrow is the primary site of hematopoiesis, giving rise to red blood cells, platelets and circulating leukocytes. A major theory posits that the hematopoietic system is hierarchical, with pluripotent hematopoietic stem cells (HSC) residing upstream in the differentiation cascade, giving rise to progenitors with progressively decreased renewal capacity but increased specificity for particular leukocytes. HSC are rare (1 in 10,000 bone marrow cells) and relatively quiescent (<5 % are in cell cycle) (Figure 1). Hematopoietic progenitor quiescence and the marrow's staggering output are delicately balanced by extrinsic components produced by niche cells collaborating with cell-intrinsic regulatory machinery. Several stromal cells, including endothelial cells, osteoblasts, macrophages and mesenchymal cells secrete messengers that bind to receptors on hematopoietic cells to regulate their proliferation, migration and lineage bias (e.g. M-CSF, GM-CSF, CXCL12 also known as SDF-1, and stem cell factor, please see Figure 2)^{17, 18}. Hematopoietic progenitors may also directly sense circulating danger signals, such as toll like receptor ligands in the setting of infection, or beta-adrenergic transmitters such as norepinephrine. In addition, adhesion molecules such as VCAM-1 and E-selectin retain HSCs in specialized hematopoietic niches. These extrinsic signals translate into HSC activity via various transcription factors. Among them, several are known to induce HSC bias towards myeloid cell production, including PU.1, C/EBP α , Egr-1, Irf8, Klf4 and Mafk¹⁹. Once monocytes and neutrophils are produced, their release into circulation is tightly regulated by a number of signals, including CCR2 ligands and CXCL12. In addition, blood monocyte numbers

oscillate according to circadian rhythms by as much as 100%, with a diurnal peak and nocturnal nadir (in humans)²⁰.

Despite our growing understanding of macrophages' importance in atherosclerosis and blood monocyte levels' impressive correlation with cardiovascular mortality^{21, 22}, less is known about how monocyte production changes in cardiovascular disease. During atherosclerosis, monocyte levels increase progressively²³, and more abruptly after acute myocardial infarction, which implies that acceleration of myelopoiesis associates with disease progression²⁴. Impaired cholesterol handling²⁵ directly increases hematopoietic progenitor activity, and ischemic heart disease alters many bone marrow regulation signals, including inflammatory molecules such as IL-1 β , toll like receptor ligands and chemokines⁴. In addition, altered sympathetic activity, as observed after myocardial infarction²⁴, substantially changes the hematopoietic bone marrow niche²⁶. However, we are only beginning to understand how cardiovascular risk factors and lifestyle may affect hematopoietic activity and location.

Splenic monocyte supply

Although the bone marrow is the primary monocyte production site in the steady state, other lymphoid organs, such as the spleen, can produce monocytes in inflammatory conditions^{24, 27–30}. During atherosclerosis, HSPC mobilize from the bone marrow in large numbers and, instead of returning to the bone marrow, settle in the spleen's red pulp, where they proliferate and differentiate, giving rise predominantly to neutrophils and monocytes. This shift of myeloid cell production from the bone marrow to the spleen, we hypothesize, may be a major factor in the systemic oversupply of inflammatory monocytes and neutrophils. We still know little about the mechanisms that drive this shift from medullary (i.e., in bone marrow) towards extramedullary hematopoiesis, but some thought-provoking clues are emerging. In response to high fat diet, increased mobilization from the bone marrow depends on IL-23 production by lipid-rich splenic macrophages and dendritic cells. According to the model, IL-23 activates a cytokine cascade that culminates in increased G-CSF, a known mobilizing factor in the bone marrow³¹. Once HSPC seed the spleen, they must anchor to the splenic parenchyma to proliferate and differentiate. The mechanisms orchestrating HSPC retention in extramedullary sites remain poorly understood but may involve SIP signaling^{32, 33}. Once retained, HSPC proliferate and give rise to monocytes and neutrophils. Factors intrinsic to HSPC, such as augmented expression of the beta common chain, the ligand for the growth factors GM-CSF, IL-3, and IL-5, promote HSPC proliferation²⁹. Likewise, factors extrinsic to HSPC may be relevant. In response to infection and atherosclerosis, a GM-CSF-producing population of B cells, the innate response activator (IRA) B cells, develops in the spleen^{34, 35} and may instigate heightened myeloid cell production in the organ³⁶. Finally, spleen-produced monocytes and neutrophils mobilize, enter the circulation, and infiltrate atherosclerotic lesions³⁰.

Many questions about spleen-derived monocytes' and neutrophils' importance to the overall myeloid response remain unanswered. It is unclear, for example, whether splenic monocytes are identical to their bone marrow-derived counterparts or whether they carry a generative signature that endows them with differential function. Are splenic monocytes a source of

inflammatory (M1-like) plaque macrophages or do they give rise to cells with an alternative resolution phenotype? To test these ideas, careful fate-mapping studies are necessary. Because the spleen is dispensable to life, it is tempting to tackle these questions with splenectomy. However, the surgical procedure removes an organ that is nevertheless important to many immunological processes. For example, removing the spleen also removes a large supply of B cells, which play vital roles in innate and adaptive immunity, not to mention macrophages known to scavenge senescent erythrocytes and, very likely, monocytes and neutrophils. Thus, removing the spleen likely obscures the true contribution of the organ's myeloid cell production.

