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Clonal Hematopoiesis: A New Step Linking Inflammation to Heart Failure

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CME/MOC/ECME Objective for This Article: Upon completion of this activity, the learner should be able to: 1) identify genetic mutations associated with increased risk of cardiovascular disease; 2) discuss the impact of clonal hematopoiesis on heart failure; and 3) discuss the current data regarding anti-inflammatory treatment for cardiovascular disease.

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HIGHLIGHTS

- Clonal hematopoiesis is a common condition in the elderly that can result from the acquisition of somatic mutations in HSPCs that confer a selective advantage and allow for clonal cell expansion.
- This clonal population of mutated HSPCs can give rise to leukocytes with altered immune properties, and this condition can adversely impact the cardiovascular system.
- Clonal hematopoiesis may represent a new causal risk factor for cardiovascular disease that can add to the predictive value of the traditional risk factors.
- Understanding the clonal hematopoiesis status of a patient could aid in the development of personalized strategies for anti-inflammatory therapies for cardiovascular disease.

ABSTRACT

Heart failure is a common disease with poor prognosis that is associated with cardiac immune cell infiltration and dys-regulated cytokine expression. Recently, the clonal expansion of hematopoietic cells with acquired (i.e., nonheritable) DNA mutations, a process referred to as clonal hematopoiesis, has been reported to be associated with cardiovascular diseases including heart failure. Mechanistic studies have shown that leukocytes that harbor these somatic mutations display altered inflammatory characteristics that worsen the phenotypes associated with heart failure in experimental models. In this review, we summarize recent epidemiological and experimental evidence that support the hypothesis that clonal hematopoiesis-mediated immune cell dysfunction contributes to heart failure and cardiovascular disease in general. (J Am Coll Cardiol Basic Trans Science 2020;5:196–207) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Heart failure is a complex and prevalent clinical syndrome that afflicts 6.2 million people in the United States alone (1). Heart failure is projected to increase to >8 million Americans by 2030 due in part to the aging of the population. Because advanced age is a major risk factor in the development of heart failure, there is a need to identify the age-related mechanisms that contribute to this pathology and investigate whether these new targets can be leveraged in the development of novel therapies.

Although much of the past research on the mechanisms that contribute to heart failure has focused on cardiomyocyte, fibroblast, and endothelial cell biology, it is increasingly recognized that immune cells and their production of cytokines have critical roles in the pathogenesis of this disease (2). Accumulating evidence shows that inflammatory cytokines can promote heart failure phenotypes. It has been well established that elevations in interleukin (IL)-1β and tumor necrosis factor (TNF)-α can promote progressive left ventricular dysfunction and remodeling in experimental models (3–5). In the patient population, levels of IL-1β and TNFα increase as heart failure worsens, and C-reactive protein levels correlate with impaired exercise capacity and predict death (6-8). Collectively, these observations suggest that immune cell infiltration and the overactivation of cytokine signaling can promote heart failure progression (9).

Clonal hematopoiesis is a condition that is associated with the acquisition of mutations in hematopoietic stem and progenitor cells (HSPCs) that can lead to clonal populations of leukocytes in the blood that exhibit altered immune properties (10,11). Although these clonal expansions in the blood have been known for decades, only recently has it been appreciated that they are associated with increases in all-cause mortality, coronary heart disease, stroke, and heart failure prognosis. Here we review the epidemiological and experimental studies that suggest that clonal hematopoiesis represents a new causal risk factor for cardiovascular disease and discuss how this could impact our view of how to treat patients with heart failure and other cardiovascular conditions.

