

## Not just clonal expansion of hematopoietic cells, but also activation of their progeny in the pathogenesis of myeloproliferative disorders

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The identification of a unique gain-of-function mutation of the Janus kinase 2 (*JAK2*) gene in patients with chronic myeloproliferative disorders<sup>1-4</sup> clearly represents a major breakthrough in the pathogenesis of these conditions. An article summary in the *New England Journal of Medicine* (Apr 28, 2005) stated that this identification opened new avenues of research, which could lead to improved diagnosis and treatment. In effect, in less than ten months an impressive number of papers have appeared that not only confirm the initial findings but also provide novel observations of biological and clinical interest.<sup>5-30</sup> By using allele-specific polymerase chain reaction (PCR), the frequency of the *JAK2* (V617F) mutation has been found to be greater than 85-90% in polycythemia vera, greater than 50% in essential thrombocythemia and around 50% in chronic idiopathic myelofibrosis. The majority of patients with polycythemia vera are therefore positive for *JAK2* (V617F), which should definitely be considered as a major diagnostic criterion for this condition. However, it should also be taken into account that there is a minority of patients with polycythemia vera, both sporadic and familial cases, in whom the mutation is absent.

A recent study<sup>16</sup> has likely clarified why the mutation is found in clonal disorders of myeloid, but not lymphoid, lineage and why it is mainly present in myeloid-lineage cells in myeloproliferative disorders. *JAK2* plays a central role in the signal transduction by homodimeric type-I cytokine receptors such as the erythropoietin receptor, granulocyte colony-stimulating factor (G-CSF) receptor and thrombopoietin receptor, and signal transduction by *JAK2* (V617F) requires a cytokine receptor as a scaffold. While myeloid-lineage cells express these type I cytokine receptors, lymphoid-lineage cells do not. This explains why the occurrence of the *JAK2* (V617F) mutation in a multipotent hematopoietic stem cell results in a selective expansion of its myeloid-lineage cell progeny.

A two-step model<sup>4</sup> for the role of *JAK2* (V617F) in the clonal evolution of myeloproliferative disorders is illustrated in Figure 1A. The available evidence suggests that this model might best apply to polycythemia vera, the condition in which the vast majority of patients carry the mutation. Recent studies in human cell lines suggest that homozygous expression of *JAK2* (V617F) may not only be the result of mitotic recombination, but also the consequence of loss and amplification of chromosome 9p.<sup>31</sup> It will be of interest to verify whether this also occurs in primary hematopoietic cells. In any case, irrespective of the underlying mechanism, progression

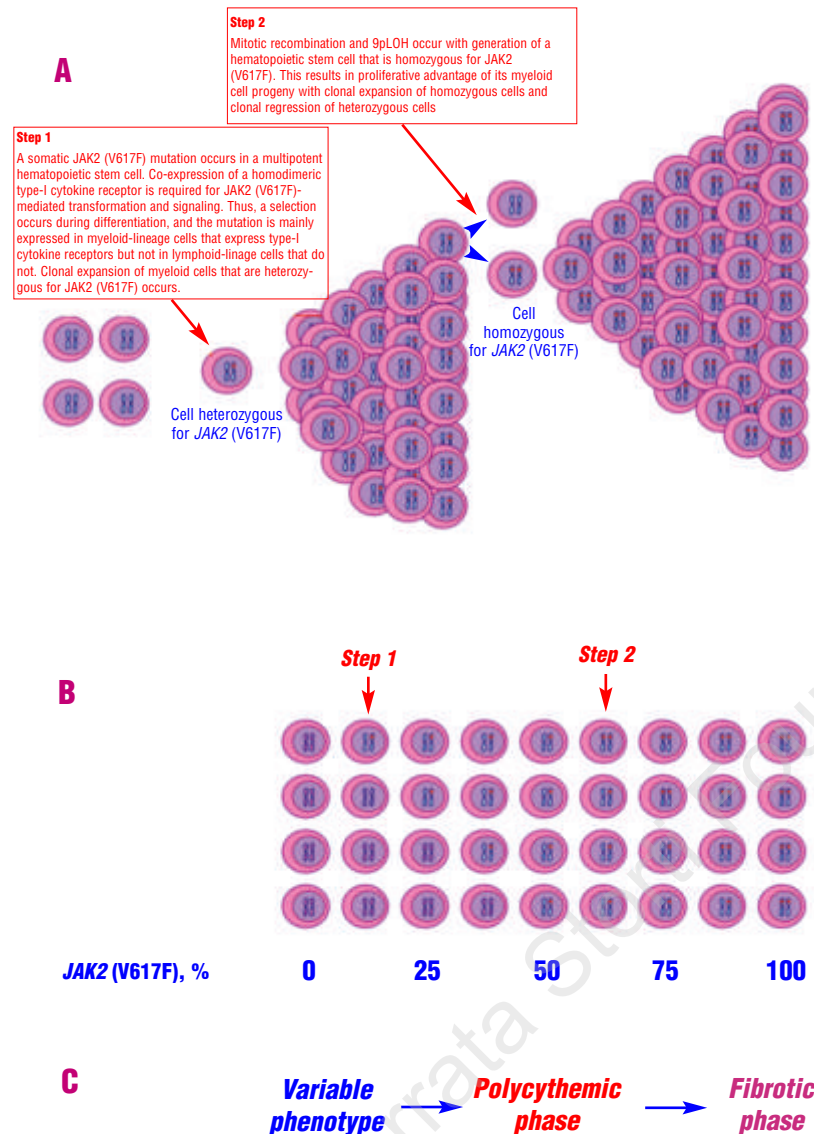
from heterozygosity to homozygosity for *JAK2* (V617F) represents an important step in disease progression in chronic myeloproliferative disorders.

