Myeloid cell contributions to cardiovascular health and disease

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Recent advances in cell tracing and sequencing technologies have expanded our knowledge on leukocyte behavior. As a consequence, inflammatory cells, such as monocyte-derived macrophages, and their actions and products are increasingly being considered as potential drug targets for treatment of atherosclerosis, myocardial infarction and heart failure. Particularly promising developments are the identification of harmful arterial and cardiac macrophage subsets, the cells’ altered, sometimes even clonal production in hematopoietic organs, and epigenetically entrained memories of myeloid progenitors and macrophages in the setting of cardiovascular disease. Given the roles of monocytes and macrophages in host defense, intricately understanding the involved cellular subsets, sources and functions is essential for the design of precision therapeutics that preserve protective innate immunity. Here I review how new clinical and preclinical data, often linking the cardiovascular, immune and other organ systems, propel conceptual advances to a point where cardiovascular immunotherapy appears within reach.

Recent conceptual and technological breakthroughs increase the resolution and depth of our knowledge on myeloid cells and their functions in cardiovascular disease (CVD). Such breakthroughs span the entire biomedical spectrum. On the clinical side, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial provided the first convincing proof that modulating inflammation improves patients’ cardiovascular health. This large-scale trial (Box 1), which examined the effects of an antibody that neutralizes the cytokine interleukin-1β (IL-1β) in atherosclerosis, launches the era of immunotherapy in CVD. The association of CVD with clonal hematopoiesis, i.e., the massive expansion of leukocytes that derive from just one hematopoietic stem cell, is another clinical discovery that creates excitement, as it is conceptually linked to translational studies on interactions among cardiovascular risk factors, hematopoiesis and inflammation in cardiovascular organs. Furthermore, rapid diagnostic technology development (Box 2) has spurred multidimensional, unbiased data collection in macrophages, triggering a deeper understanding of their functional diversity. On the fundamental science end of the spectrum, preclinical studies revealed the dichotomy of cardiovascular tissues’ innate immune cells according to their source (local proliferation versus recruitment from blood). These discoveries bridge the chasms currently dividing clinical cardiology, immunology and hematology, indicating a need for vigorous interdisciplinary exchange. Here I aim to integrate new cardiovascular, innate immunity and hematopoiesis data with a focus on monocyte and macrophage biology, which I review for a broad readership.

Uncovering the mechanisms leading to atherosclerosis and myocardial infarction (MI), i.e., coronary risk factors, markedly lowered individual cardiovascular mortality. Understanding and then treating cardiovascular risk factors (listed in Table 1) provided the foundation for this success story. However, longer survival and changes in lifestyle keep CVD at the top of worldwide mortality statistics, and managing risk factors such as high cholesterol, hypertension and diabetes prolongs life but does not necessarily provide a cure. Some of the residual cardiovascular risk is attributed to inflammation, which damages the arterial wall and myocardium. Uncovering how cardiovascular risk factors propel inflammation may provide new orthogonal therapeutic targets that could repeat what statins have achieved in the past: breaking the vicious cycle leading to often catastrophic organ ischemia. Cholesterol and macrophage deposits accumulate below the vascular endothelium. Other immune cells, including lymphocytes, dendritic cells and neutrophils, participate in arterial wall remodeling. Plaques may either erode or rupture, causing downstream ischemia. Heart failure may ensue as a consequence of extensive necrotic muscle loss. During all disease stages, monocyte-derived macrophages replace tissue-resident macrophages and modulate cardiovascular tissue health.

**Box 1 | The CANTOS trial**

This trial provides the first convincing evidence that the inflammation hypothesis translates to improving outcomes in patients. There were 10,061 patients with stable atherosclerosis, a history of prior MI and a CRP concentration >2 mg/L enrolled in this study. Patients were randomized to treatment with placebo or 50, 150 or 300 mg canakinumab, an IL-1β neutralizing antibody, which was injected subcutaneously every 3 months. The treatment led to a 15% reduction of ‘hard’ cardiovascular events and a 31% reduction of all-cause and cardiovascular mortality if the CRP declined below the median in response to the first treatment.
oxygenation in the lung. Data obtained in mice in which CD11c-expressing cells are labeled suggest that dendritic cells likewise populate arterial and valvular tissue. There is an ongoing debate on the degree to which these cells are distinct from macrophages. It is generally assumed that arterial resident macrophages support tissue homeostasis and pursue surveillance, whereas organ-specific functions have not been reported. How old age, which modifies the phenotypes of tissue-resident macrophages, and other cardiovascular risk factors affect arterial resident macrophage numbers and functions is currently unknown.