ABBREVIATIONS AND ACRONYMS

ASXL1 = additional sex combs like 1

DNMT3A = DNA methyltransferase-3A

hsCRP = high-sensitivity C-reactive protein

HSPCs = hematopoietic stem and progenitor cells

IL = interleukin

JAK2 = janus kinase 2

MPN = myeloproliferative neoplasm

PPM1D = protein phosphatase, Mg2+/Mn2+ dependent 1D

TET2 = ten-eleven translocation-2

TNF = tumor necrosis factor

TP53 = tumor protein 53

VAF = variant allele fraction

ANTI-INFLAMMATORY STRATEGIES IN THE TREATMENT OF HEART FAILURE

Evidence for the involvement of inflammation has led to an interest in evaluating anti-inflammatory therapies for the treatment of patients with heart failure (12,13). For example, several small randomized trials and short-term studies have evaluated small molecules that inhibit TNF-α for their efficacy in treating heart failure, and encouraging data have been reported (14-16). In a small placebo-controlled study evaluating TNF-α antagonism with the soluble TNF receptor etanercept, improvements were found in left ventricular ejection fraction and remodeling, and there was a trend toward improved patient functional status (17). However, a subsequent large randomized trial failed to show an effect of etanercept on clinical status, hospitalization, or death in patients with chronic heart failure (18). Similarly, the treatment of patients with heart failure with infliximab, a monoclonal antibody to TNF-α, did not improve clinical conditions, and high doses of this agent were associated with adverse events (19). These results may be explained by the complex effects of TNF-α on the heart, as it has been described to exert cardioprotective actions that counterbalance its detrimental effects on the heart (20).

There has also been interest in targeting the inflammatory cytokine IL-1β in clinical heart failure. In pilot studies of patients with ST-segment elevation myocardial infarction, the recombinant IL-1 receptor agonist anakinra was shown to have a favorable safety profile and to diminish the inflammatory response and heart failure incidence in these patients (21-23). Small proof-of-concept studies in patients with systolic heart failure showed that anakinra improved exercise capacity and quality of life (24-26). More recently, escalating doses of the neutralizing IL-1β antibody canakinumab were evaluated in the large placebo-controlled CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) trial for outcomes in patients with a previous myocardial infarction and sustained levels of high-sensitivity C-reactive protein (hsCRP) (27). Canakinumab therapy led to reductions in the primary composite outcome of myocardial infarction, stroke, or cardiovascular death at the higher doses (28). Subsequent analysis revealed that the treated cohort could be subgrouped into responders and nonresponders to the therapy. Patients who achieved lower concentrations of hsCRP (to <2 mg/l) after initiating canakinumab responded better to IL-1β inhibition in terms of cardiovascular benefits, whereas no significant benefit was observed among those with hsCRP concentrations at 2 mg/l or above (29). A similar relationship was observed with reductions in interleukin (IL)-6 levels in response to canakinumab and cardiovascular event reduction (30).

The CANTOS cohort was composed of patients with a history of heart failure (22%), and subsequent analyses revealed that therapy with canakinumab led to a dose-dependent reduction in heart failure hospitalization and the composite of hospitalization for heart failure or heart failure-related mortality (31). Similar to the results of the original study, patients who achieved reductions in hsCRP concentrations to <2 mg/l also displayed reductions in the heart failure outcomes, whereas those at 2 mg/l or above did not. Within the CANTOS trial cohort, an independent single center sub-study found that patients with heart failure treated with canakinumab had significant improvements in peak oxygen consumption at 3 months and in left ventricular ejection fraction at 12 months of therapy (32).

Collectively, these studies indicate a complex interplay between inflammation and heart failure. Thus, a deeper understanding of the complexities of the immune system may be useful for the development of effective anti-inflammatory therapies for heart failure.

AGE-RELATED CLONAL HEMATOPOIESIS

Previous human genetic research in cardiovascular disease has largely focused on the risk caused by germline variations in DNA. However, recent evidence shows that there is a surprisingly high degree of mosaicism in the human body that results from the time-dependent acquisition of somatic mutations that lead to cell-to-cell genomic differences (33). Genetic clones carrying somatic mutations have been detected across normal tissues to different extents that depend on the tissue's proliferative activity, exposure to environmental mutagens, or natural architecture and microenvironment (34). This genomic mosaicism can occasionally give rise to large clonal events, particularly in proliferative cell populations where the somatic mutation provides a selective "fitness" to the cell relative to neighboring cells. This positive selection can be driven by mutations in oncogenes or tumor suppressor genes and may represent an early stage of tumorigenesis.

