Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer?

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The morbidity and mortality of patients with the chronic Philadelphia-negative myeloproliferative neoplasms (MPNs), essential thrombocythemia, polycythemia vera, and primary myelofibrosis are mainly caused by cardiovascular diseases, thrombohemorrhagic complications, and bone marrow failure because of myelofibrosis and leukemic transformation. In the general population, chronic inflammation is considered of major importance for the development of atherosclerosis and cancer. MPNs are characterized by a state of chronic inflammation, which is proposed to be the common denominator for the development of "premature atherosclerosis," clonal evolution, and second cancer in patients with MPNs. Chronic inflammation may both initiate clonal evolution and catalyze its expansion from early disease stage to the myelofibrotic burnt-out phase. Furthermore, chronic inflammation may also add to the severity of cardiovascular disease burden by accelerating the development of atherosclerosis, which is well described and recognized in other chronic inflammatory diseases. A link between chronic inflammation, atherosclerosis, and second cancer in MPNs favors early intervention at the time of diagnosis (statins and interferon-α2), the aims being to dampen chronic inflammation and clonal evolution and thereby also diminish concurrent disease-mediated chronic inflammation and its consequences (accelerated atherosclerosis and second cancer). (Blood. 2012; 119(14):3219-3225)

Introduction

The Philadelphia-negative chronic myeloproliferative neoplasms (MPNs), essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF), are clonal hematopoietic stem cell disorders, in which the JAK2 V617F mutation is detected in more than 95% of PV patients and in approximately 50% of patients with ET and PMF. Transitions between disease entities are common, which may depict a biologic continuum from early disease to the advanced myelofibrosis stage.

Since the discovery of the JAK2V617F mutation, other mutations have been described, contributing to unregulated Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling, modulation of transcription, and accumulation of oncoproteins.

However, it is not established how all these abnormalities interact and influence disease initiation, clonal evolution, and terminal blast crisis. Several of these mutations are considered to be secondary events, whereas the initiating hit(s) and the driver of clonal evolution in these neoplasms are unknown. Elevated biomarkers of chronic inflammation and inflammatory and autoimmune diseases have been described in all disease entities, being most common in the advanced myelofibrosis stage.

Based on evidence obtained from clinical observations and experimental and molecular studies, it is argued that chronic inflammation may be both an initiator and a driver of clonal evolution in patients with MPNs. The perspectives of chronic inflammation as the underlying mechanism are discussed with particular focus on the early development of accelerating atherothrombosis (premature atherosclerosis), thrombosis, and second cancer. It is concluded that this novel interpretation of current data (in perspective) supports the contention of early therapeutic intervention with agents having the potential to dampen chronic inflammation and impair clonal evolution. Statins and IFN-α2 may be highly important in this setting, with IFN-α2 being able to induce sustained molecular remissions in a subset of patients and accordingly may reduce the risk of thrombohemorrhagic complications, myelofibrotic or leukemic transformation, and the development of second cancer.

Does chronic inflammation contribute to cardiovascular morbidity and accelerated atherosclerosis in patients with ET, PV, and PMF?

Chronic inflammation has an important role in the development of atherosclerosis and for years it has been evident that chronic inflammatory diseases (eg, rheumatoid arthritis, psoriasis, and systemic lupus erythematosus) are associated with accelerated atherosclerosis (premature atherosclerosis). In addition, chronic inflammation has an important impact on the development of premature atherosclerosis in patients with diabetes mellitus as well. Furthermore, chronic inflammation also indirectly predisposes to venous thrombosis because an association between atherosclerosis and venous thrombosis has been documented.

C-reactive protein (CRP) is a sensitive marker of inflammation. Because CRP influences endothelial function, coagulation, and fibrinolysis, oxidation of low-density lipoprotein, and plaque...
Does chronic inflammation predispose to insulin resistance and diabetes mellitus in patients with ET, PV, and PMF?

Chronic inflammation has been shown to be the link between insulin resistance, obesity, and diabetes. Indeed, activation of the inflammation cascade, endothelial dysfunction, and procoagulant imbalance are very important pathophysiologic mechanisms leading to β-cell damage, insulin resistance, and the vascular complications of diabetes.