Plaque macrophage proliferation

During atherosclerosis, monocytes continuously migrate to the vessel wall, where they can become lesional macrophages^{23, 37}. Monocyte recruitment depends on chemokine-mediated migration³⁸, which is essential to the development of atherosclerosis^{39–41}. But monocyte recruitment is not the only process that dictates macrophage accumulation in atherosclerosis. Over the years, a number of studies have argued that lesional macrophages renew locally and augment their numbers through proliferation, i.e. cell division within the plaque^{42–49}. This concept did not readily catch on, partly because it was unclear whether proliferation was important and whether the proliferating cells were indeed macrophages. More conclusive recent evidence supporting macrophage proliferation in various contexts and tissues^{50–52} provided an impetus to determine the relative contributions of monocyte recruitment and macrophage proliferation in atherosclerosis. Fate mapping and parabiosis studies have shown that macrophage proliferation is key to macrophage lesional accumulation, especially in established atherosclerosis¹⁴. To be sure, macrophages are not stem cells and probably divide no more than a few times before dying or exiting the lesion. Moreover, these studies do not negate the contribution of monocyte recruitment, and indeed, locally proliferating macrophages derive from recruited monocytes; because all macrophages can eventually be replaced with bone marrow cells argues against the idea that proliferating macrophages originate from smooth muscle cells. Rather, the studies show the nuance of the spatio-temporal dynamics of macrophage accumulation while reconciling reports arguing against the concept that monocyte recruitment is the ultimate process dictating macrophage number. A revised model proposes that lesions grow through the coordinated steps of monocyte production, recruitment and local macrophage proliferation^{53–55}. It remains unclear how locally sourced macrophages differ from macrophages that directly derive from circulating monocytes, although recent studies indicate that the local environment is a crucial determinant of eventual function^{56–60}.

The hematopoietic tree on the move: macrophage production shifts to periphery in atherosclerosis

In steady state and healthy individuals, hematopoiesis occurs in the bone marrow⁶¹. Migration of hematopoietic progenitor cells is limited, as only a few hundred are found outside the marrow³³. This rather tight control loosens during the chronic inflammation of atherosclerosis: progenitor cells now travel through the periphery in greater numbers and seed the spleen, which contributes to increased leukocyte production³⁰. Monocytes made in bone marrow and spleen circulate, extravasate and then give rise to proliferating plaque

macrophages. We speculate that this shift in hematopoietic topography from the bone marrow towards the spleen and atherosclerotic plaque promotes disease progression (Figure 3). Comparing the cells produced outside the bone marrow with those that arose inside will reveal whether peripherally sourced cells (i.e. via extramedullary hematopoiesis) are more or less inflammatory than centrally produced cells (i.e. via medullary hematopoiesis) and whether the two cell types have similar phenotypes. Currently, we do not fully understand what loosens the marrow's control and why myelopoiesis shifts to peripheral sites as atherosclerosis progresses. Uncovering these mechanisms may allow us to design interventions that tighten central control and contain hematopoiesis in the bone marrow to reduce plaque inflammation. We hypothesize that atherosclerosis risk factors, and the lifestyle modifiers discussed below, initiate the macrophage production shift to peripheral sites by promoting HSC and progenitor release from the bone marrow (Figure 3).

Healthy versus disease-promoting lifestyle

Diet/Obesity

Mice lacking crucial reverse cholesterol transport components, such as ApoE or the Ldl receptor, develop atherosclerosis while consuming a so-called Western diet. These artificial models are useful because the mice develop lesions similar to those that develop in humans. In contrast, wild type mice (i.e., with intact reverse cholesterol transport) fed a high-fat diet do not develop atherosclerosis, but they gain weight and become obese with age. Elaborating the metabolic changes that occur during obesity and the links between obesity and cardiovascular disease are beyond the scope of this review and have been extensively discussed elsewhere. Indeed, research exploring the links between obesity, metabolism, and inflammation has flourished, and we guide the reader to recent reviews^{62–66}. However, we would like to focus our discussion on a few insights that are particularly relevant to how diet/obesity may affect hematopoiesis. A landmark observation, made over 10 years ago, demonstrated that macrophages accumulate in adipose tissue⁶⁷. Although the study 'simply' showed the existence of macrophages in fat, it proved to be immensely influential as it was evidence for the concept that adipocytes, metabolism, and immunity are functionally linked. Four years later, a second major study showed that macrophages residing in obese mice differed phenotypically from those in lean mice, i.e. were more inflammatory⁶⁸. The study was particularly interesting because the data suggested, by virtue of focusing on Ccr2, the chemokine receptor for Ccl2, that macrophages accumulating in fat tissue are monocyte-derived. The idea was linked conceptually with two independent atherosclerosis studies, published in the same issue, demonstrating that inflammatory Ccr2⁺ Ly-6C^{high} monocytes accumulate in atherosclerotic lesions and give rise to macrophages^{23, 38}. More studies on the origin and function of adipose-tissue resident macrophages followed^{62–66}. One study of particular interest to our discussion concluded that adipose tissue macrophages promote monocyte production by secreting IL-1β⁶⁹. The data implied that a vicious cycle operates in obesity whereby adipose tissue macrophages beget more macrophages by releasing endocrine factors that induce the generation of monocytes in the bone marrow remotely. This increase in monocyte production, triggered by obesity, may also enlarge the systemic monocyte pool available to recruitment to other sites, especially atherosclerotic plaque. This argument provides a hypothetical connection between the lifestyle factor, obesity,