FIGURE 1 Summary of Clonal Hematopoiesis Reported in 3 Studies

(A) The frequency of clonal hematopoiesis increases with age. Shown are the time courses of clonal hematopoiesis detection in 3 studies of patient cohorts that were unselected for hematologic disorders (39,40,46). **(B)** A comparison of frequencies of known and unknown driver genes in the 3 studies. In Jaiswal et al. (39), the exome sequence of 160 candidate driver genes (i.e., genes known to be recurrently mutated in hematologic malignancies) were analyzed. In Genovese et al. (40), whole exome sequencing revealed that a substantial proportion of clonal hematopoiesis events could not be attributed to known driver genes. The study of Zink et al. (46) used a nonbiased whole genome sequencing approach. The frequency of mutations in candidate driver genes accounted for approximately 20% of the total number of clonal events. This work indicates that the occurrence of clonal hematopoiesis approaches inevitability with advancing age and that we have a limited understanding of the diverse mechanisms that can contribute to this process.

HSPCs typically reside in the bone marrow, give rise to multiple cell types in the blood, and regenerate via a self-renewal process (35). Human HSPCs acquire random somatic mutations over time (36). Although most of these mutations will have little or no effect on cellular fitness, some provide HSPCs with a competitive advantage within the bone marrow niche that can be detected as a clonal expansion in the blood and other tissues infiltrated by blood cells. These clonal expansions can occur in healthy individuals who lack overt signs of hematologic disorders, and they become prevalent with age (**Figure 1A**). This condition has been historically referred to as clonal hematopoiesis (the terminology that will be used here) and more recently as clonal hematopoiesis of indeterminate potential (37) and age-related clonal hematopoiesis (38) to distinguish it from the clonal expansions that are components of hematologic malignancies. Data suggest that single mutations in leukemia-related genes can give rise to clonal hematopoiesis, and, accordingly, individuals exhibiting this condition display an increased risk of hematologic malignancies (39,40). However, epidemiological studies show that most individuals with detectable clonal hematopoiesis never develop blood cancer because this is a

relatively rare condition requiring the accumulation of multiple additional oncogenic gene mutations within the stem cell clone (41).

It has long been appreciated that clonal hematopoiesis occurs in elderly individuals who lack a detectable hematologic disorder. Early work was based on studies analyzing the ratio of maternal to paternal X chromosome inactivation in leukocytes. For example, a study of X chromosome inactivation skewing in 1996 reported clonal expansions in 38% of healthy women over the age of 60 years, but this condition was rarely detected in young women (42). Subsequently, it was reported that X chromosome inactivation skewing is associated with mutations in the epigenetic regulator ten-eleven translocation-2 (*TET2*), a blood cancer "driver gene" (43). In 2014, 3 independent studies used exome sequencing of peripheral blood to document the widespread occurrence of clonal hematopoiesis in various cohorts (39,40,44). These studies largely focused on clonal events associated with the amplification of driver genes with mutations associated with leukemia. The most prevalent mutations occurred in 3 epigenetic regulators that have been implicated in the control of hematopoiesis (**Figure 1B**). These genes are DNA methyltransferase 3A (*DNMT3A*), *TET2*, and

additional sex combs like 1 (*ASXL1*). In addition, clonal expansions associated with mutations in tumor protein 53 (TP53); Janus kinase 2 (JAK2); and protein phosphatase, Mg2+/Mn2+ dependent 1D (PPM1D) were also prevalent (**Figure 1B**).

The detected frequency of clonal hematopoiesis depends on the leukocyte DNA sequencing methodology that is used by the study (**Figure 1**). For example, Jaiswal et al. (39) used data from relatively shallow DNA sequencing to investigate clonal somatic mutations in the exomes of 160 candidate genes that are recurrently mutated in hematologic cancer in individuals who are unselected for hematologic phenotype. They estimated that approximately 10% of individuals over the age of 70 years possessed at least 1 clonal mutation in their white blood cells. In contrast, using an ultra-deep, error-corrected sequencing of candidate driver genes in the blood capable of detecting a variant allele fraction as low as 0.03%, clonal events could be detected in 19 of 20 middle-aged individuals (45). This study also analyzed driver gene mutations in B cells, T cells, and myeloid cells and provided evidence that the same mutation was present in multiple blood lineages of the same individuals, indicating that these mutations occur in progenitor cells and are then passed on to the progeny immune cells in the blood. Collectively, these data suggest that small initiating mutations in HSPCs are ubiquitous by middle age and that the expansion of these clones occurs with advancing age in a portion of individuals.

