HHS Public Access

Author manuscript

Trends Cell Biol. Author manuscript; available in PMC 2023 September 28.

Published in final edited form as:

Trends Cell Biol. 2023 August ; 33(8): 695–707. doi:10.1016/j.tcb.2022.12.001.

Clonal hematopoiesis and inflammation – the perpetual cycle

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Abstract

Acquired genetic or cytogenetic alterations in a blood stem cell that confer clonal fitness promote its relative expansion leading to clonal hematopoiesis (CH). Despite a largely intact hematopoietic output, CH is associated with a heightened risk of progression to hematologic malignancies and with non-hematologic health manifestations, including cardiovascular disease and overall mortality. We focus on the evidence for the role of inflammation in establishing, maintaining and reciprocally being affected by CH. We describe the known pro-inflammatory signals associated with CH and preclinical studies that elucidated the cellular mechanisms involved. We review the evolving literature on early-onset CH in germline predisposition conditions and the possible role of immune dysregulation in this context.

Intrinsic and extrinsic forces and their interactions that shape the hematopoietic landscape

Adaptive behaviors underlie the dynamics of individual cells and clonal cell populations within a single organism or an organ. This adaptation is itself dynamic and is shaped from the time of development through growth and aging of the organism. In the past decade examples of relative fitness and clonally dominant states have been documented in various tissues including blood [1–3], skin [4], lung parenchyma [5], esophagus [6], colonic epithelium [7], liver parenchyma [8], and endometrium [9]. The mechanisms that lead to the establishment, selective adaptation, and growth of these genetically identified clones are yet to be determined. Mutational burden increases linearly with age in a given tissue [6,10], although the rate of mutations is different between tissues, the lowest being in blood and the highest in colonic tissue [11]. Assuming stochastic acquisition of mutations in a cell in a given tissue, it remains unclear what prompts the advantage of a clone with a somatic mutation over others. The reasons for the selective advantage of a mutant clone may be constant or may change with age or environmental stressors. What constitutes as 'fitness' in a cell at one point could potentially be detrimental for it in another condition.

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Declaration of interests

The authors declare no competing interests.

Hence, the forces shaping clonal fitness are likely to be both cell-intrinsic and cell-extrinsic, encompassing hematopoietic as well as non-hematopoietic niche components.

In this review we focus on the clonal fitness of the blood cells due to acquired somatic mutations, and we examine the literature for evidence of various influences that promote clonal fitness, emphasizing the role for inflammation. We also evaluate the phenomenon of clonal hematopoiesis (CH) in the context of germline predisposition conditions and how constitutional genetic mutations may influence the environmental landscape for blood clones with acquired mutations. The data discussed are largely based on preclinical animal models, but studies from large human cohorts are enriching our knowledge at a fast pace.

Clonal hematopoiesis and human health

CH is a non-malignant hematologic phenomenon characterized by the relative expansion of a mutant hematopoietic stem and progenitor cell (HSPC) clone associated with the acquisition of somatic mutations. Original observations of a skewed hematopoietic output were first described in the 1960s with mosaic X chromosome activity [12,13]. Decades later, with the advent of large-scale sequencing, the association of recurrent genetic mutations and CH started to emerge [1,2,14,15]. Initially coined clonal hematopoiesis of indeterminate potential (CHIP) for clonal outgrowths with leukemia-driving gene mutations, it is now recognized that CH may occur in association with mutations in genes that are not typically seen in malignancies, noncoding mutations, as well as clonal growth associated with cytogenetic abnormalities [16,17]. The leukemia-associated genes with recurrent mutations implicated in CH have grown since the original publications in 2014, however, the top three remain *DNMT3A*, *TET2*, and *ASXL1*. The other genes underlie CH with more variable incidence between cohorts, and appear at a lower frequency as they are more likely to be associated with symptomatic disease states [18]. The prevalence of CH varies depending on the assays used and the depth of sequencing achieved. By whole exome or genome sequencing, clonal growths without a ‘driver’ mutation have been identified at all ages, reaching a prevalence of up to 50% by the ninth decade [16]. Error-corrected sequencing of candidate genes that can detect clones as small as 0.03% in size also demonstrated a higher prevalence of CH with multiple gene variants [19]. Most commonly used platforms employ panels of targeted leukemia-associated genes or mutational hotspots of those genes, that typically report a CH prevalence of 30–90% by age 90 years [20,21]. The CH mutations occur in early multipotent progenitors, possibly HSPCs, because mutational analyses of lymphoid and myeloid cells have confirmed the presence of the same variants [19]. There are subgroups of CH that may have underlying biological differences from the original definition that are worth noting. The genetic landscape of therapy-related CH is characterized by disproportionately increased somatic mutations in *TP53* and *PPM1D* that likely expand in the context of genotoxic chemotherapy or radiotherapy [22–25]. In adult patients these clones are typically pre-existing and are selected for after treatment, whereas in children they appear after therapy, although sequencing depth and the low prevalence of CH in children should be taken into consideration [26]. Premalignant CH has also been identified in patients who develop lymphoid malignancies, and carries a different mutational landscape, referred to as lymphoid-CHIP to distinguish it from myeloid-CHIP which is dominated by the *DNMT3A*, *TET2*, and *ASXL1* variants [27]. Age-related mosaic

chromosomal alterations (mCAs) are also markers of clonal outgrowths [28]. Irrespective of the etiology and the underlying genetic mutation, the presence of CH is associated with a heightened risk of developing hematologic malignancies [1,2,25,27,29], and thus understanding mechanisms that establish and maintain this premalignant process could potentially lend therapeutic opportunities for prevention, combination therapy for improved outcome, and/or post-transplant prophylaxis. The discussion that follows will largely focus on myeloid CH which has been most extensively studied to date.

In addition to hematologic malignancies, a curious link between CH and systemic health consequences was first implicated in a study where the authors noted an association between clonal chromosomal mosaic events and type 2 diabetes with vascular complications [30]. This observation was repeated in subsequent reports which also added the significantly increased risk of all-cause mortality and particularly cardiovascular disease (CVD) [31,32]. Various other systemic effects have been described in association with CH, including chronic obstructive pulmonary disease and other pulmonary disease [16,33,34], and possibly sickle cell disease [35], although the latter association did not persist in a larger sequencing study where no increased rate of CH was seen [36]. A question naturally arising from these clinical studies concerns whether CH is a cause or a consequence. It is plausible that both the CH and the diseases associated with it are a result of aging and related processes.

Several elegant studies in preclinical models and some observations in human patients have pointed to a strong link between CH and pro-inflammatory states driven by the mutant clones themselves. A murine model was used by two independent groups to study the effect of both heterozygous and homozygous loss of *Tet2* [31,32]. It was shown that transplanted *Tet2*-deficient bone marrow cells had a competitive advantage over the wild-type cells. The recipients of the transplanted cells were *Ldlr* knockout mice which have high levels of cholesterol and are susceptible to developing atherosclerosis on a high-fat diet [37]. The $Ldlr^{-/-}$ recipients of *Tet2* knockout HSPCs had larger atherosclerotic plaques compared to the control mice when fed a high-fat diet; the effect in the heterozygous *Tet2* knockout was relatively milder. The difference in the plaque size was only noted in the context of being fed a high-fat diet because animals on a normal diet did not exhibit any measurable difference [31], suggesting that environmental triggers that promote and/or maintain the effects of mutant hematopoietic cells are indispensable. Both studies further tested and supported the hypothesis that mature myeloid cells, specifically macrophages, were responsible for this phenotype. The mutant macrophages expressed increased levels of pro-inflammatory cytokines, including interleukin-1β (Il-1β) and Il-6, particularly in response to low-density lipoprotein (LDL) or lipopolysaccharide (LPS) and interferon gamma (IFN-γ), potentially further promoting the inflammatory cycle. In a complementary work testing the effect of a *Jak2* mutation in a model of coronary artery ligation and heart failure, the authors showed in recipients of the mutant myeloid cells that there was larger infarct size, increased cardiac hypertrophy, and eventually increased fibrosis [38]. Similarly to the *Tet2* model, increased levels of Il-6, Il-1β, and tumor necrosis factor α (TNF-α) were observed in the infarcted area. Further details on these studies are reviewed elsewhere [39]. In a murine emphysema model induced by cigarette smoke exposure and polyinosinic:polycytidylic acid [poly(I:C)] injections to mimic heightened inflammation from smoking, the authors reported increased development of the disease in recipients of *Tet2* knockout bone marrow cells, even at donor