hematopoiesis and atherosclerosis progression that is independent of direct cholesterol deposition in the vascular wall. A second study focusing on the Tlr adaptor Myd88, has deepened the connection between adipose tissue and the periphery, demonstrating that Myd88 fosters an inflammatory (M1) macrophage phenotype in adipose tissue and associates with systemic inflammation⁷⁰. These studies are highlights that by no means represent the entire breadth of work on the subject. Nevertheless, both converge on the idea that obesity is an inflammatory disease that stimulates the production of monocytes, their recruitment to adipose tissue, and differentiation to inflammatory macrophages. In this context, then, it is tempting to speculate that obesity alters macrophage supply (i.e., the hematopoietic tree) in ways that resemble disturbances observed in atherosclerosis. Perhaps the links between obesity and atherosclerosis owe more to altered hematopoiesis than has been appreciated to date.

Another area of study with possible connections between diet, atherosclerosis, and hematopoiesis focuses on the microbiome. We are learning that diets rich in particular nutrients predispose to atherosclerosis by influencing the gut intestinal flora⁷¹. Studies on allergic airway inflammation have shown that dietary fiber content alters the composition of the gut and lung microbiota, which changes the concentration of short-chain fatty acids in the blood, thus altering hematopoiesis in the bone marrow⁷². Although these connections are still tenuous, they challenge us to reorient our understanding of atherosclerosis to include specific dietary ingredients, and think more deeply about dietary inputs and their influence on disease.

Psychosocial stress

Chronic stress is a recognized risk factor for atherosclerosis^{73, 74}. Epidemiology data show increased risk of cardiovascular events on both acute and chronic time scales. Earth quakes⁷⁵ and the World Cup soccer championship⁷⁶ increase the incidence of acute myocardial infarction, while post-traumatic stress disorder, for instance in combat veterans⁷⁷, confers increased long-term risk of atherosclerotic events. Permanent stress at work or home results in an odds ratio of 2.14 and 2.12, respectively, for myocardial infarction⁷³. The manifold effects of stress on immunity are only partially understood⁷⁸. Systemic release of stress hormones triggers organismal readiness for fight and flight. Immune modulation during and after exposure to stress prepares for injuries or infections that may arise from dangerous, stressful situations. Exposure to stress activates two major pathways: the limbic-hypothalamic-pituitary-adrenal axis, which leads to systemic release of corticosteroids⁷⁹, and the sympathetic nervous system. In particular, cortisol has numerous immunosuppressive effects on leukocytes which express the glucocorticoid receptor to varying degrees (www.immgen.org). When hypothalamic fear centers activate the sympathetic nervous system, increased levels of systemic catecholamines signal through adrenergic receptors expressed by stromal and immune cells⁸⁰. A high autonomous tone may increase cardiac output, vasoconstriction and blood pressure, and thus contribute to cardiovascular risk.

Leukocytes and hematopoietic progenitors express α - and β -adrenergic receptors. For instance, bone marrow hematopoietic progenitors⁸¹ and splenic red pulp macrophages

(www.immgen.org) express β 2-adrenergic receptors. Thus, the neurotransmitter and stress hormone norepinephrine may increase inflammatory activity of macrophages and their progenitors. In the bone marrow, norepinephrine acts via β 3-adrenergic receptors expressed on stromal cells that are part of the hematopoietic niche⁸². Sympathetic nerve fibers run along bone marrow arterioles and release norepinephrine which then acts locally on mesenchymal cells (Figure 2). These cells are a source of CXCL12, a cytokine that retains hematopoietic progenitors and neutrophils within the marrow¹⁷. CXCL12 also promotes HSC quiescence. When bone marrow levels of norepinephrine increase, CXCL12 levels decline, pushing hematopoietic stem cells into active phases of the cell cycle and likely enhancing their migratory capacities. Sympathetic tone's relationship to CXCL12 levels and HSC activity was first described in the setting of circadian rhythms⁸². The central clock located in the suprachiasmatic nuclei receives retinal cues synchronizing dark/light cycles with circadian activity of the autonomous nervous system, which then confers these rhythms to the hematopoietic marrow⁸². Sympathetic activity after acute myocardial infarction in mice also activates HSC migration, leading to extramedullary monocyte production and aggravated atherosclerosis in *ApoE*^{-/-} mice²⁴. Ischemic stroke in mice similarly triggers bone marrow activation⁸³.