A similar age-dependent increase in clonal events was detected in a study that used data from whole exome sequencing, but candidate driver mutations could only be found in approximately one-half of these patients with detectable clonal hematopoiesis (40). Notably, a study by Zink et al. (46) used a non-biased whole genome sequencing approach on peripheral blood to assess the extent of clonal hematopoiesis in 11,262 Icelanders. Unlike prior studies that focused on exome sequencing approaches, this study was able to detect clonal hematopoiesis at very high frequencies in the elderly, and they posited that this condition trended toward inevitability. Indeed, it was found that the overall frequency of mutations increased from 0.5% in individuals younger than 35 years to more than 50% in individuals older than 85 years. Interestingly, when the authors examined the frequency of mutations in candidate driver genes in this cohort, it was found that they only accounted for a small proportion (~20%) of the total number of clonal events,

suggesting there are many as-yet-unidentified driver genes or other mechanisms that give rise to clonal hematopoiesis (46) (**Figure 1B**).

THERAPY-RELATED CLONAL HEMATOPOIESIS

Therapy-related clonal hematopoiesis results from the genotoxic stress of chemotherapy or radiation therapy that creates a situation that selects for the expansion of small pre-existing clones in the hematopoietic cell pool that are resistant to these stressors. Therapy-related clonal hematopoiesis shows an overlapping but distinct mutational landscape compared with the aging-associated clonal hematopoiesis described previously (47-49). Although mutations in DNMT3A, TET2, and ASXL1 are frequently observed in age-related clonal hematopoiesis, TP53 and PPM1D mutations are predominantly enriched in the leukocytes of patients who were previously exposed to cancer treatments for either hematopoietic or solid tumors (48). The association between genotoxic stress and clonal hematopoiesis can be appreciated by comparing the mutational landscape between de novo myeloid dysplastic syndrome/acute myeloid leukemia and therapy-related myeloid dysplastic syndrome/acute myeloid leukemia in cancer survivors (50,51). These studies suggest that the cancer therapy is not mutagenic per se but that pre-existing small mutant clones expand rapidly after cancer treatment. These epidemiological findings are consistent with experimental studies showing that Tp53- and Ppm1d-mutant cells undergo clonal expansion in mice subjected to genotoxic stress including various anticancer agents (51-53). Mutations in these genes are thought to confer DNA damage resistance to the clones although some controversy exists about the exact mechanism of this competition (54).

CLONAL HEMATOPOIESIS AND CARDIOVASCULAR DISEASE: EPIDEMIOLOGICAL DATA

A number of studies have documented a positive association between clonal hematopoiesis and mortality (39,40,46,55). Although it is clear that some of these mutations increase the risk of developing blood cancer (39,40), this condition is relatively rare in the population and cannot account for the observed increase in mortality in these studies. Instead, the mortality associated with clonal hematopoiesis has been associated with large increases in the incidence of cardiovascular disease (39,56). Jaiswal et al. (39)

initially reported that individuals with clonal hematopoiesis had significantly increases in the risks of incident coronary heart disease and ischemic stroke after adjusting for age and traditional risk factors. This increased risk was notable when the variant allele fraction (VAF) of the mutation within the leukocyte pool was 0.10 or greater. In this analysis, the risk of coronary heart disease and ischemic stroke was higher in carriers of clonal hematopoiesis than for individuals with traditional risk factors such as hypertension and high body mass index. Because this was an unplanned secondary analysis, Jaiswal et al. (56) performed a follow-up investigation. For this analysis, whole exome sequencing was performed on blood samples obtained from 4 case-control studies consisting of 4,726 individuals with coronary heart disease and 3,529 control individuals. In nested, case-control analyses of 2 prospective cohorts, carriers of clonal hematopoiesis had a risk of coronary heart disease that was 1.9 times greater than noncarriers, which was comparable with what was found in the prior study. In 2 retrospective case-control cohorts for the evaluation of early-onset myocardial infarction, participants with clonal hematopoiesis had a risk of myocardial infarction that was 4.0 times as great as in noncarriers. In these analyses, mutations in *DNMT3A*, *TET2*, *ASXL1*, and *JAK2* were each individually associated with coronary heart disease (56).