chimerism of only 15% corresponding to a human variant allelic frequency (VAF) of about 7.5% [34]. Of note, clinically adverse effects in humans have been typically associated with VAFs of 10% or greater [32,34]. In this emphysema model, again, exogenous stimuli were required for the blood cell genotype difference to be observed in disease manifestation. Lastly, murine *Dnmt3a* mutant hematopoiesis has been shown promote osteoporosis and bone loss through increased osteoclastogenesis that could be reversed by bisphosphonate treatment [40]. These studies suggest that the mutant clonal process in hematopoiesis, particularly in macrophages, in the context of environmental stressors, accelerates the presentation of age-associated diseases such as CVD, type 2 diabetes, pulmonary disease, and osteoporosis. The disease process itself has been suggested to reciprocally accelerate CH [41] (Figure 1). We next examine the effects of systemic disease and CH on the HSPC compartment itself.

CH effects on hematopoiesis and initiating mutant HSPC clones: inflammation as a driver

It is not yet clear how CH is initiated and more importantly how the mutant clone is maintained leading to its expansion. Understanding these pathways can be harnessed for therapeutic purposes to derail future growth and malignant transformation. It is plausible that somatic mutation acquisition occurs by chance, perhaps early in life or *in utero*, and experiences growth over time, at times in small bursts of relative expansion compared to non-mutant clones, as inferred from one study on *JAK2* mutations in myeloproliferative neoplasms (MPNs) [42]. Analyses have shown differential fitness of variants of common CH genes where nonsynonymous variants are under stronger positive selective pressure whereas most synonymous variants are affected by neutral drift [43]. The growth of the mutant clones often differs in rate; for example, *DNMT3A* mutant clones are relatively slow-growing at ~5% per year while *SRSF2* mutants clones may grow as fast as 20% per year [21]. Corroborative studies using longitudinal data have shown that gene-specific fitness is independent of when the mutation was acquired [44]. These early data suggest that the rate for a given mutant clone is generally constant, and could potentially be altered by environmental changes or the acquisition of additional mutations that may lead to clonal bursts. However, it is not clear how these clones grow and how these various mutations confer a similar biological output of fitness. Cell-intrinsic, cell-extrinsic, and combinatorial etiologies have been proposed through a number of studies. Many of these mechanisms were expertly reviewed previously [45].

The CH mutations may lead to a relative increase in proliferative rate or an increased proliferative response to external stimuli, including systemic disease such as atherosclerosis. In murine models, *Tet2* loss, including the more clinically relevant *Tet2* heterozygous knockout state, confers increased competitive repopulating capacity in $Ldlr^{-/-}$ recipients on a high-fat/high-cholesterol diet notable both at the level of mature cells and the HSPC compartment in the marrow [31]. Although full *Tet2* loss results in HSPC self-renewal increase on normal diet recipients, *Tet2* heterozygous cells were less robust in their competitive repopulation [46]. A complementary study demonstrated increased $Tet2^{-/-}$ HSPC proliferation in both $Apoe^{-/-}$ mice on atherogenic diet after competitive

transplantation and in bone marrow samples from human patients with atherosclerosis [41]. It is not clear, however, whether this increased proliferative signal is due to the mutant or wild-type clones or both. Using mathematical modeling, the authors proposed that clones with a driver mutation have an increased average proliferation rate, thereby gaining ground in the hematopoietic output at the level both the stem cell self-renewal and mature cell differentiation [41]. *Dnmt3a* mutant models have repeatedly confirmed the heightened self-renewal of *Dnmt3a*$^{-/-}$ cells that is amplified with serial transplantation [47,48]. However, in competitive transplantation into recipient wild-type mice without additional stimuli, the advantage of *Dnmt3a* mutant cells is not significant and is only apparent in the context of chronic *Mycobacterium avium* (*M. avium*) infection [49]. *Asxl1* knockout HSPCs are less fit in competitive transplants compared to their wildtype counterparts, although aged HSPCs had increased growth advantage that was dependent on Akt/mTOR signaling [50–52]. *Ppm1d* loss confers an advantage only in the context of the selective environment of cytotoxic chemotherapy or radiation, but not in steady-state post-transplant conditions [53,54]. There is paucity of data of specifically looking at mutant HSPC proliferation in these models, and their outgrowth over wild-type cells may be related to other selective signals discussed below. Furthermore, murine models to date have relied on transplantation assays for assessing the functional consequences of CH mutations. The transplantation model, although informative, can present clonal bottlenecks, selection for other fitness characteristics, force replicative stressors otherwise not experienced by HSPC clones, and describe cellular competition in an irradiated niche. There are limited data on the endogenous mutant HSPC compartment compared to wild-type counterparts. Approaches that combine transcriptional information with genotyping for a known mutation will provide a physiological and relevant means to evaluate the nature of the mutant cells in an endogenous niche, side by side with the wild-type cells [55,56]. One such approach is genotyping of transcriptomes (GoT) that acquires the genotypic identity of a single cell in addition to transcriptional states via single-cell RNA-sequencing assays. GoT was utilized to evaluate *CALR* mutations in MPN models [56] and *DNMT3A* mutant CH [57], revealing mutant cell-specific pathways in the context of the native niche.

The differential proliferative state of the mutant HSPCs that results in increased self-renewal or increased differentiation, often in the context of exogenous stimuli, may partly explain the clonal expansion. Studies have suggested that these environmental signals are frequently pro-inflammatory cytokines that play a larger role in selecting for and promoting CH. Increased levels of inflammatory cytokines and chemokines have been recognized as a significant association with CH in human studies, even following stem cell transplantation with donor-derived CH in the hosts [20,32,58]. Elevated IL-8 and IL-1β levels have been reported in patients with *TET2* CH in the Pakistan Risk of Myocardial Infarction Study (PROMIS) [20,32], positive correlations were observed between IL-6 levels and *DNMT3A*, *TET2*, and *ASXL1* mutant CH, heightened IL-18 levels with *JAK2* and *SF3B1* [20], and with IFN-γ and other cytokines in recipients of *DNMT3A*-mutant CH upon stem cell transplantation [58]. Multivariable analysis of adults with HIV-positive status, which is associated with elevated plasma IL-6 levels, demonstrated increased prevalence of CH with *DNMT3A*, *TET2*, and *ASXL1* variants [59]. The Montreal Heart Institute Biobank study identified increased C-reactive protein levels, a nonspecific marker of systemic

inflammation, in all CH carriers irrespective of the mutated gene involved [60]. These data reflect plasma cytokine levels, and the inflammatory milieu of the bone marrow niche remains unclear. The correlative relations between CH with a particular mutation and the cytokines may be refined with more investigations, although it is possible that any combination of chronic pro-inflammatory signals could promote clonal dominance of mutant HSPCs over time. Whether an existing pro-inflammatory state augments the establishment of CH or the CH establishes its own pro-inflammatory environment remains an open question in human studies.