The steady-state myocardium. We now understand that macrophages constitute 7–8% of noncardiomyocytes in a normal adult mouse, an insight that has recently been enabled by cell-specific fluorescent reporters, optical clearing, fluorescence myocardial slab imaging and quantitative multidimensional flow cytometry of myocardial specimens. Imaging of myocardium confirms that macrophages are also present in healthy humans; FACS protocols to enumerate, isolate and phenotype human heart macrophages and their subsets are currently in development. Cardiac macrophage numbers, distribution, subsets and phenotypes are heterogeneous and change in response to age and disease in mice, and this is likely also the case in humans, although they are less well studied at this time.

During heart development, macrophages are involved in shaping the vasculature. Macrophages appear in the organ on embryonic day 12.5–14.5 in mice, and different subsets populate different locations; chemokine receptor 2 (CCR2) cells, arising from fetal monocytes, are close to the endocardium, whereas CCR2 cells, which derive from the yolk sac, reside throughout the compact myocardium and in the vicinity of developing coronary arteries. In osteopetrotic colony stimulating factor 1–mutant mice that lack myocardium and in the vicinity of developing coronary arteries, which derive from the yolk sac, reside throughout the compact myocardium16,30.

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Whether cardiac macrophage numbers and phenotypes change in the aging human heart is currently unclear, although emerging preclinical data support this idea26–31. Age-dependent pheno-
typic alteration of macrophages, including increased secretion of IL-10 (ref. 32), promote myocardial fibrosis and diastolic dysfunction in older mice. In parallel to the increased myeloid cell production in aging, the number of circulating neutrophils and monocytes rises in senescent mice, as does these cells’ recruitment to the heart11,32.

| Table 1 | Cardiovascular risk factors, current standard therapy and potential action through innate immune cells and their production (hematopoiesis) |
|---|---|---|
| Modifiable? | Therapy | Link to innate immune cells |
| Hyperlipidemia | Yes | Lifestyle modification, statins, fibrates, PCSK9 inhibitors | Increased hematopoiesis via increased macrophage phenotype |
| Obesity | Yes | Lifestyle modification | Adipose tissue harbors inflammatory macrophages, increased hematopoiesis |
| Diet | Yes | Lifestyle modification | Via microbiome? |
| Hypertension | Yes | Lifestyle modification, antihypertensive drugs | Leukocytes increase in vascular wall and in the hypertrophic myocardium |
| Physical inactivity | Yes | Lifestyle modification | Via modulation of leukocyte phenotypes and hematopoiesis? |
| Psychosocial stress | Yes | Lifestyle modification | Increased hematopoiesis via sympathetic signaling, altered leukocyte number and phenotype |
| Smoking | Yes | Lifestyle modification | Leukocytosis and altered leukocyte phenotype? |
| Diabetes | Yes | Diet, oral antihyperglycemic agents, insulin | Increased hematopoiesis via RAGE ligands, etc. |
| Age | No | Currently none, possibly targeting aging hematopoietic and immune system in future | Via altered tissue resident macrophage repertoire in heart and vasculature? |
| Sex | No | N/A | Via sex hormone signaling to HSPC and leukocytes? |
| Family history and genetics | No | Currently none, possibly gene editing in future | Via hyperlipidemia and possibly hematopoiesis, etc. |

CyTOF and single-cell RNA-seq of atherosclerotic plaque and diseased myocardium provide unbiased and much-improved definitions of cell subset repertoires in cardiovascular organs. These methods may identify harmful cell subsets that are targets for precision therapeutics.