Circulating biomarkers, such as TNF-α, IL-6, CRP (inflammation), vascular cellular adhesion molecule-1, interstitial cellular adhesion molecule-1, E-selectin, von Willebrand factor (endothelial dysfunction), plasminogen activator inhibitor-1, fibrinogen, P-selectin (procoagulant state), and adiponectin (anti-inflammation), may all be associated with development of both type 1 and type 2 diabetes. Indeed, some studies, particularly in type 2 diabetes, have demonstrated certain biomarkers to have independent predictive value. These biomarkers may also be associated with development of diabetic nephropathy and retinopathy, and, in particular, in type 2 diabetes, with cardiovascular events as well. In addition, insulin resistance has been shown to play an important role in the increased frequency of atherosclerosis in patients with rheumatoid arthritis and to be an independent risk factor for atherosclerosis in this disease. Based on the close association between chronic inflammation and insulin resistance and chronic inflammation as a potentially very important pathogenetic factor contributing significantly to cardiovascular disease burden, a disturbed glucose homeostasis might be expected to be present in MPN patients as well, an association that deserves to be explored systematically in a prospective study.

Does chronic inflammation contribute to hyperuricemia in ET, PV, and PMF?

In the general population, hyperuricemia is associated with chronic inflammation, cardiovascular disease, and renal disease. Accordingly, it might be anticipated that hyperuricemia also influences the cardiovascular disease burden in patients with MPNs. However, serum uric acid has not been considered a risk factor for cardiovascular disease in these patients but may indeed be important, elicited by chronic inflammation and the increased cell turnover. The elevated serum uric acid per se may constitute a risk factor for endothelial dysfunction, even mild hyperuricemia being a significant independent risk factor for endothelial dysfunction in subjects without the metabolic syndrome. Of note, treatment with allupurinol in patients with chronic kidney disease (CKD) has been shown to decrease CRP, slow down the progression of renal disease, and reduce cardiovascular and hospitalization risk.
Does chronic inflammation contribute to CKD ("MPN nephropathy")?

Most recently, a series of patients with MPNs and chronic renal failure has been described and previous reports reviewed. The designation "myeloproliferative glomerulopathy" was proposed for this particular entity, which may be more prevalent than previously recognized. In regard to the pathogenesis of myeloproliferative glomerulopathy, it was proposed that platelet-derived growth factor and TGF-β might probably have an important role when considering that both cytokines are elevated in patients and platelet-derived growth factor is a very potent stimulus of mesangial cell proliferation and induces extracellular matrix production by mesangial cells. The impact of chronic inflammation per se on the development of CKD also has to be considered in these patients. Thus, beyond the known factors predisposing to CKD (eg, obesity, hypertension, and diabetes mellitus), there is evidence of a pathophysiologic role for inflammation as well. Indeed, chronic inflammation in MPNs may contribute significantly to the progression of CKD by inducing an increase in several inflammatory biomarkers, including CRP and IL-6, which are considered of importance in CKD pathogenesis. Chronic inflammation in CKD also further causes mortality from cardiovascular disease by contributing to the development of vascular calcifications and endothelial dysfunction. The more frequent occurrence of CKD in patients with myelofibrosis compared with earlier disease stages (ET and PV) may reflect the long-term deleterious influence of chronic inflammation in this patient group. Accordingly, chronic inflammation per se may facilitate renal dysfunction in MPN patients. The potential of statin treatment to lower disease burden associated with this particular complication deserves to be explored because statin administration is accompanied by risk reduction in all major vascular events in patients with CKD.

Does chronic inflammation trigger clonal evolution, autoimmunity, and second cancer in ET, PV, and PMF?

Chronic inflammation is considered of major importance in the development of several cancers, including certain hematologic neoplasms. Recently, several molecular and cellular signaling circuits have been identified linking inflammation and cancer, a concept that was already described by Virchow in the 19th century when he suggested that chronic inflammation might give rise to malignancy. However, not until more recently, the link between inflammation and cancer has been acknowledged, being partly attributed to epidemiologic studies, which have generated data indicating chronic infections and inflammation as major risk factors for various types of cancer. Despite chronic inflammation being well described as having a major pathogenetic role in the development and progression of certain malignant lymphomas, chronic inflammation as a potential initiating event and a driver of clonal evolution in myeloid cancer has not been focused on. However, an increased risk of myeloid malignancies in autoimmune conditions has been documented and most recently, a large Swedish epidemiologic study concluded that chronic immune stimulation might act as a trigger for development of the myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML). It is intriguing to consider whether previous and concurrent inflammation may also contribute in the pathogenesis and development of ET, PV, and PMF. Herein, it is hypothesized that chronic inflammation with ensuing sustained immune stimulation may trigger clonal evolution and also may catalyze and drive the clone from the early disease stage toward the advanced myelofibrosis stage. Accordingly, myelofibrosis may be featured by clinical, biochemical, and molecular evidence of an aberrant and dysfunctional immune system implying a potential risk of autoimmune/autoinflammatory diseases and, in addition, an increased risk of second cancer.