In mice, psychosocial stress leads to chronically increased autonomous tone in the bone marrow⁸⁴. When mice were exposed to variable stressors such as crowding, isolation, cage tilt and changes in bedding over a time period of 3–6 weeks, norepinephrine levels rose in their femoral marrow, thereby modulating the hematopoietic microenvironment. As described for circadian signaling, the neurotransmitter acted through β 3-adrenergic receptors on niche cells, reducing CXCL12 levels in stressed mice. In return, the percentage of cycling hematopoietic stem and progenitors increased as did bone marrow production and release of monocytes and neutrophils. Consequently, their numbers in circulation rose. Mice that genetically lack the β 3-adrenergic receptor exhibited a blunted response to stress, as their CXCL12 levels and bone marrow leukocyte output were unchanged⁸⁴. These data correlate well with reports of increased myeloid cell production after stress exposure⁸⁵ and injection of epinephrine into mice⁸¹. *ApoE*^{-/-} mice exposed to stress develop a more inflamed plaque phenotype, with higher macrophage numbers and increased protease activity⁸⁴, reminiscent of vulnerable plaques in humans. Systemic effects of noradrenaline may be milder but also increase lesion size in some regions of the aorta in *ApoE*^{-/-} mice⁸⁶. The effects of stress on splenic myelopoiesis and plaque macrophage proliferation are currently completely unknown. While the arterial vasculature is richly innervated by sympathetic fibers, making local autonomous nervous system effects on plaque likely, the spleen seems to lack sympathetic innervation. In contrast, the vagus nerve readily interacts with splenic leukocytes, dampening inflammatory responses⁸⁷.

In humans, acute and chronic stress exposure increases blood leukocytes, likely through demargination and possibly also through increased cell production⁸⁴. Further, leukocyte inflammatory gene expression is influenced by stress levels⁸⁸. It is tempting to speculate that the stress-related increase in leukocytosis and inflammation promotes atherosclerotic progression and complication, as blood monocytosis predicts cardiovascular mortality²².

This hypothetical disease mechanism, especially the link to bone marrow activity, should be investigated further in human patients.

Sleep disorders

We are, or should be, asleep for at least a third of our lives. Sleep is essential to life; its acute deprivation reduces alertness and performance, impairing memory and cognition, while chronic sleep deprivation is associated with high blood pressure, stroke, myocardial infarction, and heart failure. Numerous causes of sleep deprivation range from self-imposed denial, to stress-related insomnia, sleep apnea, and mental illness. Despite the many connections between sleep deprivation and disease, we still know very little about the underlying biology and connections to disease. In light of this discussion, is there any evidence that sleep predisposes to heart disease by affecting hematopoiesis?

Arguably the most work devoted to understanding the effects of sleep deprivation has been done in the context of sleep apnea, a condition that affects 2–5% of the general population, is more prevalent in obesity, and is a risk factor for cardiovascular disease. Sleep apnea is characterized by repeated stops and starts in breathing^{89, 90}. Physiologically, the disturbance results from the collapse of the upper airway during sleep, and patients repeatedly wake up so as not to asphyxiate. Complications arising from sleep apnea, such as high blood pressure, atrial fibrillation, or congestive heart failure, likely result from sudden drops in blood oxygen. Because of this, it might be difficult to distinguish which effects are related to the multiple hypoxic events and which to sleep fragmentation that necessarily occurs during waking. Regardless of the primary cause, obstructive sleep apnea syndrome (OSAS) consistently correlates systemic inflammation with hypoxia and sleep fragmentation. Patients have elevated plasma levels of TNF α , IL-6, MIF and CRP compared to obese or healthy individuals^{8, 89–94}. Moreover, patients with moderate-to-severe OSAS contain monocytes that are more adherent to the endothelium, producing more TNF α , and more neutrophils that release superoxide^{95–98}. Additional evidence for how OSAS might promote atherosclerosis centers on vascular smooth muscle activation, increased lipid loading in macrophages, lipid peroxidation, and endothelial dysfunction⁹⁹. Although no definitive study evaluated whether OSAS associates with elevated or disturbed hematopoiesis, the connection with myeloid cells render the link plausible and worthy of investigation.

Animal studies have attempted to show how sleep fragmentation induces inflammation in the absence of hypoxia, the potential confounder in OSAS. Animals prevented from falling into deep REM sleep produce TNF α , which associates with cognitive dysfunction induced by sleep fragmentation¹⁰⁰. Using TNF α -receptor deficient mice, as well as neutralizing antibodies to TNF α , it was shown that excessive sleepiness incurred by sleep fragmentation may be due to activation of TNF α -dependent inflammatory pathways¹⁰¹. The hypothetical connections between sleep deprivation, hematopoiesis and atherosclerosis are further strengthened when considering circadian rhythms. The central circadian clock, located in the suprachiasmatic nuclei, allows organisms to coordinate their behavior and biology with daily and seasonal fluctuations in the day-night cycle. In the brain, *CLOCK* genes control transcription of a large portion of the genome. Additionally, circadian fluctuations are controlled by peripheral clocks located in cells throughout the body. It is estimated, for