More recently, clonal hematopoiesis has been associated with poor outcome in patients with heart failure. Dorsheimer et al. (57) analyzed the amplification of mutations in candidate clonal hematopoiesis genes in bone marrow–derived mononuclear cells by deep targeted amplicon sequencing in a cohort of 200 patients who underwent autologous bone marrow treatment for acute myocardial infarction. Despite the relatively young age of this cohort (median age of 65 years), the prevalence of clonal hematopoiesis was 18.5% for individuals with a VAF of 2% or greater. Most of the detected mutations occurred in *DNMT3A* and *TET2*, and patients exhibiting these forms of clonal hematopoiesis displayed significantly worse long-term clinical outcomes including death and death combined with rehospitalization for heart failure (57). When patients were grouped based on VAF, it was found that there was a dose-dependent relationship between clone size and clinical outcome, and this study indicated that small clone sizes (between 1% and 2% VAF) were associated with worse outcome. Collectively, these human data indicate a connection between clonal hematopoiesis and heart failure as well as atherothrombotic cardiovascular disease.

CLONAL HEMATOPOIESIS AND CARDIOVASCULAR DISEASE: EXPERIMENTAL DATA

Because epidemiological studies are descriptive, they generally cannot distinguish whether clonal hematopoiesis and cardiovascular disease are causally linked or epiphenomena of the aging process. Thus, potential mechanistic links between clonal hematopoiesis and cardiovascular disease have been explored in model systems (10,11). To date, these experimental studies have focused on hematopoietic cell mutations in Tet2, Dnmt3a, and JAK2, and these studies are discussed below.

TEN-ELEVEN TRANSLOCATION-2. TET2 is a multifunctional epigenetic regulator that can function as a methylcytosine deoxygenase that activates transcription via demethylation of cytosine (58-60). Its activity is context dependent, and it has pleiotropic roles in hematopoiesis including the regulation of stem cell self-renewal, lineage commitment, and terminal differentiation. For example, loss-of-function mutations of Tet2 result in enhanced self-renewal of the hematopoietic stem cells and lead to malignant transformation (61,62). Furthermore, Tet2 mutations in myeloid cell populations are associated with altered outcomes in models of cancer and sepsis (63-65).

Murine studies indicate that Tet2-mediated clonal hematopoiesis may cause the development of atherosclerosis (56,66). In the initial study of Fuster et al. (66), a competitive bone marrow transplantation approach was performed to introduce a small number of Tet2-deficient hematopoietic cells to mimic the human scenario of individuals carrying a TET2 somatic mutation that gradually expands over time. Tet2-deficient HSPCs displayed progressive expansion into all immune cell progeny with a slight bias toward cells of myeloid lineage. The expansion of the Tet2-deficient cells did not affect white blood cell numbers or impact other immunologic parameters, which is consistent with what is observed in carriers of clonal hematopoiesis in the human population. This model of Tet2-mediated clonal expansion led to a marked increase in plaque size in hyperlipidemic low-density lipoprotein receptor-deficient mice. Consistent with these observations, it was independently reported that complete bone marrow transplantation of hyperlipidemic mice with Tet2-deficient cells led to an increase in vascular plaque size and general tissue inflammation (56), although these latter features are typically not observed in individuals with clonal hematopoiesis. Finally, both

FIGURE 2   Tet2-Deficiency Activates Many Steps in the Production of IL-1β

Tet2-deficiency activates many steps in the production of interleukin (IL)-1β by macrophages. This condition increases IL-1β transcript expression by increasing histone acetylation at the *ILL1β* promoter. Tet2-deficient cells also exhibit increased NLRP3 expression and caspase-1 activity. Accordingly, Tet2 deficiency leads to elevated levels of pro–IL-1β processing and IL-1β secretion. HDAC = histone deacetylase.

studies showed that myeloid-specific ablation of Tet2 was sufficient to promote atherosclerosis development (56,66).