Rapidly expanding technology for optical imaging pushes the boundaries in image resolution and stabilization, comprehensive sampling of large areas and the number of biomarkers sampled in parallel. In particular, tissue clearing enables whole-organ microscopy in human and mouse heart, artery and hematopoietic organs. As a consequence, spatial relationships between cells—for instance macrophages and conducting cardiomyocytes or hematopoietic progenitors and stromal niche cells—that alter leukocyte supply in CVD—are revealed in large, representative tissue volumes. In vivo microscopy of cell–cell interaction in plaque, myocardium and marrow will provide dynamic information on proliferation, clonal cell expansion, leukocyte recruitment, departure, antigen presentation and phagocytosis—all processes that hold promise for therapeutic intervention. Completely noninvasive optical, magnetic resonance and PET imaging of cardiovascular inflammation in mice and humans are poised to disrupt drug development, as these modalities will report on drug targets days to weeks after therapy initiation, which is much earlier than traditional endpoints that accrue over years.

Gene editing in immune and hematopoietic progenitor cells, which eventually give rise to macrophages, accelerates our ability to test the functional relevance of proteins in animals, and first human trials indicate potential translatability to patients.
Leukocytes and cardiac conduction. Given their sensitivity to inflammatory stimuli, macrophages’ response to systemic or local danger may divert steady-state macrophage functions. Such functions, which promote organ tasks rather than host defense, may be compromised by inflammation when tissue-resident macrophages die and are replaced by monocytes, for instance after ischemic injury. Of particular relevance to CVD, because macrophages participate in electrical conduction, their depletion leads to arrhythmia and conduction abnormalities in the atria and ventricles in mice. Macrophages influence the membrane potential of conducting cardiomyocytes via electrotonic communication through connexin43 (Cx43)-containing gap junctions, which are small membrane channels that connect the plasma of two cells. This crosstalk shortens the duration of the action potential in cardiomyocytes and may enable ‘bridging’ between two cardiomyocytes that are otherwise not electrically coupled. The long macrophage dendrites observed in the heart support this bridging hypothesis. Overall, macrophages’ participation in conduction leads to more reliable atrioventricular node function. Patch clamp of cocultured mouse cardiac macrophages and ventricular cardiomyocytes documented altered membrane potentials in communicating cells. Therefore, it is likely that the gap junction–enabled charge exchange between macrophages and conducting cells occurs not only in the conduction system, but everywhere in the heart, raising the possibility that atrial and ventricular fibrillation involve altered macrophage activities. Atrial fibrillation, which leads to embolic stroke and worsens cardiac output in patients with heart failure, associates with atrial and systemic inflammatory activity in patients. Whether monocyte-derived macrophages participate in normal or aberrant conduction remains unclear.

Inflammation in coronary heart disease

The inflamed arterial wall. Most deaths from cardiovascular disease are preceded by both systemic and local arterial inflammation. Innate immune cells live in the healthy arterial wall and become more numerous and more inflammatory while atherosclerotic plaques, which are subendothelial deposits of cholesterol, cells, matrix and debris, evolve. Macrophages are the largest leukocyte population in the mouse aorta, and by extension, other arteries.

Studies mapping cellular fate in mice confirm that macrophages residing in atherosclerotic plaque derive from recently recruited monocytes, although these macrophages also proliferate within the plaque, especially if the atherosclerotic lesion is advanced. Some foam cells, which are large lipid-filled phagocytes, may also arise from local smooth muscle cells. Because of the technical challenges involved, in vivo cellular imaging of the mouse arterial wall has only recently been established at a resolution that allows meaningful in vivo data collection. This breakthrough will provide information about cell–cell interactions and plaque cell motility. Questions that can only now be addressed include whether plaque macrophages depart into the circulation or alternatively die locally in the plaque, what exit routes they take and with which cells they interact.

It is hypothesized that plaques arise because of failed efferocytosis—i.e., plaque macrophages’ impaired capability of removing apoptotic cells. The process of efferocytosis, which would counteract the growth of inflammatory lesions, depends on mitochondrial fission in macrophages and calcium-enabled phagocytosis of several apoptotic cells. It is conceivable that macrophages exit atherosclerotic plaques after they consume a meal; however, a macrophage departing an atherosclerotic plaque has never been directly visual-
Acute MI. Acute ischemia triggers rapid accumulation of millions of leukocytes in the under-perfused myocardium. These immune cells’ defense abilities, which evolved as a response to nonsterile injury, harm cardiovascular organs in acute MI. A subset of macrophages, identified through single-cell RNA-seq of infarct leukocytes on day 4 after acute MI in mice, detect danger via the interferon regulatory factor 3 (IRF3)-type 1 interferon pathway, leading to macrophage transcription of inflammatory genes usually associated with viral infection. Interferon-inducible macrophages were termed ‘IFNICs’. Global deletion of either the IRF3- or the type 1 interferon receptor–encoding gene improved post-MI recovery and survival compared to wild-type mice, thereby indicating that mitigating this danger-sensing pathway rebalances the overall innate immune response to myocardial ischemia from one optimized for killing pathogens toward repair support. IFNICs may not only be harmful, but also pursue additional beneficial roles in wound healing, such as removal of debris, regulating angiogenesis or matrix deposition. Targeting specific cell functions that are harmful may be an option to preserve those that are needed for recovery from MI.