example, that >8% of the macrophage transcriptome oscillates according to the day-night cycle¹⁰², and peripheral clock genes such as *Bmal1* dramatically alter monocyte numbers in the circulation^{20, 82}. Mice exposed to dim light, which disturbs circadian rhythms, have exaggerated inflammatory responses and gain weight^{103–106}. Moreover, the onset of acute myocardial infarction follows a circadian pattern^{107, 108}. It is therefore tempting to speculate that peripheral clock genes, which regulate macrophage function independently of the central clocks located in the suprachiasmatic nuclei, likewise regulate hematopoietic output and location of the hematopoietic progenitors. The study of sleep is mostly uncharted territory, and the recent identification of convective fluxes of interstitial fluid that clear beta-amyloids from the brain during sleep only underscores how little we know¹⁰⁹. However, it is clear that hematopoiesis, monocyte production, and migration follow nervous inputs, and thus a connection between myelopoiesis and sleeping habits should be studied experimentally.

Physical activity and exercise

Physical activity, or lack thereof, greatly influences the immune system and atherosclerosis risk^{73, 110–112}. Exercise is thought to lower cardiovascular risk by improving metabolic balance and consequently counteracting development of obesity¹¹². However, physical activity may also directly affect the immune system independent of metabolic pathways¹¹³. For instance, the acute effects observed immediately after strenuous exercise are likely independent of chronically increased energy expenditure¹¹⁴. In humans and mice, exercise can increase inflammatory cytokine levels (e.g. TNF α and IL-1 β , IL-6, among others), leukocytosis¹¹⁵ and trigger release of bone marrow cells into the blood stream^{116, 117}. Specifically, the number of circulating neutrophils, monocytes and lymphocytes rises during exercise¹¹⁵. Systemic sympathetic activity, which increases during exercise, is a candidate trigger for the observed increase in circulating leukocytes and progenitor cells. Interestingly, injection of epinephrin into humans cause similar changes in blood leukocyte levels as exercise does. Similar to stress exposure⁸⁴, acute MI²⁴, stroke⁸³ and during circadian regulation of hematopoiesis⁸², exercise may acutely elevate sympathetic tone in the bone marrow and lower bone marrow CXCL12 signaling. Decrease of this chemokine may then lead to stem and myeloid cell release into blood, and contribute, in addition to demargination of cells, to blood leukocytosis acutely after strenuous physical activity. The hypothesis that exercise triggers bone marrow release of leukocytes should be tested experimentally, in conjunction with exploring key hematopoietic niche factors after exercise.

Over longer time frames, intense exercise is thought to suppress immunity in mice and humans, although the precise mechanisms are unclear (reviewed in detail in^{115, 118}). For instance, twelve weeks of aerobic and resistance training reduced circulating inflammatory monocytes in humans¹¹⁹. Exercise may reduce the number of circulating lymphocytes and NK cells¹¹⁵, potentially contributing to immunosuppressive effects.

The available human and rodent data on exercise's influence on the hematopoietic system are mostly limited to acute effects showing increased bone marrow release of leukocytes and hematopoietic progenitors. Regarding chronic effects on leukocyte production by bone

marrow, data are currently limited to a few insightful reports^{120, 121} obtained in mice exposed to forced exercise. Running mice show increased bone marrow proliferation of lineage⁻ Sca-1⁺ C-kit⁺ hematopoietic progenitors (LSK) leading to increased LSK frequencies therein. Bone marrow transplantation into irradiated recipients resulted in more splenic colonies when bone marrow donors were subjected to forced running. Blood reconstitution was similar between sedentary and exercising bone marrow donors, suggesting a comparable efficiency of transplantation. Rigorously enumerating long-term HSC in exercising mice requires a limiting dilution stem cell assay, which has not been reported thus far. In addition, we caution that experiments described above used mild electric shocks to encourage mice to run. While this study design ensures homogenous and well-defined physical activity, it may also confer emotional stress through administration of electrical shocks. The reported LSK data may thus reflect combined exposure to physical activity and psychosocial stress. We conclude that hematopoiesis, HSC number and function should also be studied in voluntary running mice.

Regular aerobic exercise increases systemic vagal tone¹²². Increased vagus nerve activity is immunosuppressive, for instance through signaling to T cells and macrophages in the spleen¹²³. It remains unclear if vagal activity affects splenic hematopoiesis, possibly through modulated macrophage activity. This is a reasonable hypothesis, as CD169⁺ macrophages are important contributors to the hematopoietic niche¹²⁴, and because splenic macrophages express acetylcholine receptors. The hypothesis that exercise-related increase in vagal activity may reduce inflammatory macrophage activation, and thus confer protective effects on atherosclerosis, should be tested experimentally.

