

# Inflammation in Myeloid Malignancies: From Bench to Bedside

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## ABSTRACT

Myeloid malignancies, stemming from a somatically mutated hematopoietic clone, can cause a wide variety of clinical consequences, including pancytopenia in myelodysplastic syndrome, overproduction of three myeloid lineages in myeloproliferative neoplasm, and the rapid growth of immature hematopoietic cells in acute myeloid leukemia (AML). It is becoming clear that inflammation is a hallmark feature of clonal myeloid conditions, ranging from clonal hematopoiesis of indeterminate potential to AML. Fundamental findings from laboratory research on inflammation in myeloid malignancies has potential implications for diagnosis, prognostication, and treatment in these diseases. In this review, we highlighted some pertinent basic science findings regarding the role of inflammation in myeloid malignancies and speculated how these findings could impact the clinical care of patients.

## BACKGROUND

The history of acute myeloid leukemia (AML) dates back to 1827, with the first published case of leukemia described as having fever, weakness, and “pus in the blood” by French physician Alfred-Armand-Louis-Marie Velpeau. The history of myeloproliferative neoplasm (MPN) dates back to 1951, when hematologist William Dameshek proposed the common pathogenesis in the myeloproliferative disorders of polycythemia vera, essential thrombocythemia, myelofibrosis (MF), and chronic myelogenous leukemia.<sup>[1]</sup> In addition, he predicted that these diseases were hematopoietic stem cell diseases, although manifesting as an over production of predominantly one mature lineage, the disease initiating cell is a multilineage cell, the hematopoietic stem cell. The history of myelodysplastic syndrome (MDS) dates to the early 20th century when certain patients with acute myelogenous leukemia were determined to have a preceding anemic period and abnormal blood cell production, which was termed “refractory anemia” at the time. These initial observations opened up the doors for subsequent discoveries in myeloid diseases that have led to the therapies we offer patients today.

## THE MUTANT CLONE INDUCES INFLAMMATION

A common theme among myeloid mutations is the induction of inflammation (Table 1). The increased inflammation is derived from the mutant clone itself as well as the induction of an inflammatory microenvironment composed of nonmutant bystander cells adopting a proinflammatory phenotype. In MPN, a disease where chronic inflammation is prominent, the *JAK2*<sup>V617F</sup> mutant cells not only produce excessive inflammatory cytokines themselves, but also induce other bystander cells to produce inflammatory cytokines.<sup>[2]</sup> Clonal hematopoiesis of indeterminate potential (CHIP) mutations, such as *TET2*, have been shown to induce inflammation<sup>[3–5]</sup> and result in increased risk of diseases, such as cardiovascular disease. This highlights that the clinical consequences of these mutant clones reach beyond hematologic malignancies; they insidiously produce a chronic inflammatory state and likely have a much more significant impact on human health than we currently appreciate.

In AML, proinflammatory and proangiogenic factors interleukin (IL)-1 $\beta$  and basic fibroblast growth factor stimulate endothelial cells to secrete vascular endothelial growth factor (VEGF)-C, which supports blast survival

**Table 1.** Contributors to Inflammation in Myeloid Malignancies

Inflammation Feature	AML	MDS	MPN
IL-1 $\beta$ and bFGF	X		
VEGF-C	X		
TLR signaling	X	X	X
DAMPs		X	X
NLRP3		X	
NK cells	X		
p38MAPK	X		

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; IL: interleukin; bFGF: basic fibroblast growth factor; TLR: toll-like receptor.

and proliferation. High plasma levels of VEGF-A and VEGF-C correlate with adverse prognosis in all three myeloid malignancies.<sup>[6]</sup> However, VEGF inhibition with bevacizumab did not demonstrate efficacy. This had been shown in a clinical trial during which no patients on this therapy met the criteria of a partial response (i.e., clearance of at least 50% marrow blasts accompanied by increases in platelet counts and hemoglobin).<sup>[7]</sup> Another trial of bevacizumab administered after chemotherapy to adults with refractory or relapsed AML showed a favorable complete response rate and duration in adults with AML (for patients with AML resistant to traditional treatment approaches). There had even been the clearance of marrow blasts in some patients, indicating that VEGF neutralization might result directly in leukemic cell death.<sup>[8]</sup> In another study, an enzyme-linked immunosorbent assay to measure VEGF and basic fibroblast growth factor levels in plasma samples from patients with AML and MDS showed the significant prognostic use of plasma VEGF levels in patients with AML compared to those with MDS. These data suggested a biological difference between AML and MDS, and why plasma VEGF level measuring was more effective for patients with AML.<sup>[9,10]</sup> A source of adjuvant treatment might be found with targeted agents in AML and MPN that have the dual advantage of normalizing the microenvironment and removing malignant cells.<sup>[11]</sup>