Preclinical studies using *Dnmt3a*-mutant HSPC transplantations showed growth of the mutant cells in competitive transplants in the context of chronic *M. avium* infections [49]. This chronic infection model has previously been shown to be mediated by a sustained IFN-γ response [61]. *Ifngr*$^{-/-}$;*Dnmt3a*$^{-/-}$ transplanted cells lost their competitive ability upon *M. avium* infection, and IFN-γ injections promoted the growth of *Dnmt3a* mutant cells in the absence of the chronic infection, supporting that IFN-γ signaling is necessary and sufficient for the fitness of *Dnmt3a* knockout hematopoiesis. Thus, although cell-autonomous proliferation of mutant HSPCs in CH could contribute to their expansion, a major determinant of their expansion may be the positive selective environment of pro-inflammatory signaling. Chronic pro-inflammatory signaling due to chronic infections or repetitive sterile inflammation, however, has been shown to be detrimental to HSPC self-renewal, promoting loss of quiescence and differentiation [61–65]. Using histone 2B–GFP label-retention assays which use an inducible GFP pulse-then-chase system to identify slow-cycling label-retaining cells [66], the authors showed that the demise of the functional stem cell population was not due to systemic inhibitory effects of inflammation on hematopoiesis but was due to increased proliferation of HSPCs, as label-retaining long-term HSPCs did not lose their repopulating capacity with chronic inflammation [65]. They further demonstrated that while repetitive sterile inflammation did not result in major gene expression changes, it resulted in hypomethylation of promoters of inflammatory (particularly interferon) response genes, leading them to conclude that self-renewal is epigenetically regulated in this model. The attrition of the wild-type HSPC pool through increased differentiation in the context of pro-inflammation would result in a relative growth of the apparent size of the mutant HSPCs given a differential response to the inflammation, in a gradual and potentially exaggerated manner over time (Figure 1).

Innate immunity as key mediator of inflammation

Over a lifetime of an organism, repeated infections, autoimmune dysregulation, systemic disease that promotes inflammatory signals such as CVD, or other physiological stresses including metabolic stress or sleep deprivation may lead to systemic inflammation that provides cumulative, chronic positive selective pressure upon CH clones [67]. Aging itself could be an additional independent source of inflammation [68]. An important cell type that has emerged as a particularly crucial mediator of tissue-level inflammation in CH and systemic diseases associated with CH is the macrophage, an innate immune cell. *Tet2* and *Ppm1d* mutant murine models have demonstrated an increased inflammatory signature in mutant macrophages, with heightened inflammatory cytokine production upon pro-inflammatory stimulation *in vitro* [31,32,69,70]. A zebrafish model of CH which

uses CRISPR-Cas9 mutagenesis to introduce edits in common CH genes resulted in a diverse set of mutations, dominated by those in *asxl1*, *tp53*, and the *DNMT3A* ortholog *dnmt8* [71]. This model identified increased expression of pro-inflammatory cytokines, including Il1b and TNF superfamily member Tnfb, in mutant macrophages isolated from the marrow compared to macrophages from control animals [71]. This suggested that the pro-inflammatory signals are produced by the mature progeny of mutant HSPCs, acting within the environment where HSPCs reside. As discussed earlier, elevated inflammatory cytokine and chemokine levels have been identified in persons with CH [20,32,58]. Thus, it is conceivable that a local pro-inflammatory microenvironment in the marrow could be established by mutant macrophages that provide localized inflammatory signals.

Inflammation from the innate and adaptive immune systems is a critical defense system against pathogens. Inflammatory cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α produced by macrophages, are the main mediators of innate inflammation. The production of these cytokines requires a metabolic shift in mature macrophages that is associated with increased reactive oxygen species (ROS) production, expression of the pro-inflammatory transcription factor hypoxia-inducible factor (HIF)-1α, and pro-inflammatory cytokine production [72]. We speculate that CH mutations may lead to chronic macrophage activation either by inducing a cell-intrinsic pro-inflammatory profile or by promoting hyper-responsiveness and increased cytokine production to exogenous environmental stressors. The byproduct of this may be a mutation-specific inflammatory profile, as observed in a large cohort [20]. We propose that an epigenetic state established by the CH mutations maintains this program.

Microbial infections can result in epigenetic memory in innate immune cells, a process termed trained innate immunity (TII) [73–75]. The essential concept of TII is that an initial immune response in myeloid cells results in epigenetic rewiring such that a subsequent trigger leads to a heightened immune response. Studies have shown that TII occurs not only in mature myeloid cells, as previously believed, but also in immature progenitors, and thus can be long-lived [74,76]. TII results in changes in metabolic states in long-term HSPCs, changes in gene expression associated with pathways of cholesterol biosynthesis, changes in ROS production by myeloid cells, and changes in inflammatory signaling [76,77]. It is possible that CH mutations alter the epigenetic landscape of the HSPCs and their progeny, particularly macrophages, in a way that would more readily lead to the establishment of TII. Elegant *Tet2* and *Dnmt3a* mutant models of murine hematopoiesis and others will be crucial contexts in which to test this hypothesis and to evaluate the specific epigenetic signature in HSPCs and their myeloid progeny that could lead to TII by promoting and intensifying the inflammatory signals with every environmental trigger. This maladaptive form of TII would provide the necessary selective force for CH clones. However, if chronic/repetitive inflammation is detrimental to HSPC self-renewal and function in the long term, it is still puzzling how the mutant HSPCs survive and even thrive in this environment.

The role of adaptive immune cells in CH is an active area of research. The CH mutations occur in HSPCs, and thus also result in mutant lymphoid cells, including T cells, which are also capable of altering the immune microenvironment. The role of CH mutations in epigenetic factors in T cell biology has been explored in the context of chimeric antigen

receptor T cells (CAR-T cells) used in treatment of hematologic cancers. One of the first observations was in a case report of a patient treated with CD19-directed CAR-T cells where the lentiviral vector integrated and disrupted the *TET2* locus with a hypomorphic second allele [78]. The T cells with dysfunctional TET2 had an abnormal epigenetic landscape consistent with loss of TET2 and demonstrated functional changes with increased production of TNF-α, IL-2, IL-6, and other cytokines upon stimulation. Another study evaluated the effect of DNMT3A loss on CAR-T cells and showed that these cells had an increased ability to proliferate without acquiring a canonical exhaustion phenotype in the context of chronic stimulation [79]. *DNMT3A*$^{-/-}$ T cells had increased production of IL-10 and showed an exhaustion-resistant phenotype. These studies highlight the plasticity of CH mutations, their consequential effects on various types of immune cells, and how those effects may shape the evolution of CH.

Survival pathways in mutant HSPC clones in CH

In addition to cell-intrinsic causes of increased self-renewal capacity, it is imperative to understand the prosurvival pathways utilized by mutant HSPCs to promote clonal expansion. Human myelodysplastic syndrome (MDS) HSPCs have shown significant innate immune system dysregulation and resistance to the pro-proliferative effects of inflammation [69,80,81]. Similar evidence of a differential response to inflammation of CH HSPCs as a means to increased fitness has been limited. *In vitro* studies using murine *Tet2*$^{-/-}$ lineage marker-negative marrow cells with 24 days of TNF-α exposure showed decreased apoptosis [69], suggesting that the immature cells may better survive prolonged inflammatory stimuli. Complementary work explored the mechanism behind this phenotype and identified an antiapoptotic long non-coding RNA called *Morrbid* that mediates downregulation of Bim, downstream of a hyperactivated SHP2–STAT3 signaling axis in *Tet2*$^{-/-}$ HSPCs [82]. This again would suggest that mutant HSPCs have the capacity to downmodulate inflammatory signals going through the STAT3 axis that would typically be detrimental for wildtype HSPC survival. How *Tet2* loss leads to *Morrbid* overexpression is not known. This could be mediated by direct regulation of the promoter or indirectly through elevated phosphorylated STAT3, as proposed by the authors [82].