There are no human data on exercise's influence on bone marrow hematopoiesis, and physical activity's effects on many of the typical niche factors that regulate hematopoietic stem cell proliferation have not been explored. Future studies should also verify that exercise indeed increases bone marrow HSC activity. If this were the case, one wonders how to reconcile increased HSC proliferation, which should lead to systemically increased levels of leukocytes, with the reported immunosuppression induced by strenuous exercise¹²⁵ and the protective effects of exercise against cardiovascular disease. More specifically, what happens to the bone marrow of voluntary running *Apoe*^{-/-} mice, which have increased HSC proliferation due to compromised reverse cholesterol transport²⁵, even when they are sedentary, as all mice housed in the small cages of current research facilities could be considered sedentary? Does the liberation of HSC after bouts of strenuous exercise accelerate extramedullary hematopoiesis in the spleen? Alternatively, does exercise reduce macrophage supply, as suggested by decreased plaque protease activity measured in this setting¹²⁶? Does systemic HSC proliferation decrease if atherosclerotic individuals exercise, because an increased vagal tone dampens splenic myelopoiesis? Is the level of exercise important, as some authors suggested a u-shaped relationship between exercise and immune function¹¹⁵, similar to what has been described for alcohol consumption and cardiovascular mortality¹²⁷? Addressing these questions experimentally will also further our understanding of the hematopoietic system's overall role in the progression of cardiovascular disease.

Conclusions

Decades of meticulous epidemiological research provides us with rich information on atherosclerosis progression's association with various risk factors. Some risk factors' causal pathways were identified and harnessed therapeutically. Because atherosclerosis is far from being cured, we ought to take a closer look at lesser-understood risk factors and explore whether their pathways of action are neural, inflammatory, metabolic or a combination thereof. We already adhere to this concept when feeding mice a high-fat diet, one of the lifestyle factors discussed above. Emerging data on the immune and hematopoietic systems suggest that both may have sizable roles in promoting cardiovascular disease. This is an opportune time to explore causalities linking lifestyle, hematopoiesis and atherosclerosis, because studying lifestyle factors and their influence on plaque macrophage supply and phenotype may reveal unknown disease pathways amenable to therapeutic interventions. For instance, if disease-propagating lifestyle factors such as stress, sleep disorders and unhealthy diet converge on a specific pathway that accelerates atherosclerosis, while positive modifiers such as physical activity have an opposing effect, this pathway should be explored as a therapeutic target to reduce macrophage supply to atherosclerotic lesions. A merger of several research fields would expedite this work, as the discussed pathways may span multiple systems, including the nervous, immune, cardiovascular, metabolic and hematopoietic. Scientific exchange among experts in these diverse areas would better enable tracking disease mechanisms that ignore the traditional — and somewhat artificial — borders between historically grown research fields. Interdisciplinary training, funding opportunities and research environments that encourage cross fertilization among seemingly disparate disciplines would likely accelerate this progress.

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Non-standard Abbreviations and Acronyms

Apoe	apolipoproteine
BrdU	bromodeoxyuridine
Ccl	C–C chemokine ligand
Ccr	C–C chemokine receptor
CD	cluster of differentiation
Cxcl	C-x-c chemokine ligand
GM-CSF	granulocyte macrophage colony stimulating factor
HDL	high density lipoprotein
HSC	hematopoietic stem cell

HSPC	hematopoietic stem and progenitor cell
IL	interleukin
IRA	innate response activator
Ldl	low density lipoprotein
LSK	Lineage negative Sca-1 positive c-kit positive
Ly-6C	lymphocyte antigen 6C
OSAS	obstructive sleep apnea syndrome
Mac1	macrophage antigen 1
MCSF	macrophage colony stimulating factor
MIF	macrophage inhibitory factor
NK	natural killer
REM	rapid eye movement
SDF	stromal cell derived
TGF	tissue growth factor
Tlr	toll like receptor
TNF	tumor necrosis factor
VCAM-1	vascular cell adhesion protein

