Comment on Li et al, page 1528

JAK2 impedes stem cell function?

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In this issue of Blood, Li et al report that JAK2-V617F increases DNA damage and impedes hematopoietic stem cell function in a conditional knock-in mouse model of JAK2-V617F–positive essential thrombocythemia.1

Several mouse models have been generated to mimic the myeloproliferative neoplasm (MPN) phenotypes observed in patients. Retroviral transduction of bone marrow cells with JAK2-V617F cDNA resulted in a transplanted MPN phenotype with polycythemia vera (PV) and variable degrees of myelofibrosis.2,3,4 Transgenic mouse models expressing the JAK2-V617F displayed a phenotype resembling essential thrombocythemia (ET) or PV, depending in part on the relative expression levels of mutant and wild-type Jak2.7,9 Three groups have now generated knock-in mice by targeting the JAK2 locus to introduce the V617F mutation.1,10,11 These 3 models differ in the targeting strategy and, interestingly, also in the observed phenotypes (Table 1). Two of them are inducible using the Cre-loxP system,1,10 but only 1 allows breeding the mice to homozygosity by providing a wild-type Jak2 transcript in the noninduced configuration.10 In 2 studies, the mouse Jak2-V617F was used and in both cases a strong PV phenotype with high hematocrit, thrombocytosis (3- to 5-fold normal) and neutrophilia was observed.10,11

In contrast, Li and colleagues inserted a human JAK2-V617F cDNA into the start codon of the mouse Jak2 gene.1 The peripheral blood phenotype was the mildest of all the models made so far, with a modest increase in platelets (1.3-fold normal) and hematocrit with no signs of myelofibrosis. Over 26 weeks, approximately 10% of the mice developed a more pronounced increase in hematocrit resembling PV with a concomitant decrease in platelets; in 1 mouse myelofibrosis was noted. The reasons for the variations in phenotype within the cohort of JAK2-V617F knock-in mice are unclear. Thus, the genotype-phenotype correlations remains puzzling even after having 3 knock-in mouse strains at hand. There could be differences between the human and mouse Jak2-V617F in respect to how they signal in mouse hematopoietic cells, which may explain the mild ET phenotype of the human JAK2-V617F in the model by Li and colleagues.1 However, at similar expression levels, the human JAK2-V617F transgene caused a more pronounced phenotype ranging from ET to PV.7 Surprisingly, the 2 knock-in models that used the mouse Jak2 had similar phenotypes, despite JAK2-V617F expression levels of only 50% of wild-type Jak2 in the report by Akada.10 A 1:1 ratio between mutant and wild-type Jak2 was found by Marty and colleagues.11 A side-by-side comparison of the different mouse strains will be necessary to resolve open issues.

Li and colleagues have gone a step further in the analysis of their mouse model and examined stem cell function and accumulation of DNA damage. Unexpectedly, they found reduced numbers of Lineage^-Sca-1^-c-Kit^ (LSK) cells, which contain the early progenitors and the hematopoietic stem cells. No difference was present at 6 weeks, but a 50% reduction became apparent at 26 weeks.1 At this stage, the hematopoietic progenitors were also decreased. Bone marrow transplantation into sublethally irradiated hosts resulted in the same mild phenotype as in nontransplanted mice, but competitive bone marrow transplantation showed a reduced capacity of the JAK2-V617F bone marrow cells to outgrow wild-type competitor cells. This difference was even more pronounced when secondary transplantsations were performed, suggesting that the JAK2-V617F stem cells show signs of senescence and exhaustion. Several questions arise: why does the mouse model with the mildest phenotype show such a severe reduction of self-renewal potential in the stem cell compartment? The Jak2-V617F mouse model by Akada et al actually showed a 4-fold increase in LSK numbers.1 Because the genetic background of the knock-in mice by Li and colleagues was not uniform, it remains possible that weak performance in the competitive repopulation of sublethally irradiated hosts could be due to immunologic rejection.

Since JAK2-V617F has been reported to induce DNA damage, Li et al measured γ-H2AX levels, a histone variant that is used as an indicator for the presence of double-strand DNA breaks. The JAK2-V617F has been shown to increase homologous recombination and genetic instability.12 Indeed, the LSK cells from JAK2-V617F mice showed increased γ-H2AX levels at 26 weeks and also reduced numbers of apoptotic cells, as measured by annexin-V/7-aminoactinomycin D. These results are very interesting and merit further investigations. Whether mutations in additional genes found to be mutated in MPN, such as TET2, ASXL1, CBL, IDH1 and IKZF1, may provide additional clonal advantage and how they influence MPN stem cell function remains to be determined. The mouse models will be important tools to dissect these functional relationships.