A zebrafish model of CH using an approach called TWISTR (tissue editing with inducible stem cell tagging via recombination) identified a survival pathway within the mutant HSPCs involving inflammatory modulators [71]. The approach combined CRISPR-Cas9 mutagenesis with color labeling of HSPCs at birth during the development of zebrafish embryos. This allows the identification of color-labeled mutant clones that expand and read out as dominant clones in fluorescence analysis of the multicolored marrow. This work focused on the intracellular changes of mutant HSPCs, particularly on differential gene expression. When comparing the transcriptional profile of HSPCs in mutant zebrafish to control animals, the authors found overexpression of several molecules, such as *socs3a*, *atf3*, and *nr4a1*, that were critical for regulating the intracellular inflammatory responses. NR4A1 and other NR4A family members are known for their immunomodulatory role in mature cell types, most commonly in T cell and macrophages [83,84]. Deletion of *Nr4a1* and *Nr4a3* resulted in loss of self-renewal of HSPCs [85,86]. The role of NR4A family members in HSPCs with respect to inflammatory pathway regulation is not known. The authors

hypothesized that upregulation of NR4A1 and other immunomodulatory proteins may regulate the level of chronic inflammatory signaling that mutant HSPCs are exposed to, thus lowering the degree of their proliferative response relative to wild-type cells, and preserving their stemness and fitness. Using TWISTR, the authors showed that homozygous loss of *nr4a1* in *asxl1* mutant clones prevented clonal growth, suggesting that NR4A1 is necessary for clonal expansion [71]. How mutant ASXL1 induces NR4A1 expression is not known. C-terminally truncated ASXL1 binds to the *Nr4a1* locus in murine marrow cells, suggesting a possible direct mechanism of regulation (analysis of data in [51]). Given the correlations between particular CH mutations and pro-inflammatory signals, we posed the hypothesis of engagement of a mutation-dependent anti-inflammatory pathway that resulted in this prosurvival phenotype of the mutant HSPCs. Whether the pro-inflammatory phenotype in macrophages and the anti-inflammatory phenotype in HSPCs is due to the same pathway dysregulation by the acquired mutation or involves engagement of cell type-specific mechanisms remains to be determined. Our hypothesis predicts that mutant HSPCs in CH establish the proinflammatory environment via their mature progeny (e.g., macrophages), where they survive better than the wild-type HSPCs. A similar positive feedforward model of competitive outgrowth of mutant cells has been shown in intestinal stem cells where *Apc* mutant clones secrete WNT antagonists that promote the differentiation of wild-type clones [87]; because *Apc*-mutant cells themselves are insensitive to WNT modulation, this leads to their expansion and adenoma formation.

Local inflammatory signals provided by the mutant myeloid cells could potentially create microniches in the marrow where the prosurvival phenotype of resistance to inflammation would be selected for. As such, this physically restricted signal would limit the relative growth of the mutant HSPCs compared to wild-type cells. It has been observed in several cohorts that the VAFs of CH clones are typically small and do not expand significantly without additional transforming events [20,88]. This physical limitation could be due to proximity to limited niche signals including inflammatory signals [89], differential antigen presentation that has been shown be important for T cell-mediated HSPC clearance [90], or other so far undiscovered selective pressures. Dysregulation of niche inflammation could also contribute to cancerous transformation of CH clones [91]. Understanding the local environment around mutant and neighboring wild-type HSPCs in model systems would be an important next step towards uncovering the chronic selective forces underlying the establishment, maintenance, and malignant transformation of CH clones. An important context for studying the dynamics of mutant clones and the premalignant biology of CH is germline predisposition conditions where the various selective pressures intersect with and are potentially altered by the germline genetic pathology (Box 1).

Therapies to restore immune balance as a means to control clonal growth in CH

CH has found itself at the interface of hematopoiesis, CVD, and other systemic diseases [88]. Despite its hematologically silent nature, CH has been associated with increased mortality and morbidity [1,32]. Therapies to halt or reverse CH, particularly CH associated with a high risk of malignant transformation, is a central goal in the field. Approaches

could include potentiating wild-type cells for increased competition, altering the selective forces that provide an advantageous environment to the mutant cells, or both. Using the *Tet2*$^{-/-}$ murine model, it was found that the Il-6/*Morrbid* axis through STAT3/SHP2 is a crucial pathway used by the mutant cells in competitive transplantation assays [82]. The authors then blocked the pathways with chemical inhibitors of both NF-κB and STAT3 (E3330), or SHP2 (SHP099), and showed that either of these compounds abrogates the competitive growth of aged *Tet2*$^{-/-}$ cells and reduces serum IL-6 levels [82]. Anti-IL-6 antibodies reduced the growth of *Tet2*$^{-/-}$ cells in culture and reduced number of myeloid cells *in vivo*, although it remains unclear whether such therapy alters their competitive growth advantage [92]. Interventions to alter the metabolic state of mutant cells have also been explored using vitamin C [93,94]. An intriguing role for mitochondrial stress and cGAS signaling has been proposed in *DNMT3A*- and *TET2*-related CH, suggesting a role for cGAS inhibitors and blockade of IFN-γ signaling [95]. A clinical trial has tested the effect of neutralizing pro-inflammatory IL-1β signaling in CVD [Canankinumab Anti-Inflammatory Thrombosis Outcomes Trial (CANTOS); ClinicalTrials.gov NCT01327846] [96]. Participants were treated with three different doses of anti-IL-1β antibody once every 3 months, and cardiovascular events were measured as the primary outcome. An exploratory study within the CANTOS trial evaluated the role of CH in this outcome, although the conclusions remain somewhat limited given the number of patients with CH. A modest decrease of cardiovascular events was observed in patients with TET2 CH, with a hazard ratio of 0.38 (95% confidence interval, 0.15–0.96), which was not seen in patients with non-*TET2* CH. A complementary approach of IL-1β level control could be through blocking the NLRP3-mediated production of the cytokine through the inflammasome, which has been tested with MCC950, an inhibitor of NLRP3, in the *Tet2*$^{-/-}$ murine transplant model [97]. Possible therapeutic avenues may also focus on the inflammatory defense mechanisms of the mutant stem cells, for example by targeting immune modulators such as NR4A1 and SOCS3. Longer and larger randomized studies will be necessary to draw definitive conclusions about the benefits of anti-inflammatory therapies not only to reduce CVD risk in the short term but also to reduce the development of hematologic malignancies by reducing the growth of mutant clones. Ongoing efforts to better define high-risk CH that has a higher potential of resulting in adverse health outcome are crucial and will help to define the populations for interventional studies. These efforts may identify an ‘adaptive CH’ phenomenon where acquired mutations do not have negative health outcomes or are protective in certain clinical circumstances.

Concluding remarks

CH has emerged as a critical interface of between hematopoiesis and systemic disease largely mediated by innate cell-driven inflammation based on research in preclinical models. As expanding research in patients educates us about the mutational landscape of various subtypes of CH, such as myeloid, lymphoid, therapy-related, and more recently germline predisposition-related CH, it will further enrich our knowledge of specific mutation-associated health consequences. The identification of high-risk CH that increases the risk of hematologic cancers, compared to those that do little to the hematopoietic output but increase cardiovascular risks, would assist clinicians to accurately follow and intervene

with therapies. Understanding the mechanism of mutant HSPC fitness that results in CH will be crucial in finding therapeutic opportunities to block the expansion of mutant clones and their transformation. These mechanisms might be shared but also different for CH in the context of germline predisposition conditions that affect children and adults. Technological advances using single-cell assays will aid the rigorous research in CH. These span emerging approaches such as multi-omic assays that combine gene expression, epigenetic profiling, and mutational profiling at a single-cell level, which currently suffer from low recovery rates for accurate mapping of mutant versus wild-type cells in the same sample, and are limited to small-scale investigations that constrain drawing generalizable conclusions. In addition, lineage-tracing technologies could also expand our ability to construct the clonal dynamics over decades [98]. Continued exploration via all these fronts and integrating clinical information through the lifetime of an individual will be valuable in our understanding of the pathobiology that leads to CH and its effects on health (see Outstanding questions).