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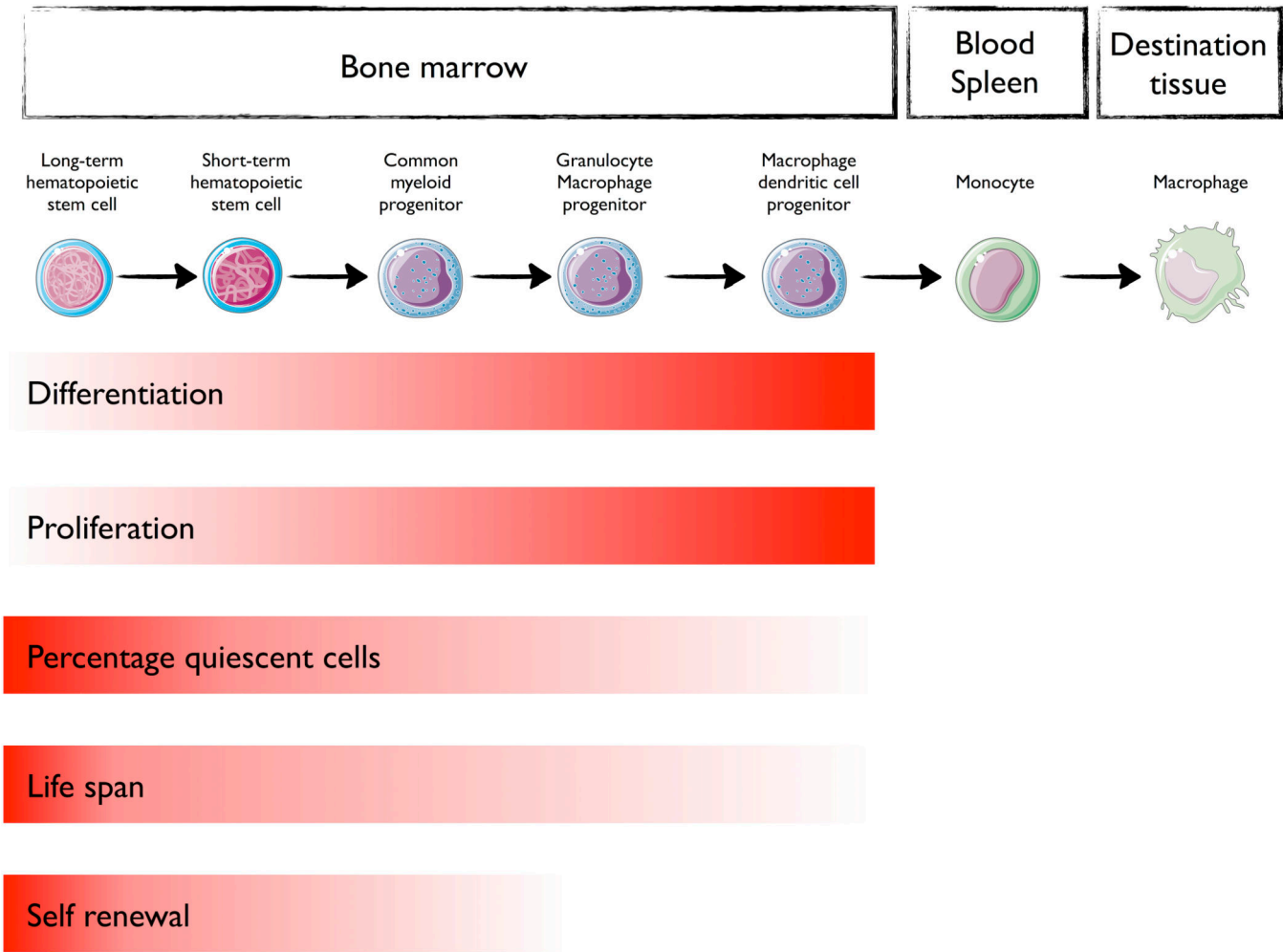


Figure 1.
The hematopoietic tree and properties of myeloid progenitors as a function of their differentiation.

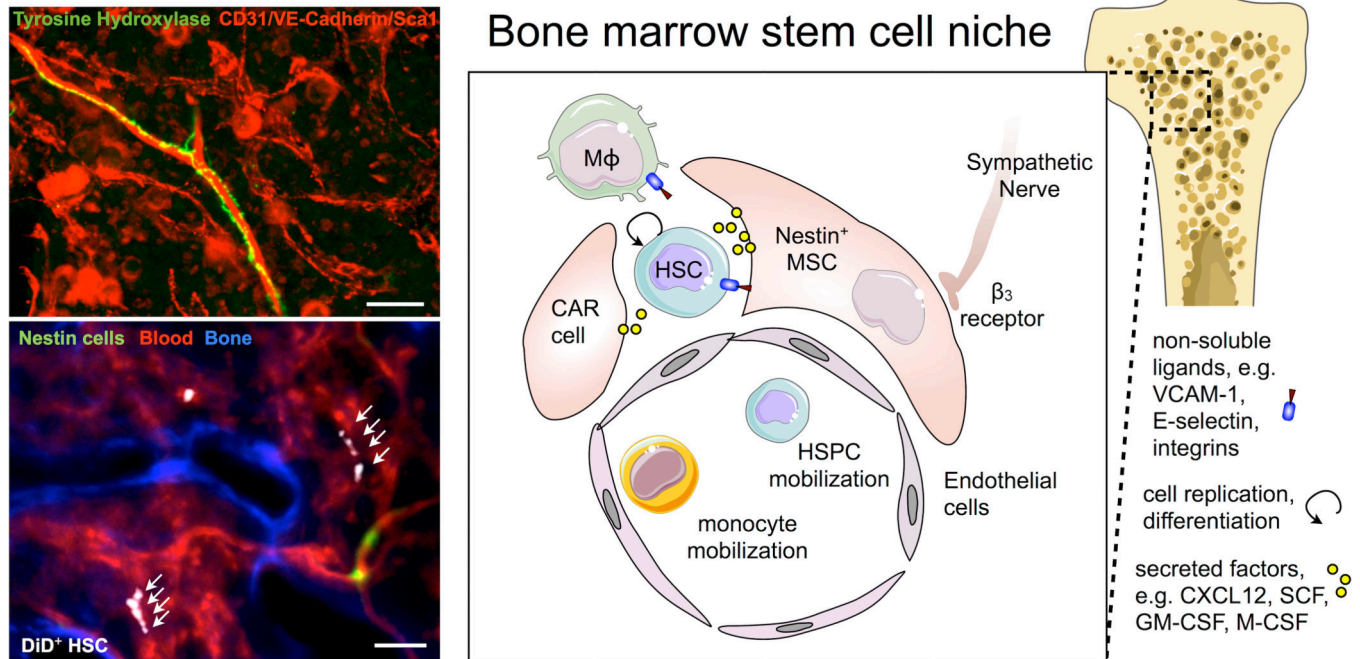


Figure 2.