Recently, we provided experimental evidence that Tet2-mediated clonal hematopoiesis could have a causal role in the development of heart failure (67,68). To assess the impact of Tet2-mediated clonal hematopoiesis on cardiac pathology, experimental ischemic and nonischemic heart failure models were achieved by the surgical ligation of the left anterior descending artery of the heart and transverse aortic constriction, respectively (67). In a separate study, Tet2 loss of function was evaluated in a model of heart failure that was induced by the continuous infusion of angiotensin II (68). In all of these models, mice displayed greater cardiac dysfunction under conditions in which mice received a competitive bone marrow transplant with Tet2-deficient cells. The cardiac dysfunction caused by the hematopoietic Tet2 mutation was associated with structural changes to the heart, including enhanced hypertrophy and fibrosis. As had been seen in the atherosclerosis model, these cardiac effects could be recapitulated in mice deficient for Tet2 in myeloid cells, suggesting a

contribution of myeloid cell–mediated inflammation to this condition (67,68).

Multiple lines of evidence suggest that TET2-mediated clonal hematopoiesis contributes to the pathogenesis of cardiovascular disease through macrophage proinflammatory activation that involves IL-1β/NLRP3 inflammasome activation (**Figure 2**). Stimulation of macrophages from Tet2-deficient mice with lipopolysaccharide and interferon gamma leads to the activation of cytokines and chemokines including IL-1β and IL-6 (66). Elevations of IL-1β protein and transcript could be detected in the injured myocardium (67) and atherosclerotic plaques (66) of mice implanted with Tet2-deficient bone marrow. Consistent with a previous report (69), it was found that Tet2 regulates IL-1β expression through its ability to modulate histone acetylation rather than DNA methylation (66). Evidence for this comes from the observations that the *Il1β* promoter displays greater histone H3 acetylation in Tet2-deficient macrophages, and Tet2 overexpression in macrophages can suppress IL-1β expression independent of its catalytic activity to oxidize 5-methylcytosine. Tet2 deficiency under these

CENTRAL ILLUSTRATION Clonal Hematopoiesis Can Alter Immune Cell Function and Contribute to Heart Failure

HSPC   Somatic
mutation   Clonal
hematopoiesis

Prevalent in the "healthy elderly" and
patients subjected to genotoxic stress

↑Inflammation
↑Fibrosis
↓Cardiac
function

Differentiated
immune cells in the
systemic circulation

Infiltration of the heart
by mutant cells and
modulation of inflammation

Mutated gene	Potential mechanisms
Tet2	Upregulation of IL-1ß, IL-6 NLRP3 inflmmasome
Dnmt3a	Upregulation of CXCL1, 2 and IL-6 ↑Th17/T reg ratio
Jak2 V617F	Upregulation of Stat-1 signaling, IL-1ß, IL-6, TNFα ↑NET formation

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Hematopoietic stem and progenitor cells (HSPCs) acquire random somatic mutations over time. Occasionally, these mutations occur in driver genes **(yellow circles)** that provide the HSPC with a competitive advantage, and this leads to clonal expansion in a limiting bone marrow niche. This process is referred to as clonal he-matopoiesis, and it can be prevalent in individuals who lack overt signs of hematologic disorders. These mutant HSPCs give rise to progeny immune cells that harbor the mutant gene. This condition may alter the phenotype of the leukocyte and contribute to heart failure.

conditions also led to the up-regulation of NLRP3 expression and caspase-1 activity, a component of the inflammasome that converts pro-IL-1β to its active form. Finally, treatment with a small molecule NLRP3 inflammasome inhibitor reversed the accelerated heart failure (67) and atherosclerosis (66) that are caused by the expansion of Tet2-deficient hemato-poietic cells.

The findings from these experimental studies may have relevance for our understanding of the CANTOS trial outcomes. As discussed previously, responders who achieved hsCRP reduction to <2 mg/l displayed decreased outcomes of myocardial infarction, stroke, or cardiovascular death, whereas those who did not achieve substantial reductions in hsCRP showed little or no benefit from the drug (29). A similar depen-dence between event and mortality reduction on the

magnitude of hsCRP reduction was also observed in the heart failure cohort within this trial (31). What could determine this differential response to canaki-numab therapy? Based on the mechanistic analyses described previously, the possibility was raised that individuals within TET2-mediated clonal hemato-poiesis would be better responders to canakinumab (66). In an exploratory analysis, Svensson et al. (70) assayed for candidate clonal hematopoiesis driver gene mutations in 3,964 patients enrolled in the CANTOS trial. In concordance with the experimental work, it was found that individuals with somatic TET2 mutations exhibited a better response to canakinu-mab therapy than patients with no detectable clonal hematopoiesis. These findings indicate the clinical utility of analyzing clonal hematopoiesis in the pa-tient population (i.e., that can be predictive of a

patient's response to a drug), and they suggest a precision medicine approach for the application of anti-inflammatory therapies for patients with cardiovascular disease.