Acknowledgments

This work was supported by RUNX1 Research Program, Alex's Lemonade Stand Foundation (S.A.), and the Howard Hughes Medical Institute (L.I.Z.). The authors acknowledge Brandon Gheller for critical reading of the manuscript.

References

1. Jaiswal S et al. (2014) Age-related clonal hematopoiesis associated with adverse outcomes. N. Engl. J. Med. 371, 2488–2498 [PubMed: 25426837]
2. Genovese G et al. (2014) Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N. Engl. J. Med. 371, 2477–2487 [PubMed: 25426838]
3. Lee-Six H et al. (2018) Population dynamics of normal human blood inferred from somatic mutations. Nature 561, 473–478 [PubMed: 30185910]
4. Martincorena I et al. (2015) High burden and pervasive positive selection of somatic mutations in normal human skin. Science 348, 880–886 [PubMed: 25999502]
5. Yoshida K et al. (2020) Tobacco smoking and somatic mutations in human bronchial epithelium. Nature 578, 266–272 [PubMed: 31996850]
6. Martincorena I et al. (2018) Somatic mutant clones colonize the human esophagus with age. Science 362, 911–917 [PubMed: 30337457]
7. Lee-Six H et al. (2019) The landscape of somatic mutation in normal colorectal epithelial cells. Nature 574, 532–537 [PubMed: 31645730]
8. Brunner SF et al. (2019) Somatic mutations and clonal dynamics in healthy and cirrhotic human liver. Nature 574, 538–542 [PubMed: 31645727]
9. Moore L et al. (2020) The mutational landscape of normal human endometrial epithelium. Nature 580, 640–646 [PubMed: 32350471]
10. Cagan A et al. (2022) Somatic mutation rates scale with lifespan across mammals. Nature 604, 517–524 [PubMed: 35418684]
11. Manders F et al. (2021) The dynamics of somatic mutagenesis during life in humans. Front. Aging 2, 802407 [PubMed: 35822044]
12. Lyon MF (1962) Sex chromatin and gene action in the mammalian X-chromosome. Am. J. Hum. Genet. 14, 135–148 [PubMed: 14467629]
13. Beutler E et al. (1962) The normal human female as a mosaic of X-chromosome activity: studies using the gene for C-6-PD-deficiency as a marker. Proc. Natl. Acad. Sci. U. S. A. 48, 9–16 [PubMed: 13868717]
14. Busque L et al. (2012) Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat. Genet. 44, 1179–1181 [PubMed: 23001125]

15. Xie M et al. (2014) Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat. Med. 20, 1472–1478 [PubMed: 25326804]
16. Zink F et al. (2017) Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood 130, 742–752 [PubMed: 28483762]
17. Zekavat SM et al. (2021) Hematopoietic mosaic chromosomal alterations increase the risk for diverse types of infection. Nat. Med. 27, 1012–1024 [PubMed: 34099924]
18. Sperling AS et al. (2017) The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. Nat. Rev. Cancer 17, 5–19 [PubMed: 27834397]
19. Young AL et al. (2016) Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat. Commun. 7, 12484 [PubMed: 27546487]
20. Bick AG et al. (2020) Inherited causes of clonal haematopoiesis in 97,691 whole genomes. Nature 586, 763–768 [PubMed: 33057201]
21. Fabre MA et al. (2022) The longitudinal dynamics and natural history of clonal haematopoiesis. Nature 606, 335–342 [PubMed: 35650444]
22. Gibson CJ et al. (2017) Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J. Clin. Oncol. 35, 1598–1605 [PubMed: 28068180]
23. Coombs CC et al. (2017) Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell 21, 374–382 [PubMed: 28803919]
24. Wong TN et al. (2018) Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. Nat. Commun. 9, 455 [PubMed: 29386642]
25. Bolton KL et al. (2020) Cancer therapy shapes the fitness landscape of clonal hematopoiesis. Nat. Genet. 52, 1219–1226 [PubMed: 33106634]
26. Spitzer B et al. (2022) Bone barrow surveillance of pediatric cancer survivors identifies clones that predict therapy-related leukemia. Clin. Cancer Res. 28, 1614–1627 [PubMed: 35078859]
27. Niroula A et al. (2021) Distinction of lymphoid and myeloid clonal hematopoiesis. Nat. Med. 27, 1921–1927 [PubMed: 34663986]
28. Liu X et al. (2021) Genetics of autosomal mosaic chromosomal alteration (mCA). J. Hum. Genet. 66, 879–885 [PubMed: 34321609]
29. Desai P et al. (2018) Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat. Med. 24, 1015–1023 [PubMed: 29988143]
30. Bonnefond A et al. (2013) Association between large detectable clonal mosaicism and type 2 diabetes with vascular complications. Nat. Genet. 45, 1040–1043 [PubMed: 23852171]
31. Fuster JJ et al. (2017) Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 355, 842–847 [PubMed: 28104796]
32. Jaiswal S et al. (2017) Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N. Engl. J. Med. 377, 111–121 [PubMed: 28636844]
33. Buscarlet M et al. (2017) DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. Blood 130, 753–762 [PubMed: 28655780]
34. Miller PG et al. (2022) Association of clonal hematopoiesis with chronic obstructive pulmonary disease. Blood 139, 357–368 [PubMed: 34855941]
35. Pincez T et al. (2021) Clonal hematopoiesis in sickle cell disease. Blood 138, 2148–2152 [PubMed: 34521115]
36. Liggett LA et al. (2022) Clonal hematopoiesis in sickle cell disease. J. Clin. Invest. 132, e156060 [PubMed: 34990411]
37. Ishibashi S et al. (1993) Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J. Clin. Invest. 92, 883–893 [PubMed: 8349823]
38. Sano S et al. (2019) JAK2 V617F-mediated clonal hematopoiesis accelerates pathological remodeling in murine heart failure. JACC Basic Transl. Sci. 4, 684–697 [PubMed: 31709318]
39. Tall AR and Fuster JJ (2022) Clonal hematopoiesis in cardiovascular disease and therapeutic implications. Nat. Cardiovasc. Res. 1, 116–124 [PubMed: 36337911]