As in generic wound healing, ischemia of the heart triggers a temporally defined inflammatory response that is dominated by divergent leukocyte phenotypes. First, cardiac resident macrophages die alongside ischemic myocytes. Massive cell death recruits neutrophils and inflammatory monocytes (Ly6C hi monocytes in the mouse, CD16+ monocytes in humans) that patrol the endothe-

Failing myocardium. Cardiac inflammation is not limited to ischemic myocardium. Despite the wealth of studies describing innate immune pathways in the failing myocardium, cellular resolution of such data is only now emerging. Using flow cytometry, it is possible to isolate pure cardiac cell populations, and cell-specific promoters enable gene deletion in all major cell types, including macrophages. Leukocyte quantification through a sensitive flow cytometry assay reveals that even shortly after acute MI, the nonischemic remote myocardium recruits inflammatory cells, peaking around day 10 after ischemia in mice. This process progressively continues as the left ventricle remodels. The local macrophage population expands because of both local macrophage proliferation and monocyte recruitment from the blood and hematopoietic organs. Recruited macrophages’ net effect on remodeling nonischemic myocardium post-MI is likely detrimental, as using RNA silencing to inhibit monocyte recruitment, starting 1 week after MI, reduces left ventricular fibrosis and dilation.

Heart failure with preserved ejection fraction (HFnEF) is a condition with rising incidence and lack of treatment options. HFnEF does not compromise the contraction of the heart muscle but rather its diastolic filling with blood. In mice in which HFnEF is triggered by hypertension, aldosterone infusion and renal failure, cardiac macrophage numbers rise because of local macrophage proliferation and monocyte recruitment. In addition, cardiac macrophage phenotypes shift, as these cells produce significantly more IL-10, an interleukin that promotes fibrosis. Macrophage-specific deletion of IL-10 improves diastolic dysfunction in mice, indicating that macrophages are causally involved in deleterious matrix build up. However, macrophages likely continue to pursue salutary functions that promote myocyte health. For instance, macrophages provide angiogenic factors that regulate capillary density adaption to hyper trophy. It will therefore be important to therapeutically target a subset of detrimental macrophages, or, if such a subset remains elusive, to target only injurious macrophage functions. When choosing such targets, potential side effects are relevant. For instance, neutralizing IL-10 may lead to enhanced production of inflammatory cytokines.

While myeloid cells are the most numerous leukocyte population in the healthy, ischemic and failing myocardium, others, including mast cells and lymphocytes, may either directly influence fibrosis, angiogenesis and hypertrophy or regulate macrophage phenotypes and supply. Systemically expanding B lymphocytes trigger monocyte bone marrow release and recruitment via chemo- kine (C–C motif) ligand 7 (CCL7) in mice with MI. This discovery led to a clinical trial (NCT03072199) that explores repurposing the CD20 B cell–depleting antibody rituximab, a drug approved for autoimmune disease and lymphoma, as a therapy for acute MI. In CVD, it is well known that nonleukocytes, such as cardiomyocytes, fibroblasts and endothelial cells, participate decisively in inflam-
The bone marrow harbors hematopoietic tissues, which produce billions of blood cells every day. Some of these cells, especially if supplied in excess, promote CVD. Hematopoietic organs are also exceptionally well vascularized; however, very little is known about how the hematopoietic system adapts to and drives cardiovascular pathology. The marrow contains two major cell types: (i) HSPCs that give rise to circulating blood cells and (ii) niche cells that provide the environment in which HSPCs thrive, proliferate and differentiate into blood cells. HSPCs and their progeny resemble a pyramidal hierarchy with about 10,000 bona fide hematopoietic stem cells on top[15]. HSPCs are equipped with Toll-like, cytokine, growth factor and adrenergic receptors, which can sense the circulating signals that rise while CVD develops. HSPCs also rely on niche cell–derived information passed on by bone marrow endothelial cells, macrophages, mesenchymal cells, osteoblasts and perivascular cells[11,213]. Upon integrating such signals, HSPCs adapt their activity to either remain quiescent or produce mature blood cells. The type of cells they give rise to depends on their lineage commitment, which they assume as a result of incoming signals and epigenetic modification[36].