DNA METHYLTRANSFERASE 3A. DNMT3A is an enzyme that modulates gene transcription by catalyzing DNA methylation (71). As discussed previously, mutations in DNMT3A are prevalent in individuals who display clonal hematopoiesis with no overt hematologic disorder (39,40). DNMT3A mutations are generally believed to result in loss of its enzymatic activity (71). These mutations result in the enhanced self-renewal of HSPCs, leading to their clonal expansion (72). Accumulating evidence suggests that DNMT3A mutations in HSPCs will also affect the phenotypes of their blood cell progeny including mast cells, macrophages, and T cells (73-75). Recently it was reported that patients with aortic valve stenosis harboring a DNMT3A mutation demonstrated a significantly elevated ratio of the pro-inflammatory TH17 cells over the anti-inflammatory regulatory cells (76).

Our recent study assessed the impact of HSPC Dnmt3a mutations on experimental heart failure (68). In this study, Dnmt3a deficiency in lineage-negative hematopoietic cells was achieved by CRISPR/Cas9 editing, and these cells were then transplanted into irradiated mice. In contrast to the disruption of Tet2 by CRISPR/Cas9 gene editing, Dnmt3a disruption did not result in the detectable expansion of the mutant cells. These observations are consistent with prior studies of Dnmt3a (77), and they illustrate the gene-specific actions of Tet2 and Dnmt3a in the context of clonal hematopoiesis. Despite this lack of cell expansion, a low level of hematopoietic cell Dnmt3a deficiency led to greater cardiac hypertrophy, fibrosis, and dysfunction in the angiotensin II infusion model (68). The enhanced pathology caused by the Dnmt3a-deficient state was accompanied by greater macrophage infiltration and the elevation of T-cell markers in the myocardium. Collectively, these data provide the first experimental evidence that DNMT3A mutations in hematopoietic cells can contribute to cardiovascular disease.

JANUS KINASE 2. JAK2 is a nonreceptor tyrosine kinase that transmits intracellular signals downstream of various cytokine receptors. Although JAK2 is broadly expressed, the activating mutation JAK2 G1849T (V617F) in hematopoietic cells is commonly associated with rare myeloproliferative neoplasms (MPNs) including polycythemia vera, essential thrombocytopenia, and myelofibrosis (78,79). These diseases can contribute to an increased incidence of

myocardial infarction, stroke, and deep vein thrombosis due to increases in blood viscosity, clotting, leukocytosis (80), and neutrophil extracellular trap (NET) formation (81,82). In addition, it appears that individuals exist with $JAK2^{V617F}$-mediated clonal hematopoiesis in the absence of a hematologic malignancy (i.e., in the absence of overt changes in erythrocytes, platelets, or leukocytes) (40,46,56). However, the transformation potential of $JAK2^{617F}$ in the erythroid and megakaryocyte progenitors makes it difficult to establish animal models of clonal hematopoiesis because they are confounded by the MPN phenotype (81,83,84). For example, using LyzM-Cre mice to restrict the expression of $JAK2^{V617F}$ to myeloid cells will give rise to MPN phenotypes because this system permits the ectopic expression of this mutation in a small fraction of HSPCs. Furthermore, because of the transformation activity of the mutation, there is controversy as to whether $JAK2^{V617F}$-mediated clonal hematopoiesis represents a discrete clinical entity or an early stage of an MPN (85-87).