Table 1. Mouse model targeting strategy and observed phenotype

<table>
<thead>
<tr>
<th>First author</th>
<th>Construct</th>
<th>JAK2-V617F</th>
<th>IoxP</th>
<th>Hb, g/L</th>
<th>WBC, ×10^9/L</th>
<th>Plt, ×10^9/L</th>
<th>Myelofibrosis</th>
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<tr>
<td>Li1</td>
<td>Knock-in</td>
<td>Human cDNA</td>
<td>Yes</td>
<td>150-170*</td>
<td>10-12</td>
<td>1500-1800</td>
<td>No†</td>
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<tr>
<td>Akada10</td>
<td>Knock-in</td>
<td>Mouse gene</td>
<td>Yes</td>
<td>200-230</td>
<td>20-35</td>
<td>1300-2000</td>
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<tr>
<td>Marty11</td>
<td>Knock-in</td>
<td>Mouse gene</td>
<td>No</td>
<td>na</td>
<td>50-70</td>
<td>3500-4500</td>
<td>Yes</td>
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<tr>
<td>Tiedt7</td>
<td>JAK2-BAC</td>
<td>Human gene</td>
<td>Yes (MxCre)</td>
<td>180-220</td>
<td>10-40</td>
<td>3000-6000</td>
<td>Yes</td>
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<tr>
<td>Tiedt7</td>
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<td>Human gene</td>
<td>Yes (VavCre)</td>
<td>140-170</td>
<td>15-25</td>
<td>6000-14 000</td>
<td>Yes</td>
</tr>
<tr>
<td>Shide8</td>
<td>H2K prom.</td>
<td>Mouse cDNA</td>
<td>No</td>
<td>na</td>
<td>10-50</td>
<td>1000-5000</td>
<td>Yes</td>
</tr>
<tr>
<td>Xing9</td>
<td>Vav prom.</td>
<td>Mouse cDNA</td>
<td>No</td>
<td>180 ± 14</td>
<td>11 ± 3</td>
<td>2700 ± 700</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Hb indicates hemoglobin; WBC, white blood count; Plt, platelet count; BAC, bacterial artificial chromosome; and na, not available.

*Approximately 10% of mice developed higher hemoglobin values at 26 weeks.
†Except in 1 mouse.
**PHAGOCYTES & GRANULOCYTES**

Comment on Meissner et al, page 1570

**NOX-free inflammasome activation**

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In this issue of Blood, Meissner and colleagues discover that immune cells from CGD patients that have defective phagocyte oxidases show hyperactive inflammasome activation. These findings implicate that ROS down-regulate rather than enable caspase-1 activation and identify anti-IL-1 strategies as a potential therapy for the disproportionate inflammatory responses associated with CGD.

In recent years, much has been learned about how the highly proinflammatory cytokines of the interleukin-1β (IL-1β) cytokine family are regulated. These cytokines differ from other cytokines in that they are produced as inactive proform in the cytosol upon transcriptional induction. In a second step, the protease caspase-1 mediates the cleavage and release of the IL-1β family of cytokines. Caspase-1 itself is produced as an inactive proform and also requires proteolytic activation, which is mediated by cytosolic multimolecular protein complexes that are termed inflammasomes. Among these, the NLRP3 inflammasome appears to be especially important as it is activated by a large variety of danger-associated signals including high concentrations of extracellular adenosine triphosphate, microbial pore-forming toxins, and aggregated materials. NLRP3 has been implicated in the pathogenesis of many inflammatory diseases; however, little is known about how the NLRP3 inflammasome can sense such a broad range of stimuli (see figure). The work by Meissner and colleagues contributes to our understanding of how caspase-1 is regulated and identifies IL-1 cytokines as potential therapeutic targets for inflammatory complications that are associated with chronic granulomatous disease (CGD).

The primary immunodeficiency disease CGD is based on mutations in any of the components of the nicotinamide dinucleotide phosphate (NADPH) oxidase complex, which include the membrane-bound gp91phox and p22phox glycoproteins and the cytoplasmic components p47phox and p67phox. The functional outcome, and a feature that aids in the diagnosis of CGD, is that phagocytes of affected persons fail to generate the reactive oxidant superoxide anion and its metabolites, hydrogen peroxide, hydroxyl anion, and hypohalous acid. Because these highly reactive molecules can function in the phagosome to attack ingested microbes, it is not too surprising that CGD patients are hypersusceptible to certain bacteria and fungi and suffer from recurrent life-threatening infections. A second, etiologically less obvious hallmark of CGD is the frequent appearance of inflammatory lesions, such as chronic colitis or lupus-like symptoms. It is known that the inflammatory symptoms in CGD are of noninfectious origin and—in most cases—respond to immunomodulatory therapy such as glucocorticoids. However, the molecular basis for this hyperinflammatory state in CGD patients remains to be elucidated.

The current study by Meissner together with 2 other recent reports identify a likely mechanism that could explain why CGD patients frequently suffer from sterile inflammation. Meissner et al demonstrate that active caspase-1 is elevated in immune cells from asymptomatic CGD patients leading to increased secretion of biologically active IL-1β. Furthermore, immune cells derived from CGD patients presenting with hyperinflammatory conditions released copious amounts of IL-1β. This response as well as clinical symptoms of hyperinflammation could be counteracted with the IL-1 receptor antagonist anakinra. These results indicate that a functional phagocyte oxidase is not essential in human cells for caspase-1 activation and IL-1β secretion and that reactive oxygen species (ROS) down-modulate IL-1β rather than activating it in these cells.

In the past, the role of NADPH oxidases and ROS in the activation of caspase-1 has been quite controversial. In a previous, technically very elegant study, the same group reported that SOD1-deficient macrophages, which fail to detoxify reactive superoxide species and thus have much higher ROS levels, secrete much less active IL-1β upon inflammasome stimulation. The reason for this defective IL-1β cytokine response is that constitutively elevated ROS levels in SOD1-deficient cells decrease the cellular redox potential and reversibly oxidize and glutathionylate cellular components including caspase-1. In vivo, SOD1-deficient mice are—similar to caspase-1-deficient mice—profoundly resistant to endotoxic shock. Consistent with these studies, a hyperinflammatory response is observed in mice that lack functional NADPH oxidases and caspase-1 activation is not impaired. Together, these studies provide persuasive evidence that ROS have an inhibitory effect on inflammasome activation.

**REFERENCES**

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