**Box 3 | Hematopoiesis**

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**Cardiovascular risk factors, comorbidities and leukocyte supply.** In addition to hyperlipidemia, other cardiovascular risk factors may influence hematopoiesis (Fig. 1 and Table 1). Diabetes associates with increased circulating leukocytes in patients[68]. In mice, hyperglycemia enhances myeloid cell production[69,70] via neutrophil release of calprotectin (S100A8 and S100A9), a ligand that binds the receptor for advanced glycation end products (RAGE) on HSPCs. Diabetic mice lacking RAGE fail to develop leukocytosis[70]. As neutrophil depletion reduced monocyte counts in diabetic mice, a self-enhancing vicious cycle in which increased myeloid cell levels further enhance myelopoiesis and thus inflammatory myeloid cells expand has been proposed[70].

**Diabetes also affects bone marrow niche cells**[69]. Specifically, it reduces mesenchymal stem cells’ responsiveness to β-adrenergic signaling, and as a consequence, the growth factor G-CSF. By impairing niche cells’ ability to reduce the retention factor CXCL12 after G-CSF injection, diabetes inhibits HSPC mobilization from the marrow with consequences for the clinical transplantation setting[69]. Although this explains why many diabetic patients are ‘poor mobilizers’, the implications of diabetes for the hematopoietic niche, and hence on leukocyte production in CVD, are still mostly unexplored. In mice, long-term exposure to diabetes causes microangiopathy and may thus exhaust the HSPC pool[71]. Further strengthening the argument that metabolic syndrome may affect CVD via leukocyte...
production, mice with diet-induced obesity have increased myelopoesis\(^2\). In obese mice, visceral adipose tissue macrophages release IL-1β into the systemic circulation. This cytokine then stimulates monocyte and neutrophil production by binding HSPC receptors\(^3\).

Psychosocial stress is yet another CVD risk factor\(^{25}\) contributing to inflammation. In mice, adrenergic signaling, which regulates hematopoietic circadian rhythms\(^{31}\), shapes the hematopoietic niche environment after exposure to psychosocial stress. Acting via β3-adrenergic receptors on bone marrow niche cells, stress-induced noradrenaline lowers the quiescence-promoting cytokine CXCL12 and thus accelerates HSPC proliferation and leukocyte supply\(^{36,44}\). HSPC and mature leukocytes also express β-adrenergoreceptors; thus, catecholamines may directly act on hematopoietic cells. Human data indicate that, as in mice, chronic stress activates hematopoiesis\(^7\) and increases leukocyte blood counts\(^6\).

Despite these promising beginnings, how CVD and its risk factors modulate hematopoiesis and blood cell composition and to what degree this determines outcomes in humans remain mostly unclear. For instance, aging, smoking and hypertension associate with increased myeloid cell counts and with CVD; however, we lack mechanistic studies into causal relations. Rheumatoid arthritis and other autoimmune diseases that associate with CVD may also partly exert their adverse influence on cardiovascular health via reshaping immune cell supply and leukocyte phenotypes. It is unclear if and how health-promoting behavior—or its absence—alters hematopoiesis; i.e., the influence of regular exercise, sleep, nutrition and microbiota on the hematopoietic system are mostly unexplored in the setting of CVD.