The hematopoietic niche. The cartoon depicts components that indirectly regulate the activity of hematopoietic stem cells. The upper panel on the left shows an original microscopy image of the mouse bone marrow, illustrating sympathetic nerve fibers traveling along bone marrow vessels. The lower left panel was obtained in the bone marrow of a mouse after ischemic stroke, and shows replicating hematopoietic progenitor cells (arrows). Both images are taken from Courties G. et. al.⁸³.

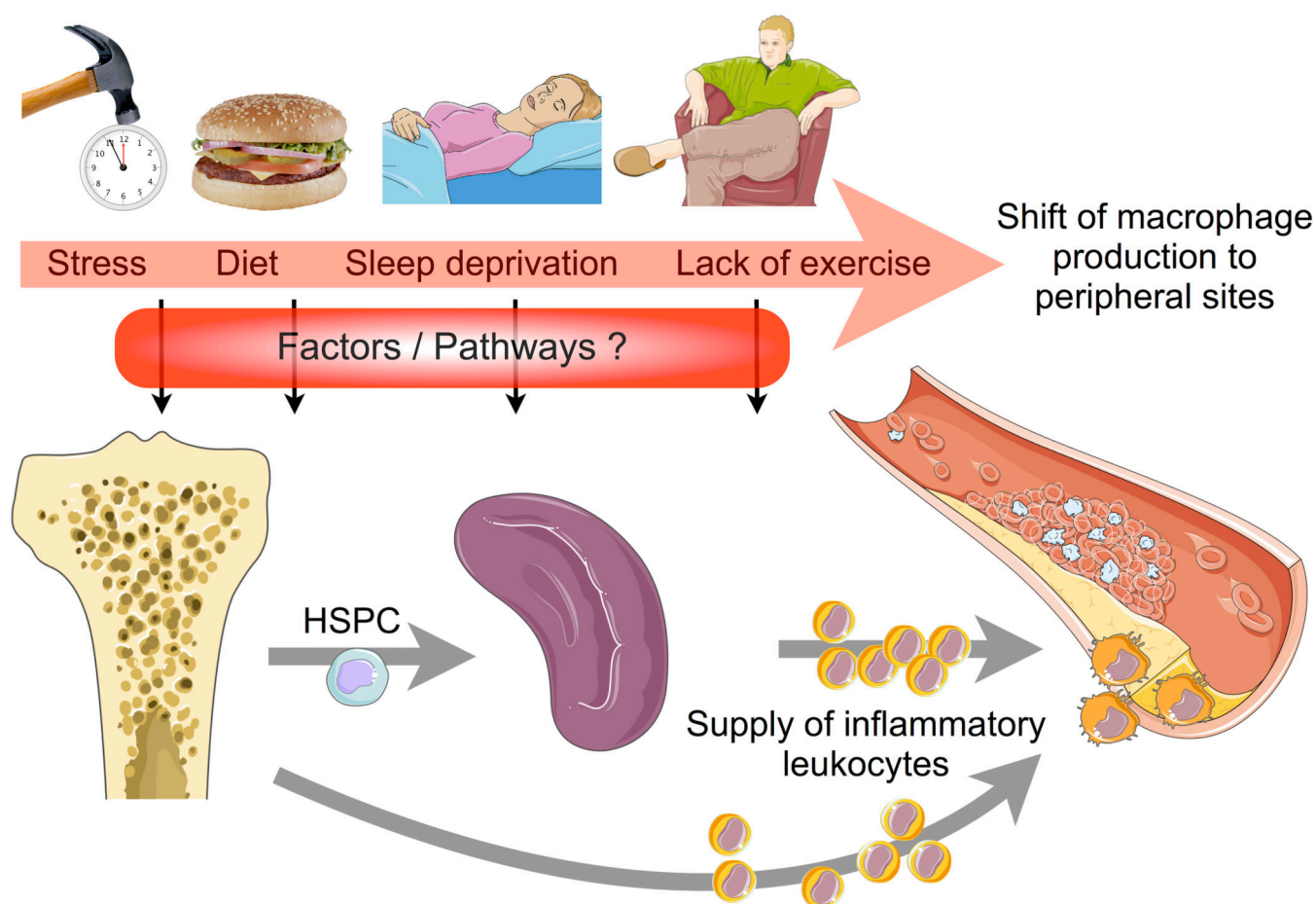


Figure 3. The hematopoietic tree on the move. The cartoon illustrates the hypothetical contribution of life style factors towards shifting the macrophage supply peripherally and associating with atherosclerosis progression and complication.