Several observations are supportive for this hypothesis. First, several studies have shown that inflammatory diseases may precede or develop during the course of ET, PV, and myelofibrosis. In the Swedish study, a prior history of any autoimmune disease was associated with a significantly increased risk of myeloproliferative neoplasm. The "inflammatory" diseases included, among others, Crohn disease, polymyalgia rheumatica, and giant cell arteritis, and the "autoimmune" diseases included immune thrombocytopenic purpura and aplastic anemia. An association between autoimmunity and myeloid cancer has been known for several years in patients with MDS, and various autoimmune phenomena have also been reported in MPN patients, including immune activation in the bone marrow, circulating immune complexes, complement activation, and autoimmune cytophenias. Second, most recent studies have shown that patients with MPNs have an increased risk of second cancer: both hematologic and nonhematologic. This risk may be inherent and associated with the particular JAK2 V617F haplotype. Other pathogenetic mechanisms might be related to the JAK2V617F mutation per se, which has been shown to be associated with an increased risk of second cancer. Third, vascular endothelial growth factor and TGF-β are potently immunosuppressive cytokines, which are elevated in the circulation and markedly expressed in the bone marrow in MPNs. Both cytokines may induce qualitative and quantitative alterations in immune cells, being responsible for intact tumor immune surveillance (eg, dendritic cells, cytotoxic T cells, regulatory T cells, NK cells). Fourth, based on the association between antecedent chronic inflammatory or autoimmune diseases and MPNs and the link between chronic inflammation, autoimmunity, and development of other myeloid cancers (MDS and AML) it is tempting to speculate whether chronic inflammation may also elicit clonal myeloproliferative cancer rather than merely being a consequence of clonal myeloproliferation. Most recently, the link between chronic inflammation with elevated levels of several cytokines, including TNF-α, and clonal evolution has been substantiated by the findings that TNF-α is capable of facilitating clonal expansion of JAK2V617F-positive cells in myeloproliferative neoplasms. Accordingly, chronic inflammation may drive clonal expansion from early disease stage to the burnt-out myelofibrosis stage. Fifth, the hypothesis that chronic inflammation may precede and predispose to the development of MPNs is further supported by the findings that chronic inflammatory diseases (eg, Crohn disease and certain myeloid cancers, including MPNs, share an increased frequency of the JAK2 46/1 haplotype of chromosome 9q, which is present in approximately 45% of the normal population. The JAK2 46/1 haplotype may be a "marker of inappropriate myelomonocytic response to cytokine stimulation leading to increased risk of inflammation, myeloid neoplasms, and impaired defense against infection," which most recently has been excellently described. Indeed, the observations by Tefferi et al, that the JAK2 46/1 haplotype confers inferior survival in PMF and increases the risk of myelofibrotic transformation in patients with
PV,71 may reflect enhanced chronic inflammation, contributing to clonal evolution consequent to increased genetic instability (JAK2 46/1 predisposes to additional mutations in the JAK2 gene; JAK2 activation and the JAK2V617F mutation per se induces genetic instability73) but also to cytokine-mediated expansion of the malignant clone.59,64,73 Importantly, the epidemiologic evidence of an association between antecedent chronic infections and inflammatory diseases and the development of other myeloid cancers (MDS and AML)46,47 is suggestive of a similar association in myeloproliferative cancer. Lastly, several studies have shown elevation of a number of cytokines involved in inflammation and immunoregulatory cancer.46,49 and most recently gene expression profiling studies have also evidenced a marked deregulation of several inflammation and immune genes in patients with MPNs,50,51 all supporting chronic inflammation and immune stimulation/deregulation to be involved in the pathogenesis of these neoplasms.

**NF-κB, the common pathway for chronic inflammation, atherosclerosis, and cancer?**

The causal relationship at the cellular level between chronic inflammation and atherosclerosis and between chronic inflammation and cancer development includes several pathophysiologic processes,74 all being linked by inflammation and a single transcription factor, NF-κB.42,75 As other cancers, the MPNs are caused by defective tumor immune surveillance and signal-transduction mechanisms.74 Among these, the NF-κB pathway has a key role in inflammation and innate immunity75 but also in promoting tumor development.76,77 Accordingly, the NF-κB pathway may provide the link between chronic inflammation, atherosclerosis, and cancer in patients with MPNs. Furthermore, NF-κB may have a major role in controlling the ability of the malignant clone to resist apoptosis propagated by tumor-surveillance mechanisms. In this context, the impact of TNF-α as a tumor promoter77,78 and its ability to facilitate clonal expansion of JAK2V617-positive cells may be of crucial importance.64