### Clonal hematopoiesis

The odds ratio for developing CVD doubles in people with clonal hematopoiesis and even quadruples for early-onset MI in these individuals\(^{24}\). In clonal hematopoiesis, HSPCs deriving from one ancestor cell give rise to a variably large share of the blood leukocytes (Fig. 3). The phenomenon is a premalignant condition, and its association with CVD was discovered by studying the exome sequence in blood cells for genes that are mutated in leukemia\(^2,3\). For a person with clonal hematopoiesis, the risk of developing hematologic cancer is ten times higher, at about 1% per year, if the clone size is >20% (ref. \(^{75}\)). If cancer develops, malignant cells carry the mutation previously observed in the clone, thereby indicating that the clone indeed gives rise to cancer. Loss-of-function mutations in DNMT3A, TET2, ASXL1, TP53 and JAK2, among other genes, are frequently found in the expanded cell clones. These driver mutations affect genes encoding epigenetic regulators of cell proliferation; the TET2 gene encodes an enzyme that catalyzes DNA hydroxymethylation\(^5\), an intermediate stage toward demethylation. TET2 deletion increases aortic lesion size, while circulating leukocyte counts remained unchanged. LysM\(^{-}\)–driven deletion of TET2 in myeloid cells and their hematopoietic progenitors likewise caused larger aortic plaques and inflammatory macrophage phenotypes, including increased production of, yet again, IL-1β (ref. \(^{77}\)).

Given that CVD’s association with clonal hematopoiesis is a recent discovery, interesting questions remain open. For instance, some clinical and preclinical studies state that clonal hematopoiesis, despite increasing macrophage numbers in the arterial wall and other organs, may not lead to blood monocytosis\(^{27}\). The number of available data points in the clinical study\(^7\) may be too small to exclude monocytosis for some mutations, and monocytosis could evolve at later time points. A whole-exome sequencing study in Icelanders\(^{66}\) does report an association of clonal hematopoiesis with blood monocytosis but does not mention an association with CVD. Prior data document an association between leukocytosis and CVD in patients\(^{56}\). If there is a hematopoietic clone with an advantage, why does this not increase blood monocyte levels? Does this observation imply that unknown counter-regulatory mechanisms, which are disabled in individuals with leukocytosis, tightly control blood monocyte numbers? Interestingly, TET2 deficiency acts on two different processes: increasing HSPC proliferation and nudging plaque macrophages toward more inflammatory phenotypes\(^7\).
working model is that HSPCs lacking TET2 expand and give rise to monocyte clones and plaque macrophages that have higher inflammasome activation and are thus more atherogenic. TET2 deficiency also impairs mouse recovery from acute MI. We do not yet know what is causing the disease acceleration: clonal hematopoiesis, the inflammatory phenotype of the progeny or a combination of both. Mutation in Tet2 is currently the only factor that has been tested for causally increasing atherosclerosis in mice. Future experimentation will determine whether other driver mutations are also causal for CVD, and why. We must still determine whether clonal hematopoiesis without driver mutations increases CVD risk and what mechanisms lead to clonal hematopoiesis in the absence of a driver mutation. Finally, how clonal hematopoiesis affects other blood cells that promote CVD, especially neutrophils and platelets, should be explored.

Trained immunity

Whether the arterial wall and myocardium are inflamed depends not only on the number of locally present leukocytes, but also on their phenotypes. As most cells in an individual contain the same DNA sequence, the vastly different cellular phenotypes—for instance, between an endothelial cell and a myocyte or an inflammatory monocyte-derived macrophage and a tissue-resident macrophage—are caused by epigenetic regulation of gene expression. Epigenetic DNA modification also regulates cell production rates, fate decisions during myelopoiesis and phenotypes of mature macrophages residing in different tissues. DNA methylation and histone-tail modifications, such as methylation and acetylation, are the most well-studied epigenetic alterations that regulate DNA accessibility and, consequently, gene expression. Such epigenetic marks, which are passed on during cell division, are modulated by environmental stimuli.

Some cardiovascular risk factors represent environmental triggers that entrain memory to the innate immune system via epigenetic DNA modification. The trained immunity concept, which describes how monocytes’ differential reactions to a recall stimulus depend on their prior exposure to (or ‘training with’) either lipopolysaccharide (LPS) or β-glucan, components of the bacterial and fungal cell walls, respectively, has been extended to CVD-relevant signals. Oxidized low-density lipoprotein (LDL), long known to propel atherosclerosis by damaging the arterial wall, leads to epigenetic modification, specifically lower histone 3 lysine 4 trimethylation (H3K4me3), which renders monocytes more inflammatory upon a secondary stimulus. Such epigenetic modification of monocytes may enhance plaque inflammation. Investigating monocytes’ long-lived hematopoietic progenitors and downstream tissue macrophages may reveal more durable effects. Indeed, Ldlr<sup>−/−</sup> mice that switched from a western-type to normal diet retained epigenetic modifications in granulocyte–macrophage progenitor cells for weeks, resulting in more inflammatory innate immune cell phenotypes despite normalized blood cholesterol levels. IL-1β and GM-CSF mediate trained immunity on the HSPC level. These studies considerably broaden the trained immunity concept, extending it to triggers such as cholesterol, cytokines and growth factors acting on hematopoietic progenitor cells rather than mature myeloid cells. The resulting epigenetic marks may persist in circulating monocytes and macrophages recruited to sites of inflammation, including atherosclerotic plaques.