## ABERRANT INNATE IMMUNE SIGNALING IN MYELOID DISEASES

Aberrant innate immune activation plays a central role in the pathogenesis of MDS. Toll-like receptors (TLRs) are expressed on hematopoietic stem and progenitor cells (HSPC) and help mediate emergency hematopoiesis.<sup>[12,13]</sup> TLR signaling is found to be constitutively active with deletion of chromosome 5,<sup>[14]</sup> a common cytogenetic abnormality found in MDS. Increased expression of TLR4 on HSPC seen in MDS results in an increase in apoptosis of CD34 $^{+}$  cells. TLR2 signaling also leads to CD34 $^{+}$  cell death via  $\beta$ -arrestin 1.<sup>[15]</sup> Downstream TLR signaling can lead to loss of progenitor cell function.<sup>[16]</sup> Potential clinical applications of TLR investigations could include therapeutic targeting of different TLRs, such as TLR2 in MDS

CD34 $^{+}$  cells (potentially leading to increased erythroid differentiation).<sup>[13]</sup>

In AML, patients with myelomonocytic and monocytic subtypes have higher expression of TLR2 and TLR4 in the bone marrow, particularly in those who fail induction therapy. This higher expression of TLR2 and TLR4 was correlated with shorter overall survival.<sup>[17]</sup> Activation of TLR2 leads to the expansion and increased survival of leukemic cells, promoting differentiation of these cells.<sup>[18]</sup>

Excessive TLR signaling is also seen in MPN as well as CHIP. Monocytes from patients with MPN persistently produce tumor necrosis factor-alpha (TNF) in response to exposure to the TLR4 agonist lipopolysaccharide.<sup>[19]</sup> TET2 knockout macrophages have increased production of TNF upon stimulation with lipopolysaccharide,<sup>[5]</sup> this increased TLR signaling likely plays an important mechanistic role in the nonhematologic sequelae of CHIP, such as accelerated atherosclerosis.

Damage-associated molecular patterns, or alarmins, are released during inflammation or cell death, which modulate the innate immune system through TLR signaling. Damage-associated molecular patterns, such as S100A8 and S100A9, drive mitochondrial dysfunction, oxidative stress, and DNA damage response activation in HSPCs, leading to bone marrow failure and leukemia predisposition syndrome (e.g., MDS).<sup>[20]</sup> Circulating S100A8 is elevated in patients with MDS.<sup>[21]</sup> It was proposed that S100A8/9 expression screening could be used as an important determinant of disease outcome, especially for those high-risk patients, and guide therapeutic decision-making in MDS. Moreover, the inhibition of the pyroptosis pathway via the inhibition of alarmins has been shown to promote effective hematopoiesis in MDS HSPCs and has potential future applications in the clinic.<sup>[22]</sup>

Expression of the S100A8/S100A9 alarmin complex in mesenchymal stem cells marks progression toward the fibrotic phase in MPN; moreover, treatment with a S100A9/S100A9 small molecule inhibitor tasquinimod reduces fibrosis in MPN mouse models.<sup>[23]</sup> S100A is also increased in plasma and granulocytes of patients with MPN and correlates with JAK2<sup>V617F</sup> allele burden.<sup>[24]</sup> This preclinical data highlights that S100A8/9 is a putative therapeutic target in MPN.