40. Kim PG et al. (2021) Dnmt3a-mutated clonal hematopoiesis promotes osteoporosis. J. Exp. Med. 218, e20211872 [PubMed: 34698806]
41. Heyde A et al. (2021) Increased stem cell proliferation in atherosclerosis accelerates clonal hematopoiesis. Cell 184, 1348–1361 [PubMed: 33636128]
42. Williams N et al. (2022) Life histories of myeloproliferative neoplasms inferred from phylogenies. Nature 602, 162–168 [PubMed: 35058638]
43. Watson CJ et al. (2020) The evolutionary dynamics and fitness landscape of clonal hematopoiesis. Science 367, 1449–1454 [PubMed: 32217721]
44. Robertson NA et al. (2022) Longitudinal dynamics of clonal hematopoiesis identifies gene-specific fitness effects. Nat. Med. 28, 1439–1446 [PubMed: 35788175]
45. Challen GA and Goodell MA (2020) Clonal hematopoiesis: mechanisms driving dominance of stem cell clones. Blood 136, 1590 [PubMed: 32746453]
46. Moran-Crusio K et al. (2011) Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell 20, 11–24 [PubMed: 21723200]
47. Challen GA et al. (2011) Dnmt3a is essential for hematopoietic stem cell differentiation. Nat. Genet. 44, 23–31 [PubMed: 22138693]
48. Jeong M et al. (2018) Loss of Dnmt3a immortalizes hematopoietic stem cells In vivo. Cell Rep. 23, 1–10 [PubMed: 29617651]
49. Hormaechea-Agulla D et al. (2021) Chronic infection drives Dnmt3a-loss-of-function clonal hematopoiesis via IFNγ signaling. Cell Stem Cell 28, 1428–1442 [PubMed: 33743191]
50. Abdel-Wahab O et al. (2013) Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. J. Exp. Med. 210, 2641 [PubMed: 24218140]
51. Nagase R et al. (2018) Expression of mutant Asxl1 perturbs hematopoiesis and promotes susceptibility to leukemic transformation. J. Exp. Med. 215, 1729–1747 [PubMed: 29643185]
52. Fujino T et al. (2021) Mutant ASXL1 induces age-related expansion of phenotypic hematopoietic stem cells through activation of Akt/mTOR pathway. Nat. Commun. 12, 1826 [PubMed: 33758188]
53. Kahn JD et al. (2018) PPM1D-truncating mutations confer resistance to chemotherapy and sensitivity to PPM1D inhibition in hematopoietic cells. Blood 132, 1095–1105 [PubMed: 29954749]
54. Hsu JI et al. (2018) PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell Stem Cell 23, 700–713 [PubMed: 30388424]
55. van Galen P et al. (2019) Single-cell RNA-seq reveals AML hierarchies relevant to disease progression and immunity. Cell 176, 1265–1281 [PubMed: 30827681]
56. Nam AS et al. (2019) Somatic mutations and cell identity linked by genotyping of transcriptomes. Nature 571, 355–360 [PubMed: 31270458]
57. Nam AS et al. (2022) Single-cell multi-omics of human clonal hematopoiesis reveals that DNMT3A R882 mutations perturb early progenitor states through selective hypomethylation. Nat. Genet. 54, 1514–1526 [PubMed: 36138229]
58. Gibson CJ et al. (2022) Donor clonal hematopoiesis and recipient outcomes after transplantation. J. Clin. Oncol. 40, 189–201 [PubMed: 34793200]
59. Dharan NJ et al. (2021) HIV is associated with an increased risk of age-related clonal hematopoiesis among older adults. Nat. Med. 27, 1006–1011 [PubMed: 34099923]
60. Busque L et al. (2020) High-sensitivity C-reactive protein is associated with clonal hematopoiesis of indeterminate potential. Blood Adv. 4, 2430–2438 [PubMed: 32492156]
61. Matatall KA et al. (2016) Chronic infection depletes hematopoietic stem cells through stress-induced terminal differentiation. Cell Rep. 17, 2584–2595 [PubMed: 27926863]
62. Pietras EM et al. (2016) Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. Nat. Cell Biol. 18, 607–618 [PubMed: 27111842]
63. Essers MAG et al. (2009) IFNalpha activates dormant haematopoietic stem cells in vivo. Nature 458, 904–908 [PubMed: 19212321]

64. Pietras EM et al. (2014) Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons. J. Exp. Med. 211, 245–262 [PubMed: 24493802]
65. Bogeska R et al. (2022) Inflammatory exposure drives long-lived impairment of hematopoietic stem cell self-renewal activity and accelerated aging. Cell Stem Cell 29, 1273–1284 [PubMed: 35858618]
66. Wilson A et al. (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135, 1118–1129 [PubMed: 19062086]
67. Florez MA et al. (2022) Clonal hematopoiesis: mutation-specific adaptation to environmental change. Cell Stem Cell 29, 882–904 [PubMed: 35659875]
68. Kovtonyuk LV et al. Inflamm-aging of hematopoiesis, hematopoietic stem cells, and the bone marrow microenvironment. Front. Immunol. 7, 502 [PubMed: 27895645]
69. Abegunde SO et al. (2018) An inflammatory environment containing TNFα favors Tet2-mutant clonal hematopoiesis. Exp. Hematol. 59, 60–65 [PubMed: 29195897]
70. Yura Y et al. (2021) The cancer therapy-related clonal hematopoiesis driver gene ppm1d promotes inflammation and nonischemic heart failure in mice. Circ. Res. 129, 684–698 [PubMed: 34315245]
71. Avagyan S et al. (2021) Resistance to inflammation underlies enhanced fitness in clonal hematopoiesis. Science 374, 768–772 [PubMed: 34735227]
72. Nonnenmacher Y and Hiller K (2018) Biochemistry of proinflammatory macrophage activation. Cell. Mol. Life Sci. 75, 2093–2109 [PubMed: 29502308]
73. Netea MG et al. (2020) Defining trained immunity and its role in health and disease. Nat. Rev. Immunol. 20, 375–388 [PubMed: 32132681]
74. Kaufmann E et al. (2018) BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell 172, 176–190 [PubMed: 29328912]
75. Cirovic B et al. (2020) BCG vaccination in humans elicits trained immunity via the hematopoietic progenitor compartment. Cell Host Microbe 28, 322–334 [PubMed: 32544459]
76. Mitroulis I et al. (2018) Modulation of myelopoiesis progenitors is an integral component of trained immunity. Cell 172, 147–161 [PubMed: 29328910]
77. de Laval B et al. (2020) C/EBPβ-dependent epigenetic memory induces trained immunity in hematopoietic stem cells. Cell Stem Cell 26, 793 [PubMed: 32386557]
78. Fraietta JA et al. (2018) Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature 558, 307–312 [PubMed: 29849141]
79. Prinzing B et al. (2021) Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. Sci. Transl. Med. 13, eabh0272 [PubMed: 34788079]
80. Barreyro L et al. (2018) Chronic immune response dysregulation in MDS pathogenesis. Blood 132, 1553–1560 [PubMed: 30104218]
81. Trowbridge JJ and Starczynowski DT (2021) Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. J. Exp. Med. 218, e20201544 [PubMed: 34129017]
82. Cai Z et al. (2018) Inhibition of inflammatory signaling in Tet2 mutant preleukemic cells mitigates stress-induced abnormalities and clonal hematopoiesis. Cell Stem Cell 23, 833–849 [PubMed: 30526882]
83. Boulet S et al. (2022) Role of NR4A family members in myeloid cells and leukemia. Curr. Res. Immunol. 3, 23–36 [PubMed: 35496823]
84. Odagiu L et al. (2021) Role of the orphan nuclear receptor NR4A family in T-cell biology. Front. Endocrinol. 11, 1107
85. Land RH et al. (2015) The orphan nuclear receptor NR4A1 specifies a distinct subpopulation of quiescent myeloid-biased long-term HSCs. Stem Cells 33, 278–288 [PubMed: 25284014]
86. Freire PR and Conneely OM (2018) NR4A1 and NR4A3 restrict HSC proliferation via reciprocal regulation of C/EBPα and inflammatory signaling. Blood 131, 1081 [PubMed: 29343483]
87. van Neerven SM et al. (2021) Apc-mutant cells act as supercompetitors in intestinal tumour initiation. Nature 594, 436–441 [PubMed: 34079128]

88. Kar SP et al. (2022) Genome-wide analyses of 200,453 individuals yield new insights into the causes and consequences of clonal hematopoiesis. Nat. Genet. 54, 1155–1166 [PubMed: 35835912]

89. Miao R et al. (2022) Competition between hematopoietic stem and progenitor cells controls hematopoietic stem cell compartment size. Nat. Commun. 13, 4611 [PubMed: 35941168]

90. Hernández-Malmierca P et al. (2022) Antigen presentation safeguards the integrity of the hematopoietic stem cell pool. Cell Stem Cell 29, 760–775 [PubMed: 35523139]

91. Yeaton A et al. (2022) The impact of inflammation-induced tumor plasticity during myeloid transformation. Cancer Discov. 12, 2392–2413 [PubMed: 35924979]

92. Meisel M et al. (2018) Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. Nature 557, 580–584 [PubMed: 29769727]

93. Cimmino L et al. (2017) Restoration of TET2 function blocks aberrant self-renewal and leukemia progression. Cell 170, 1079–1095 [PubMed: 28823558]