**Do monocytes link atherosclerosis and cancer in patients with MPNs?**

Monocytes are key cells of the immune system, which is indispensable for the development and progression of both atherosclerosis6,7 and cancer.79 Circulating monocytes increase in number as the atherosclerosis worsens and monocyte migration to the vessel wall is a key event in the growth of atherosclerotic lesions. When accumulating in the vessel wall, monocytes differentiate into macrophages and lipid-rich foam cells, which are of utmost importance in disease complications.79

Monocytosis is common in patients with myelofibrosis, in whom it is an independent prognostic factor implying inferior survival.80 It is intriguing to consider whether monocytosis in myelofibrosis, in addition to clonal myeloproliferation, may reflect cardiovascular disease burden as well. Finally, several studies in solid tumors have shown that tumor-associated macrophages (TAMs) in the tumor microenvironment promote cancer,81,82 It remains to be elucidated whether the monocytosis in myelofibrosis is associated with an increased density of TAMs within the bone marrow, which may further add to the poor prognosis.

**Discussion and perspectives**

The morbidity and mortality of patients with MPNs are closely associated with cardiovascular disease burden and clonal evolution with myelofibrotic and/or leukemic transformation.1,4,24,28,83 Most recently, epidemiologic studies have evidenced that these patients are exposed to an increased risk of both hematologic and nonhematologic cancer as well.52,53 In the context of cardiovascular disease burden, the general concept has been that these complications were primarily attributed to abnormal rheology consequent to the raised hematocrit, leukocytosis, and thrombocytosis, and in vivo activation of leukocytes, thrombocytes, and endothelial cells.24,28 Consequently, activated clonal cells release several proinflammatory products, which altogether elicit a state of chronic inflammation.25 However, another supplementary mechanism may imply that chronic inflammation per se elicits and drives clonal evolution toward the burnt-out myelofibrosis phase. This novel concept is supported by the known link between chronic inflammation and cancer being recognized for several years and most recently also substantiated in myeloid cancer by the association between chronic inflammatory/autoimmune diseases and the subsequent development of MDS and AML.47 Furthermore, several reports have described the concurrence of chronic inflammatory or autoimmune disease and MPNs, in particular in patients with myelofibrosis.4,5,55-58

Chronic inflammation and cancer share a common pathway, the NF-κB. In cancer, including the MPNs, NF-κB is misregulated and constitutively active. As such, NF-κB turns on the expression of many genes whose products mediate inflammatory and immune responses and genes that sustain cell proliferation and inhibit apoptosis, potentially promoting cancer development.75,77 Of note, many of these cytokines (eg, IL-1, IL-6, IL-8, and TNF-α) are elevated in patients with MPNs, in particular in myelofibrosis.81 In the bone marrow, the expression of genes for E-selectin, IL-8, and TNF-α may result in neutrophil activation and TNF-α-facilitated clonal expansion.64 In the context that NF-κB controls many genes involved in inflammation and chronic inflammation has a major role in the development of atherosclerosis, this pathway is activated in atherosclerosis as well.84 Importantly, key regulators of NF-κB are associated with elevated mortality, especially from cardiovascular disease.85,86

The NF-κB pathway has been shown to be activated and implicated in the abnormal release of TGF-β in PMF, implying an important role of NF-κB activation in the development of bone marrow fibrosis.87,88 As noted previously, TGF-β is highly immunosuppressive, impairing the functionality of several immune cells (dendritic cells, NK cells, and cytotoxic T cells) involved in tumor immune surveillance.61,63 Accordingly, by enhancing TGF-β release in the bone marrow, NF-κB activation may not only promote fibrosis but also indirectly promote expansion of the malignant clone by suppressing immune cells.

The JAK2V617F-mutation induces constitutive activation of downstream signaling pathways, including STAT3/STAT5 with ensuing induction of cell proliferation (STAT5) and neutrophil activation (STAT3).90 By triggering the NF-κB and JAK pathways, STAT3 also activates the production of enzymes (metalloproteinases), cytokines (IL-6, IL-10, IL-17, and IL-23), and growth factors (vascular endothelial growth factor, fibroblast growth factor). Accordingly, STAT3 may be a key regulator of cancer-associated inflammation in MPNs eliciting and sustaining angiogenesis and metastatic potential (egress of CD34+ cells from the bone marrow). In the context of tumor inflammation and immunity,
STAT3 has a dual function because STAT3 both promotes oncogenic inflammatory pathways, including NF-κB and IL-6, and also opposes STAT1-and NF-κB-mediated T helper 1 antitumor immune responses.91