NOD-like receptor protein 3 inflammasome activation is a central driver of inflammation in MDS. This is activated by S100A8/A9 and oxidized mitochondrial DNA. NOD-like receptor protein 3 signaling leads to the activation of caspase-1 and the eventual promotion of cation entry, cell swelling, pyroptosis, and cell death in MDS.<sup>[22]</sup>

## IMMUNE ESCAPE STRATEGIES EMPLOYED BY AML CELLS

AML cells co-opt physiologic mechanisms to evade phagocytosis. AML cells upregulate CD47 on the cell

surface which relays a “do not eat me” signal to macrophages, allowing the leukemia cells to evade phagocytosis.<sup>[25]</sup> Therapeutic blockade of CD47 with the anti-CD47 antibody magrolimab leads to engulfment of leukemic cells and has the potential to be a potent treatment for myeloid malignancy cells that can overexpress CD47 to escape phagocytosis.<sup>[26,27]</sup>

Natural killer (NK) cells have also been implicated in AML. This occurs via the mechanisms of immune escape from NK cell-mediated recognition. On these dysfunctional NK cells, there is an imbalance of receptor expression (more NK inhibitory, such as PD-1, than activating receptors, such as NKG2D). Furthermore, AML cells will overexpress NK inhibitory receptor ligands (compared with activating receptor ligands). Finally, the tumor microenvironment can be populated by myeloid-derived suppressor cell and Treg cells, increasing the interference with NK functions.<sup>[28]</sup> This all allows for the propagation of the AML phenotype and is why the field of NK therapies to treat AML is of significant interest.

## INFLAMMATION AS A PREDISPOSING FACTOR TO DEVELOP HEMATOLOGIC MALIGNANCIES

There is evidence to suggest that inflammation may increase one's risk of hematologic malignancies. There is an increase of MDS and AML in those with a history of infections or autoimmune disorders.<sup>[29,30]</sup> A prior history of any autoimmune disease was associated with an increased risk of MPN (odds ratio = 1.2).<sup>[31]</sup> The addition of therapeutic agents used in autoimmune diseases could also potentially impact development of a myeloid malignancy, as azathioprine exposure was associated with a statistically significant 7-fold risk for myeloid neoplasms in a case-control study of more than 40,000 patients.<sup>[32]</sup> A cohort of patients with ulcerative colitis was found to have an increased rate of CHIP with *DNMT3A* and *PPM1D* mutations.<sup>[33]</sup> In addition, inflammation as a result of infection may also impact the development of myeloid malignancies, as there is an increased risk of myeloid malignancies in those with community acquired infections.<sup>[34]</sup> In addition, lifestyle choices that induce inflammation, such as smoking behavior, may increase the risk of MPN<sup>[35]</sup> as well as CHIP.<sup>[3,36]</sup> This highlights the potential use of anti-inflammatory interventions to decrease or prevent the development of hematologic malignancies, particularly in those who are genetically predisposed.

## INFLAMMATION CREATES AN ENVIRONMENT CONDUCIVE TO MUTANT CLONAL HEMATOPOIESIS

A common theme is emerging among mutant hematopoietic clones, they are selected for during chronic inflammation. Chronic inflammation leads to exhaustion of normal HSC; however, HSC with myeloid

disease-associated mutations may be resistant to inflammation-induced exhaustion. TNF selects for *JAK2<sup>V617F</sup>* mutant cells<sup>[37]</sup> as well as *TET2* mutant cells.<sup>[38]</sup> Specific types of inflammatory stressors may select for specific mutations; for example, smoking is associated with emergence of *ASXL1* CHIP but not *TET2* CHIP.<sup>[36]</sup> This offers opportunities to therapeutically alter selective pressures such that mutant clones no longer have an advantage and could possibly offer the opportunity to deduce selective pressures present based on which specific clones are selected. Although preclinical work suggests that anti-inflammatory therapies reduce mutant clones, such as *TET2*,<sup>[39]</sup> interventional studies in humans prospectively investigating the impact of anti-inflammatory agents in clonal hematopoiesis are forthcoming.

In AML, inflammation caused by increased IL-1 expression leads to the expansion of myeloid progenitors (and suppresses the growth of normal progenitors). This is done by IL-1 enhancing p38MAPK phosphorylation and promoting secretion of inflammatory cytokines and various growth factors. This study showed the importance of screening to identify pathways to target in these myeloid malignancies for the development of therapeutics.<sup>[40]</sup>

The Canakinumab Anti-inflammatory Thrombosis Outcome Study<sup>[41]</sup> looked at the use of IL-1 $\beta$  inhibitor canakinumab to test for reduced cardiovascular disease risk. The success of this trial had led for the use of canakinumab to determine if there is a CHIP-specific response to IL-1 $\beta$  inhibition. The administration of canakinumab had decreased relative risk of major cardiovascular events by 64% in carriers of acquired *TET2* CHIP mutations. This result indicates the promising method of IL-1 $\beta$  inhibition in treating myeloid malignancies with increased IL-1 $\beta$  expression.