94. Agathocleous M et al. (2017) Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. Nature 549, 476–481 [PubMed: 28825709]

95. Cobo I et al. (2022) DNA methyltransferase 3 alpha and TET methylcytosine dioxygenase 2 restrain mitochondrial DNA-mediated interferon signaling in macrophages. Immunity 55, 1386–1401 [PubMed: 35931086]

96. Svensson EC et al. (2022) TET2-driven clonal hematopoiesis and response to canakinumab: an exploratory analysis of the CANTOS randomized clinical trial. JAMA Cardiol. 7, 521–528 [PubMed: 35385050]

97. Sano S et al. (2018) Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1β/NLRP3 inflammasome. J. Am. Coll. Cardiol. 71, 875–886 [PubMed: 29471939]

98. Ludwig LS et al. (2019) Lineage tracing in humans enabled by mitochondrial mutations and single-cell genomics. Cell 176, 1325–1339 [PubMed: 30827679]

99. Churpek JE et al. (2015) Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood 126, 2484–2490 [PubMed: 26492932]

100. Brown AL et al. (2020) RUNX1-mutated families show phenotype heterogeneity and a somatic mutation profile unique to germline predisposed AML. Blood Adv. 4, 1131–1144 [PubMed: 32208489]

101. Six KA et al. (2021) B-cell acute lymphoblastic leukemia in patients with germline RUNX1 mutations. Blood Adv. 5, 3199–3202 [PubMed: 34424323]

102. Liggett LA et al. (2021) Precocious clonal hematopoiesis in down syndrome is accompanied by immune dysregulation. Blood Adv. 5, 1791–1796 [PubMed: 33787858]

103. Lundgren S et al. (2022) Somatic compensation of inherited bone marrow failure. Semin. Hematol. 59, 167–173 [PubMed: 36115694]

104. Kennedy AL et al. (2021) Distinct genetic pathways define pre-malignant versus compensatory clonal hematopoiesis in Shwachman–Diamond syndrome. Nat. Commun. 12, 1344 [PubMed: 33637722]

105. Tan S et al. (2021) Somatic genetic rescue of a germline ribosome assembly defect. Nat. Commun. 12, 5044 [PubMed: 34413298]

106. Pressato B et al. (2012) Deletion of chromosome 20 in bone marrow of patients with Shwachman–Diamond syndrome, loss of the EIF6 gene and benign prognosis. Br. J. Haematol. 157, 503–505 [PubMed: 22295858]

107. Schratz KE et al. (2021) Somatic reversion impacts myelodysplastic syndromes and acute myeloid leukemia evolution in the short telomere disorders. J. Clin. Invest. 131, e147598 [PubMed: 34343137]

108. Sahoo SS et al. (2021) Clinical evolution, genetic landscape and trajectories of clonal hematopoiesis in SAMD9/SAMD9L syndromes. Nat. Med. 27, 1806–1817 [PubMed: 34621053]

109. Avagyan S and Shimamura A (2022) Lessons from pediatric MDS: approaches to germline predisposition to hematologic malignancies. Front. Oncol. 12, 813149 [PubMed: 35356204]

110. Iskander D et al. (2019) Impaired cellular and humoral immunity is a feature of Diamond-Blackfan anaemia; experience of 107 unselected cases in the United Kingdom. Br. J. Haematol. 186, 321–326 [PubMed: 30980390]

111. Khan S et al. (2008) Some cases of common variable immunodeficiency may be due to a mutation in the SBDS gene of Shwachman–Diamond syndrome. Clin. Exp. Immunol. 151, 448–454 [PubMed: 18190602]

112. Jyonouchi S et al. (2011) Dyskeratosis congenita: a combined immunodeficiency with broad clinical spectrum – a single-center pediatric experience. Pediatr. Allergy Immunol. 22, 313–319 [PubMed: 21284747]

113. Wagner CL et al. (2018) Short telomere syndromes cause a primary T cell immunodeficiency. J. Clin. Invest. 128, 5222–5234 [PubMed: 30179220]

114. Spinner MA et al. (2014) GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood 123, 809–821 [PubMed: 24227816]

115. Donadieu J et al. (2018) Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. Haematologica 103, 1278–1287 [PubMed: 29724903]

116. de Jesus AA et al. (2020) Distinct interferon signatures and cytokine patterns define additional systemic autoinflammatory diseases. J. Clin. Invest. 130, 1669–1682 [PubMed: 31874111]

117. Furutani E et al. (2020) Inflammatory manifestations in patients with Shwachman–Diamond syndrome: a novel phenotype. Am. J. Med. Genet. A 182, 1754–1760 [PubMed: 32293785]

118. Zhou C et al. (2022) Germline ETV6 mutation promotes inflammation and disrupts lymphoid development of early hematopoietic progenitors. Exp. Hematol. 112/113, 24–34 [PubMed: 35803545]

Highlights

Clonal hematopoiesis (CH) is associated with elevated pro-inflammatory cytokines which act as a driving force for clonal expansion.

Recurrent or chronic infections and sterile inflammation may predispose to CH, particularly in cases of germline predisposition.

Blocking inflammation may be a viable therapeutic approach for high-risk CH and associated hematopoietic and non-hematopoietic health sequelae.

Box 1.

Germline predisposition conditions and early-onset CH

CH is typically associated with advancing age. It is the single strongest correlate to CH incidence, whether assessed by targeted CH gene panels or by whole genome sequencing platforms [16,20]. The fraction of samples with detected CH assessed by targeted gene panel sequencing in persons younger than 25 years of age is very small [16]. The prevalence of CH in young patients following chemotherapy for childhood cancers was also low [26]. In asymptomatic young individuals with germline conditions with predisposition to hematologic malignancies, the prevalence of early-onset CH may be higher [99–102], although no systemic assessment has been published to date. In asymptomatic individuals with *GATA2* deficiency and *RUNX1* familial platelet disorder (*RUNX1*-FPD), clonal growths with typical CH gene mutations can be found in otherwise well adolescents and young adults, although the landscape of mutations may not mirror that found in the general population.

Two important distinctions about CH in the context of germline predisposition should be acknowledged. First, asymptomatic patients with germline predisposition often do not have abnormal blood counts and/or bone marrow findings that are characteristic of the specific underlying genetics. For example, *RUNX1*-FPD is often associated with thrombocytopenia and bleeding symptoms, *GATA2* deficiency may be associated with hypocellular marrow or low monocyte count, and Fanconi anemia and telomere biology disorders may be associated with various cytopenias with dysplasia or atypical marrow findings inconsistent with malignancy. Thus, the strict definition of CH as a clonal growth with normal hematologic parameters may not apply. Second, the germline genetic lesion in HSPCs likely presents a selective pressure for somatic genetic changes that may alleviate the detrimental effects of the germline mutation on HSPC survival. These types of events, termed somatic compensations [103], often result in clonal growths and likely represent a separate path to CH. Examples of this phenomenon have been described in Shwachman-Diamond syndrome (SDS) where clones with deletion of 20q (del20q) or mutations in *EIF6* on chromosome 20q arise [104–106]. Similarly, mutations in the shelterin complex gene POT1 have been described in telomere biology disorders as a means of somatic genetic reversion [107]. Somatic compensations may be adaptive, as in the case of del20q in SDS, or maladaptive, such as mutations in *TP53* in SDS or the acquisition of monosomy 7 in *SAMD9* and *SAMD9L* deficiency, which are associated with a higher risk of malignant transformation of the altered clone [104,108]. CH could also be a byproduct of HSPC pool attrition secondary to the germline mutations important for HSPC development, allowing a relatively larger size of the somatic mutant clone by virtue of reduced total HSPC numbers. The latter hypothesis has not been formally tested by careful clonal analysis of the endogenous HSPC population in patients with germline predisposition. Nevertheless, early-onset CH, independently of the compensatory nature of the acquired mutation, carries a risk of malignant transformation occurring in germline conditions [104], and may present a unique opportunity to study the mechanisms involved in the process. Early-onset CH may mirror aging-related CH processes in an accelerated manner, but most likely is subject to other selective pressures.