Tissue-resident macrophages in cardiovascular organs could also be modulated by trained immunity, although this has not been tested experimentally. In the steady state, macrophages’ tissue-context-dependent phenotypes are a product of epigenetic modifications. Likely, lifestyle-related risk factors and inflammatory comorbidities affect macrophages in parallel, and these epigenetic modifications could have consequences for macrophage activity, tissue integrity and an individual’s propensity to develop CVD. Thus, even if exposure to cardiovascular risk factors ends, epigenetic modifications that may persist in HSPC, and macrophages could serve as therapeutic targets to reduce inflammatory cells in the arterial wall and myocardium. Experiments using genetic disruption of the epigenetic mechanisms involved in trained immunity are needed to document the causality and specificity of connections between this compelling concept and the role of innate immune cells in CVD.

Therapeutic strategies

Immunotherapy. The CANTOS trial is the first successful immunotherapy trial in CVD (Box 1). A neutralizing antibody (canakinumab) against IL-1β, an inflammatory cytokine made by myeloid cells, reduced CVD events by 15% (ref. 1). All-cause and cardiovascular mortality declined by 31% in responders identified by a prespecified decline in C-reactive protein (CRP), a circulating inflammation biomarker, after the first canakinumab dose. This trial changes concepts of clinical cardiovascular care, which currently does not target inflammation. As the targeted cytokine is involved in epigenetic regulation of hematopoiesis, trained immunity and clonal hematopoiesis, canakinumab’s effects may extend to the phenotypes and numbers of systemic leukocytes.

CANTOS demonstrates that inflammation is a worthwhile drug target in CVD and provides a blueprint for future trials. The higher treatment efficacy in patients with drug-induced decline of CRP suggests that screening for patient subsets with high inflammatory activity is effective, as such screening achieves larger drug effects that can be detected in precisely defined, more economically sized patient populations. The concept of concise patient selection may be extended to other criteria—for instance, presence of clonal hematopoiesis or of somatic mutations in circulating leukocytes. Embracing surrogate endpoints, such as CRP or imaging of inflammation in cardiovascular organs, for instance by immuno–positron emission tomography (immuno-PET) with radioisotope-labeled antibodies or nanoparticles, provides another cost-saving strategy, which may afford more pilot studies, scaled one or two orders of magnitude smaller, and thus de-risk the following outcome trial. Such measures are commonplace in oncology, in which imaging shortly after first drug application identifies nonresponders. Likely, CANTOS will motivate a search for other safe, efficacious targets and other applications for canakinumab—for instance, in the setting of acute MI, wherein the drug may not only reduce reinfarction, which is particularly common during the inflammatory surge post-MI, but also improve myocardial healing. Preclinical studies document that IL-1β increases myelopoiesis after MI and that neutralizing IL-1β reduces post-MI heart failure. As CANTOS monitored hundreds of MI while patients were treated with canakinumab, safety concerns specific to infarct rupture are now addressed. CANTOS reported a higher rate of infections with canakinumab treatment. The experience with similar therapeutics in patients with autoimmune and malignant disease indicates that such risk can be managed, but it will likely require careful risk–benefit analyses and patient selection.