## ACQUISITION OF DRIVER MUTATIONS COMMONLY OCCURS VERY EARLY IN LIFE

A common question asked by patients with hematologic malignancies is “when did I acquire this mutation, was I born with it?” Recent elegant studies from multiple groups demonstrate that MPN driver mutations are acquired decades before clinical presentation. Using whole genome sequencing of more than 800 hematopoietic colonies from patients with MPN, Williams et al<sup>[42]</sup> were able to reconstruct the phylogenetic tree of mutation acquisition and with it deduce the timing during each patient's life. The *JAK2<sup>V617F</sup>* mutation was acquired on average 30 years before diagnosis, and in many was acquired in utero or shortly after birth. Another study by Van Egeren et al<sup>[43]</sup> found similarly that the *JAK2<sup>V617F</sup>* mutation was acquired long before clinical manifestation of MPN. CHIP mutations may also occur very early in life, as identical CHIP mutations can be found in monozygotic twins.<sup>[44]</sup>

These intriguing studies highlight the long slow tempo of MPN and conceivably offer a long period of time (decades) to intervene with preventive strategies that steer evolution of hematopoiesis to favor healthy HSC over mutant HSC. It also suggests that the presence of *JAK2*<sup>V617F</sup> mutant cells may be much more common than we currently appreciate, and that the important factor may not be the acquisition of these mutations per se, but the selection of the mutant HSC.

## IMPACT OF NUMBER OF MUTATIONS AND ORDER OF ACQUISITION

The order in which mutations are acquired can impact the clinical manifestations of MPN. Patients with MPN who first acquired a *JAK2* mutation before a *TET2* mutation were significantly more likely to have polycythemia vera, an increased risk of thrombosis, and increased *in vivo* sensitivity to the JAK inhibitor ruxolitinib. Also, there is a difference in cellular expansion versus cellular differentiation depending on the order of acquisition of mutations.<sup>[45]</sup>

A study determined that patient outcome is determined by the number of driver mutations for oncogenic genes (not for *TP53* or *SF3B1* mutations). The most common mutated genes that cause MDS are *ASXL1*, *DNMT3A*, *RUNX1*, *TET2*, and genes that form the spliceosome.<sup>[46]</sup> It has been estimated that the median leukemia-free survival for patients with 1 oncogenic mutation or cytogenetic lesion was 49 months, dropping to 42, 27, 18, and 4 months for patients with 2, 3, 4 to 5, and 6 or more mutations, respectively. These data are determined to match with observations than MDS-to-AML transformation (or relapse of *de novo* AML) is driven by clonal evolution associated with acquisition of new driver mutations.<sup>[47-49]</sup>

Also, there is the study of acquisition to determine if a specific mutation had been acquired early or late in the life of a patient. The acquiring is likely caused by either “constraint” (mutations must occur in a specific order) or “opportunity” (likelihood that mutations happen in any order).<sup>[46]</sup>

The genetic screening for AML-causing mutations may have theoretical advantages of attacking an identifiable clone early. However, this may lead to the development of a therapy-resistant clone population. A workaround for this would be the use of an adaptive therapy that is continuously adjusted to maintain a balance between chemo-sensitive cells and resistant cells, avoiding the rise of an uncontrollable and resistant clonal population.<sup>[8]</sup>

## INFLAMMATION DRIVES DIFFERENT SYMPTOMS OF MYELOID MALIGNANCIES

Many of the symptoms of myeloid malignancies are reminiscent of symptoms commonly encountered with chronic autoimmune disease and infections. These include fever, fatigue, redness, pain, and enlarged spleen.

Inflammatory signaling has been shown to cause these symptoms.<sup>[50,51]</sup> In MPN, where improvement of symptom burden is a key therapeutic goal, JAK inhibitors reduce inflammatory cytokines coincident with improvement in symptom burden.<sup>[52,53]</sup> Therefore, inflammation not only promotes the development of myeloid malignancies but also has a significant impact on quality of life.