To evaluate the impact of $JAK2^{V617F}$-mediated clonal hematopoiesis on the pathogenesis of heart failure in the absence of confounding MPN phenotypes, we established a mouse model in which myeloid-restricted $JAK2^{V617F}$ is expressed in leukocytes that differentiate from lineage-negative bone marrow cells that are transduced with the mutant transgene (88). These mice do not show blood cell count abnormalities or detectable signs of MPN. In addition to unperturbed hematopoiesis in the unchallenged condition, these mice do not display cardiac abnormalities at baseline. This is in contrast to the baseline cardiac hypertrophy that is seen in mice that express $JAK2^{V617F}$ from the *vav-1* promoter, suggesting that the polycythemia vera and essential thrombocythemia phenotypes that occur in this model can contribute to heart failure per se (84). Although mice with myeloid-restricted $JAK2^{V617F}$ expression displayed no baseline phenotype, they exhibited enhanced features of heart failure in models in which the myocardium was injured (88). Depending on the model, myeloid-restricted $JAK2^{V617F}$ expression was associated with enhanced macrophage infiltration of the myocardium and elevated levels of transcripts encoding for IL-1β and IL-6. These results raise the possibility that the acquisition of mutations within monocyte/neutrophil-restricted progenitor cells could account for $JAK2^{V617F}$ clonal hematopoiesis in the absence of MPN phenotypes and thereby result in elevated cardiovascular disease incidence (85).

$JAK2^{V617F}$ is constitutively active and can regulate downstream targets without the requirement for

cytokine stimulation. However, binding to a cytokine receptor scaffold is still required for JAK2^{V617F} to transmit signals, and the scaffold that confers this function in myeloid cells was unknown. In our study, it was shown that interferon gamma receptor 1 (IFNGR1) is required for STAT1 phosphorylation and the up-regulation of several interferon-responsive genes (88). Based on these data, we propose that JAK2^{V617F} promotes proinflammatory characteristics in myeloid cells through the activation of the IFNGR1-JAK2-STAT1 signal transduction pathway. These studies further illustrate the mechanistic differences between JAK^{V617F} and Dnmt3a and Tet2 driver gene mutations.

PERSPECTIVE AND FUTURE DIRECTIONS

Accumulating evidence suggests that clonal hematopoiesis is a new causal risk factor for heart failure and atherothrombotic disease that shares features with hematologic malignancies. It represents a new mechanism of cardiovascular disease involving the clonal expansion of HSPCs that results in part from their acquisition of somatic mutations in pre-leukemic driver genes. These driver gene mutations are passed on to the leukocyte progeny of the HSPCs, altering their immunomodulatory properties and impacting cardiovascular tissues (Central Illustration). Because of the prevalence of this condition, it may be warranted to evaluate an individual's clonal hematopoiesis status in trials that evaluate anticytokine therapies for cardiovascular disease.

Clonal hematopoiesis research is in its infancy, and there are many unresolved questions that can be addressed by additional research. Although the data are currently lacking, it is reasonable to assume that clonal hematopoiesis will impact multiple pathological processes, beyond cardiovascular disease and hematologic cancer, that involve alterations in immune cell function. Presumably, gene-specific and disease-specific effects will be observed when comparing the different clonal hematopoiesis drivers due to inherent differences in the functions of the proteins encoded by the mutated genes. Data from a limited number of experimental studies have documented numerous mechanistic differences in how mutant forms of Tet2, Dnmt3a, and JAK2 differentially impact the hematopoietic cells and cardiovascular remodeling. In this regard, it is possible that some clonal hematopoiesis driver genes will be highly pathological, whereas others will be relatively benign with regard to their effects on disease progression. Thus, benign forms of clonal hematopoiesis would effectively represent false-positive events in terms of how individuals are typically grouped in epidemiological studies. Furthermore, it is becoming clear that the incidence of clonal hematopoiesis is underestimated in studies that only focus on driver gene candidates that are recurrently mutated in hematologic malignancies (i.e., the "clonal hematopoiesis of indeterminate potential" genes). Thus, it is likely that many individuals with clonal expansions in hematopoietic cells are falsely identified as negative due to our limited understanding of the mechanisms that contribute to these phenomena. In toto, these considerations indicate that additional epidemiological and mechanistic studies are required to address these issues and increase our understanding of how clonal hematopoiesis contributes to cardiovascular diseases.

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KEY WORDS DNMT3A, Il-1β inflammasome, JAK2, TET2, TNF-α

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