Supporting infarct healing. Timed myeloid cell–depletion experiments in mice indicate that the phenotype transition between inflammatory and reparatory myeloid cells is functionally important for infarct healing. Since a delayed transition compromises tissue repair and recovery from MI, there is an ongoing effort to identify factors that usher in resolution of inflammation. These factors may include macrophage-intrinsic programs, macrophage phenotype alteration as a result of effector cytokines, autonomic tissue innervation, resolvins, macrophage interaction with lymphocytes and/or continued provision of inflammatory Ly6C<sup>hi</sup> monocytes from hematopoietic organs. Because reparatory macrophages support tissue rebuilding and regeneration (via VEGF, IL-10 and others), it is desirable to augment them. One way of doing...
so is curbing over-recruitment of inflammatory leukocytes, which improves outcomes in mice\textsuperscript{21,39}. Alternatively, macrophages could be nudged toward reparative phenotypes by manipulating their gene expression\textsuperscript{40}. In patients, heightened and prolonged inflammation associates with increased post-MI heart failure\textsuperscript{41}. Theoretically, many options exist to dampen inflammatory activity or cell supply, including modulating macrophage phenotypes, inhibiting overproduction and decreasing cell recruitment.

**Regenerating myocardium.** Newborn mice regenerate injured myocardium\textsuperscript{42}, and anecdotal evidence indicates this may occur in newborn humans\textsuperscript{43}. This observation suggests that if we can recreate the yet-to-be-discovered newborn conditions that give rise to myocardial regeneration in adult and elderly myocardium, we may be able to heal failing hearts. Because the immune system changes after birth and macrophages are involved in many organs’ homeostasis and repair, including salamander limb regeneration\textsuperscript{44} and mouse hematopoiesis\textsuperscript{45,46}, investigating the cells’ role in newborn heart repair may provide clues regarding how to therapeutically shape post-MI innate immunity. Infarct macrophage numbers are higher in mouse neonates, and their phenotypes differ from adults\textsuperscript{47}. In newborn mice, macrophage depletion abolishes scar-free regeneration, which associates with a lower angiogenic response to ischemia\textsuperscript{48}. Macrophages may also influence myocyte proliferation\textsuperscript{49}. After myocyte necrosis, the mouse neonatal heart does not recruit monocytes, but rather expands the MHCII$^\text{+}$CCR2$^\text{+}$ resident macrophage subset by local proliferation\textsuperscript{50}. Which unique properties of neonate macrophages support heart regeneration are not known, and their relative contribution to regeneration remains unclear. The data obtained in neonates support the hypothesis that post-MI progenitor cell therapy may modulate macrophages\textsuperscript{51}, as it is now commonly accepted that intramyocardially injected progenitor cells do not survive. Phagocytosis of apoptotic cells, for instance neutrophils, dampens inflammatory macrophage activity\textsuperscript{52}. Whether progenitor cells injected into the heart elicit a specific reparative macrophage phenotype that is distinct from the one triggered by dying myocytes or neutrophils remains to be formally tested.

**Technologies on the horizon.** Emerging therapeutic technologies include gene editing and RNA interference to modulate immune cell production and phenotypes. Both methods were recently applied in promising preclinical studies\textsuperscript{53,54} and early studies on human cells, including CRISPR gene editing in HSPC\textsuperscript{55,56}. As HSPCs are routinely collected and retransplanted in humans, it is conceivable that HSPCs could be edited to correct genetic diseases, such as $\beta$-thalassaemia\textsuperscript{57}, to repair driver mutations causing malignancies or clonal hematopoiesis and to alter inflammatory genes in HSPC-derived leukocytes. Using nonviral delivery strategies\textsuperscript{58}, any gene in immune\textsuperscript{59,60} or endothelial\textsuperscript{61} cells, including multiple or previously difficult to reach targets such as transcription factors, could be edited, deleted or silenced in vivo.

At this time, the intersection of CVD and immunology is highly dynamic, with successful inroads being made from several directions. Clinically, the proof of concept for CDV immunotherapy revealed in CANTOS will inspire subsequent trials targeting inflammation. Fundamentally, the field of CVD research has identified and advanced on forward-looking problems, including cell identity, source, fate, communication and subversion of steady-state tasks by inflammatory responses that compromise cardiovascular health. Embracing these conceptual and technological advances will generate precise drug targets on a large scale. Historically disconnected research areas are closing ranks, as immunology and hematology increasingly focus on residual cardiovascular risk. The rigorous standards of these fields—for instance how to identify leukocytes and their progenitors—are embraced across the board. Harnessing the improved understanding of immunity’s role in oncology already saves lives. Immunotherapy is now poised to accomplish similar feats for CVD, as the required interdisciplinary effort emerges to tackle this task.

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