## ANEMIA OF INFLAMMATION IN MYELOID MALIGNANCIES

As damage-associated molecular patterns proteins (e.g., S100A8/9) have increased expression, this helps mediate a differentiation defect caused by the common 5q chromosome deletion seen in MDS, leading to increased inflammation, ineffective erythropoiesis, and anemia.<sup>[54]</sup> The loss of certain micro RNAs also led to inflammation and anemia.<sup>[55,56]</sup>

Anemia in MF is commonly severe enough to require blood transfusions and is associated with a poor quality of life, transfusion dependency leads to an additional disease complication of iron overload.<sup>[57]</sup> The anemia in MF is an example of anemia of chronic inflammation (AI), which is seen in other conditions, including chronic kidney disease, chronic inflammatory diseases, malignancy, obesity, and aging. Chronic inflammation elevates hepcidin. Hepcidin is the master iron regulator and a significant contributor to AI. Hepcidin binds and degrades ferroportin, which prevents export of intracellular iron from the cells into the bloodstream. Although iron stores are abundant in AI, this iron cannot be used for red blood cell production because it is sequestered inside the cells of the reticuloendothelial system. Hepcidin levels are elevated in patients with MF, suggesting that AI is in part driving anemia in MF.

Hepcidin inhibitors relieve anemia in animal models of AI. Momelotinib inhibits the bone morphogenic protein receptor kinase activin A receptor type 1 to lower serum hepcidin. Momelotinib normalized hemoglobin in a rat model of chronic inflammation induced by streptococcal peptidoglycan-polysaccharide injection.<sup>[58]</sup> Momelotinib is a JAK inhibitor actively being investigated in MF clinical trials. Momelotinib's ability to improve anemia in MF is unique among JAK inhibitors and is attributed to hepcidin inhibition.<sup>[59]</sup>

## USING CYTOKINE SIGNATURES AS A DIAGNOSTIC/PROGNOSTIC TOOL

Specific cytokine signatures are likely characteristic of MPN subtypes and so could potentially be used as a diagnostic tool in this disease. In a comprehensive serum cytokine profile of more than 400 MPN patient samples Øbro et al<sup>[60]</sup> found an ET-specific inflammatory cytokine signature consisting of eotaxin, GRO- $\alpha$ , and EGF. Moreover, GRO- $\alpha$  levels were predictive of MF progression in patients with essential thrombocythemia, sug-

gesting that potentially longitudinal sampling of cytokines could be used to monitor for transformation to MF. Reduction in inflammatory cytokines by ruxolitinib, specifically TNF, correlated with a reduction in spleen size.<sup>[17]</sup>

In MF cytokines also have a prognostic impact. In treatment naïve patients, increased levels of IL-8, IL-2R, IL-12, IL-15, and IP-10 were independently predictive of inferior survival.<sup>[61]</sup> Although quantification of plasma cytokines is not used clinically in patients with MPN at the present time, it is possible that in the future cytokine quantification could be incorporated into the clinical care of patients with MPN, aiding in diagnosis, prognostication, and to monitor treatment and progression.

Patients with AML were found to have higher TNF, IL-6, and IL-10 levels. For the patients over the age of 65, there were further increased levels of IL-4, IL-5, and IL-12p70. For the patients younger than 65, IL-8 was increased. This study concluded that decreased IL-6 and increased IL-10 correlated with favorable prognostic factors for survival in patients with AML.<sup>[62]</sup>

While AML has similar cytokine and chemokine expression as in MDS, there are some differences between them. AML has increased levels of IL-8 and IL-13, while MDS has increased levels of VEGF-A.<sup>[63]</sup> In MDS, an analysis of inflammatory cytokines demonstrated higher levels of TNF, IL-6, and IL-8 in patients with MDS than in a control population. The study concluded that these inflammatory cytokine profiles change along the progression of the disease.<sup>[64]</sup>

Also, in MDS and AML expression of cytokines and chemokines have been classified into eight recurrent cytokine signatures (expression patterns) that can be used to predict patient prognosis independent of other factors.<sup>[63]</sup> The eight signatures can be combined to form the following three outcome groups: favorable, intermediate, and unfavorable. The presence of these eight distinct signatures suggests that there are interactions between leukemic cells and the surrounding environment, leading to specific protein expression signatures. These eight signatures can be used as sources to target with therapeutics.<sup>[63]</sup>