In the general population, leukocytosis is a well-known risk factor for cardiovascular disease,92 and most recently leukocytosis has been shown to be an important risk factor for the development of thrombosis in the MPN population as well.28,29 Furthermore, leukocytosis has a detrimental effect on survival in patients with “ET,” when these patients are validated strictly according to WHO criteria, implying significantly different survival of patients with WHO-defined ET and early/prefibrotic PMF.93 In regard to atherosclerosis, a heavy burden of cardiovascular morbidity may be seen already at the time of diagnosis, albeit the risk of cardiovascular death is but moderate compared with the high risk of death from noncardiovascular causes (mainly hematologic transformations) in a large cohort of patients with PV.83 It is tempting to speculate, however, whether these patients may actually have an increased risk of developing premature atherosclerosis, considering the link between chronic inflammation and atherosclerosis.5-13,16-18 Because biomarkers of chronic inflammation are elevated in MPN patients,3 chronic inflammation may not only have an impact on the risk of thrombosis but also facilitating clonal expansion mediated via high levels of TNF-α in the bone marrow.64

Most recently, gene expression profiling studies have unraveled massive deregulation of a number of genes involved in immune regulation and inflammation,50,51 adding to the concept that chronic inflammation and immune deregulation may be important in the pathogenesis and progression of these neoplasms.50,51 Irrespective of the underlying mechanisms for aberrant inflammatory and immune function, deregulation of inflammation and immune genes may drive clonal myeloproliferation and evolution toward the myelofibrotic burnt-out phase of the disease. Ultimately, the abnormal immune homeostasis may imply a defective tumor immune surveillance, which may contribute to the increased frequency of second cancer as well.52,53 The impressive efficacy of JAK1-2 inhibitor and HDAC-inhibitor treatment in reducing huge splenomegaly, alleviating hypermetabolic symptoms, and partly also reducing leukocyte and platelet counts34,56 may primarily reflect the very potent anti-inflammatory effects of both agents. In this context, the rapid resolution of large splenomegaly may be consequent to elimination of reactive immune cells in the spleen more than a direct antitumor effect, being supported by no or minor reduction in JAK2V617F-allele burden despite a pronounced reduction in inflammatory cytokines, including TNF-α and IL-6.64

The concept of chronic inflammation as a likely important contributing factor in MPN pathogenesis may have several implications. In regard to treatment, this concept certainly argues for initial treatment to eliminate leukocytosis, a risk factor for thrombosis in the general population and undoubtedly also in this patient group probably an important factor for cardiovascular disease. Statins are standard medications used in cardiovascular diseases, not only because of their cholesterol-lowering effects but also because statins possess several pleiotropic effects, including very potent anti-inflammatory properties,97 which collectively (by dampening inflammation, activation of leukocytes, platelets, and endothelial cells) may benefit patients with MPNs and accordingly should be considered already at the time of diagnosis.97 Furthermore, in the context of chronic inflammation as a potential promoter of cancer development and progression and given that these patients indeed have an increased risk of second cancer as well,52,53 it is also for these reasons important to alleviate the chronic inflammatory drive, which is perpetuated by the clonal myeloproliferation per se. The potential role of statins in the treatment of patients with MPNs97 has most recently been substantiated by the findings that statins decrease primary MPN cell colony formation and cooperate with JAK inhibitors (both JAK1 inhibitor I and JAK1-2 inhibitor ruxolitinib) with an enhanced reduction of JAK2-V617F-positive cell growth over single-agent treatment.98 Likewise, combination therapy with IFN-α may be a rational approach99,100 with the potential of inhibiting clonal expansion and thereby interrupting the inflammation-driven increase of several cytokines (eg, TNF-α, IL-6), which otherwise might facilitate clonal expansion, release of proinflammatory products from the expanding clone and accordingly further enhancement of chronic inflammation, and rise in inflammatory cytokines.

In conclusion, in the perspective that chronic inflammation and impaired tumor immune surveillance may be important factors in the pathogenesis and progression of these neoplasms, it seems rational, in addition to statins, to initiate immune-enhancing treatment with IFN-α at the time of diagnosis when the tumor burden is the least and accordingly the outcome of IFN-α likely the very best. By restoring potentially impaired tumor immune surveillance, IFN-α may prohibit clonal expansion, myelofibrotic and leukemic transformation, and development of second cancer as well.99,100

Authorship

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