Our understanding about the selective pressures that could promote early CH in germline predisposition conditions is limited. It is possible that inflammatory dysregulation and/or a higher baseline inflammatory state nurture early establishment of CH. Many of the associated immunological phenotypes of germline predisposition conditions have been reviewed elsewhere [109]. A recurrent theme includes a degree of immunodeficiency due to neutropenia, lymphopenia, or immune dysregulation that results in frequent complicated or uncomplicated infections and thus chronic repeated exposure to inflammatory stimuli. The immune defects may be in the cellular or humoral arms, or in both, as seen in Diamond-Blackfan anemia [110], SDS [111], telomere biology disorders [112,113], and *GATA2* deficiency [114,115]. Furthermore, immune dysregulation with a spectrum of autoimmune disorders is being recognized to be disproportionately high in some germline predisposition conditions. These include eczema and allergies in *RUNX1*-FPD [100], and autoinflammatory diseases in *SAMD9L* deficiency [116] and SDS [117]. One study documented an altered protein composition in the plasma of six patients with SDS, and many were consistent with increased inflammatory pathways by gene ontology analysis [117]. Germline *ETV6* loss leads to altered progenitor maturation and increased pro-inflammatory cytokine production resulting in aberrant B cell differentiation [118]. Heightened chronic inflammatory states could potentially be the common thread between aging-related CH and early-onset CH in germline predisposition. It is possible that the underlying germline mutations may alter the rate of sporadic genetic alterations that would be selected for, inevitably accelerating the onset of CH and subsequent blood malignancies by decades.

Early-onset CH occurs in germline predisposition and may play an important role in the pathogenesis of pediatric and young adult hematologic malignancies, either directly by being part of the malignant clone or indirectly by inducing hematopoietic stress on the remaining clones. Future studies on disease pathogenesis in the context of specific germline conditions will be necessary to understand the clonal dynamics and clonal evolution affected by the clinical course of patients and other environmental and physiological stressors.

Outstanding questions

Understanding the local environmental effects in the marrow around the mutant cells is crucial – what is the inflammatory milieu and what is the role of the hematopoietic as well as non-hematopoietic niche cells in establishing this environment?

Are cells other than the innate myeloid lineage involved in shaping CH?

The interaction of CH mutations with environmental stressors (diet, psychological stress, aging, toxins, others) and how that potentiates the hematopoiesis by mutant cells is not well understood. How do somatic mutations that occur with time in other tissues, such as cardiac muscle or others, alter the CH effect on extramedullary health?

What is the tipping point for a CH clone to undergo transformation, and why? Is this gradual or an off–on switch?

How best to stop or reverse CH from progressing through transformation to malignancy?

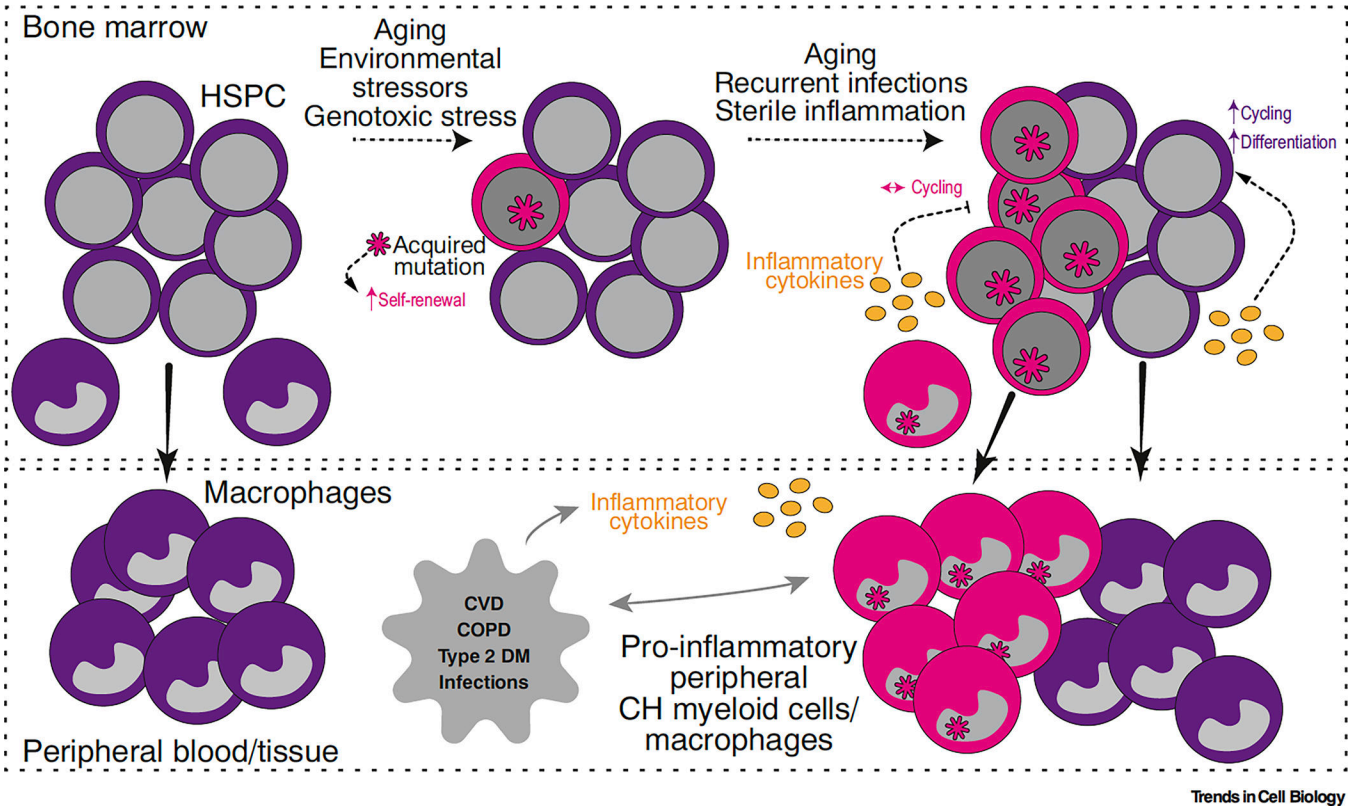

Figure 1. Establishment and maintenance of clonal hematopoiesis (CH).

Schematic of CH from early establishment to clonal expansion. HSPCs which reside in the bone marrow maintain lifelong hematopoiesis, including the production of innate immune cells such as macrophages that reside in the marrow space and the peripheral tissues. Aging and/or various organismal and environmental stressors induce random mutations in HSPCs, some of which confer fitness over time due to cell-intrinsic or cell-extrinsic effects or both. The selective pressures include further aging-associated cellular and organismal stressors, chronic/recurrent infections, or sterile inflammation. Inflammatory cytokines implicated in CH are typically pro-inflammatory in nature and are produced by mutant macrophages, although an additional role for adaptive immune cells is possible. Pro-inflammatory signaling in wild-type HSPCs leads to increased cycling and differentiation, whereas in mutant HSPCs it is modulated to resist its effects. Mutant macrophages provide these inflammatory cytokines in the local microenvironment of the HSPCs in the marrow, and similarly induce inflammation in peripheral tissues that leads to the various non-hematologic health outcomes associated with CH. Reciprocal exacerbation of inflammation by these CH-associated age-related disease processes is also possible. Abbreviations: COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DM, diabetes mellitus; HSPC, hematopoietic stem and progenitor cell.