## INFLAMMATION AS A THERAPEUTIC TARGET

Appreciating is mounting for inflammation as a common therapeutic target in hematologic malignancies (Table 2). Multiple drugs in use or under investigation for myeloid malignancies have anti-inflammatory properties. JAK inhibitors are the only Food and Drug Administration-approved class of drugs for MF. As a drug class, JAK inhibitors are also widely used in autoimmune diseases.<sup>[65]</sup> Although JAK inhibitors do not reduce the mutant neoplastic clone, their benefits lie in reduction of spleen size and improvement in symptoms, these benefits may be a consequence of its anti-inflammatory potential.<sup>[52,53]</sup>

**Table 2.** Ongoing Interventional Clinical Trials Targeting Inflammatory Pathways

Clinical Trial	AML	MDS	MPN
Bevacizumab (VEGF inhibitor) <sup>[7,8]</sup>	X	X	
Magrolimab (CD47 inhibitor) <sup>[26,27]</sup>		X	
Mediterranean dietary intervention			X
Momelotinib (JAK1/2 and hepcidin inhibitor) <sup>[58,59]</sup>			X
BET inhibitors <sup>[68-68]</sup>			X
Pacritinib (IRAK1 and JAK1/2 inhibitor) <sup>[69-72]</sup>	X	X	X
CA-4948 (IRAK4 inhibitor) <sup>[73]</sup>	X	X	

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; VEGF: vascular endothelium growth factor.

BET inhibitors are also in clinical trials for MPN. BET inhibitors reduce NF-κB induced inflammation and reduce fibrosis when combined with JAK inhibitors in mouse models.<sup>[66]</sup> The BET inhibitor CPI-0610 is being investigated in MF in multiple settings, including ruxolitinib-naïve<sup>[67]</sup> patients as well as an add on to ruxolitinib.<sup>[68]</sup>

Targeting of inflammatory pathways is also an active area of clinical investigation in MDS and AML. The use of IRAK1 and JAK1/2 inhibitor pacritinib for patients with MF has shown promising results in early phase trials with limited myelotoxicity and clinical responses.<sup>[69-71]</sup> Mutations in U2AF1 in MDS and AML induce expression of IRAK4 isoforms leading to chronic innate immune signaling.<sup>[72]</sup> An IRAK4 inhibitor CA-4948 is currently in phase 1 clinical trials for MDS and AML.<sup>[73]</sup>

Immunosuppressive therapy is used for certain subtypes of MDS in which immune mediated pathogenesis is suspected, such as hypocellular MDS. Scoring systems leveraging factors including HLA-DR15, age, and duration of red cell transfusion dependence can be helpful to predict those more likely to have a response to immunosuppressive therapy.<sup>[74]</sup> Antithymocyte globulin has been used for the bone marrow failure associated with myelodysplastic syndromes.<sup>[75]</sup> Immunosuppressive therapy using alemtuzumab, a monoclonal antibody that can bind CD52-lymphocytes and target them for destruction, has been shown to be a viable therapeutic option.<sup>[76]</sup>

Agents that reduce inflammation could also be employed in the future for CHIP, not only as a preventive measure to block progression to hematologic malignancies as well as prevent the other sequelae of clonal hematopoiesis, such as cardiovascular disease. Tocilizumab, an IL-6 blocker, was found to reduce the TET2 mutant clonal burden in a rhesus macaque model of CHIP.<sup>[39]</sup> For preventive measures, however, low cost, low risk, widely available anti-inflammatory may be a more feasible approach.

## CONCLUSION

Overall, there is large body of mounting evidence demonstrating an important role for inflammation in

the development, pathogenesis, and clinical presentation of myeloid malignancies. We are only beginning to leverage the scientific findings from preclinical laboratory research into improvements in clinical care for patients with myeloid malignancy. Theoretically, inflammatory markers could be exploited for early detection, diagnosis, and prognostication in myeloid malignancies. Therapeutics targeting inflammation could be used for a wide range of applications, including prevention, symptom management, and targeting the malignant clone and deranged microenvironment.

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