The model reported in Figure 1 also illustrates how quantitative evaluation of granulocyte *JAK2* (V617F) mutant alleles may have clinical relevance in polycythemia vera (Figure 1B-C). In fact, clonal dominance of cells that are homozygous for *JAK2* (V617F) appears to be closely associated with constitutive mobilization of CD34<sup>+</sup> cells and fibrotic transformation.<sup>19</sup> We previously concluded that gain of function and loss of control appear to be the essential features of the excessive myeloproliferation associated with *JAK2* (V617F).<sup>32</sup> Gain of function results in increased production of mature blood cells. The mutated *JAK2* protein, in fact, improves signaling efficiency of several myeloid growth factors (erythropoietin, granulocyte colony-stimulating factor, thrombopoietin) determining increased production of mature blood cells. Loss of control means that the normal mechanisms that regulate circulating cell counts (for instance, transcriptional feedback regulation by erythropoietin) are no longer effective. In addition, loss of control means that activated mature blood cells – the progeny of expanded hematopoietic cells – have major effects on disease phenotype.

We recently reported that patients with myeloproliferative disorders have granulocyte activation patterns similar to those induced by granulocyte colony-stimulating factor.<sup>13,19</sup> These patients also have variably elevated circulating CD34<sup>+</sup> cell counts.<sup>19</sup> A *JAK2* (V617F) gene dosage effect was observed on both CD34<sup>+</sup> cell counts and granulocyte activation in polycythemia vera, in which abnormal patterns were mainly found in patients carrying >50% mutant alleles. These observations suggest that *JAK2* (V617F) may constitutively activate granulocytes and by this means mobilize CD34<sup>+</sup> cells.

In this issue of the journal, Arellano-Rodrigo and co-workers report studies aimed at investigating the role of platelet and leukocyte activation in the pathogenesis of thrombosis in essential thrombocythemia.<sup>33</sup> Their findings suggest that platelet and monocyte activation may play a role in the pathogenesis of thromboembolic complications in essential thrombocythemia. In particular, the *JAK2* (V617F) mutation appears to be associated with higher platelet activation.

Taken together, the above studies indicate that clinical manifestations of myeloproliferative disorders are not only related to increased numbers of mature blood cells, but also to their activation. While *JAK2* (V617F) can directly activate nucleated cells such as granulocytes and monocytes, activation of platelets is likely a result



**Figure 1.** Two-step model for the role of *JAK2* (V617F) in the clonal evolution of myeloproliferative disorders. **A.** The first step consists of a G-to-T transversion (red dot) that changes a valine to a phenylalanine at position 617 in one *JAK2* allele on chromosome 9p: this gives rise to a clone that is heterozygous for *JAK2* (V617F) which expands to replace hematopoietic cells without the *JAK2* mutation. The second step consists of a mitotic recombination in a hematopoietic cell that is heterozygous for *JAK2* (V617F); this generates uniparental disomy and loss of heterozygosity of 9p (9pLOH) in one of the two daughter cells. The daughter cell that is homozygous for *JAK2* (V617F) gives rise to a new clone that expands and replaces the previous heterozygous clone (clonal dominance). Both the transition from normal hematopoiesis to a clonal population of heterozygous cells, and that from heterozygous to homozygous clonal dominance are continuous processes characterized by mixed populations of cells. **B.** Expected percentages of *JAK2* (V617F) mutant alleles in the mixed cell populations generated by the above described clonal evolutions. These calculations are based on the assumption that the mitotic recombination (step 2) occurs once clonal dominance of cells that are heterozygous for *JAK2* (V617F) is fully established. Should step 2 occur earlier, the result would be a mixed population of normal, heterozygous and homozygous cell, and proportions of these cell types might vary considerable. For instance, 50% of mutant alleles might also reflect the following cell population: 25% normal cells, 50% heterozygous cells and 25% homozygous cells. **C.** Relation between different stages of polycythemia vera and granulocyte *JAK2* (V617F) mutation status. A small proportion of mutant alleles may be associated with variable phenotype. On the other hand, transition from heterozygosity to homozygosity for *JAK2* (V617F) appears to be associated with evolution from the polycythemic to the fibrotic phase of the disease, which is characterized by constitutive mobilization of CD34<sup>+</sup> cells into peripheral blood.

of the effect of *JAK2* (V617F) in megakaryocytes. These observations not only contribute to clarifying the pathophysiology of myeloproliferative disorders, but also provide useful diagnostic and prognostic information. For instance, sequential evaluation of the percentage of *JAK2* mutant alleles and enumeration of circulating CD34<sup>+</sup> cells appear potentially very useful for disease monitoring in polycythemia vera.<sup>19</sup>

Activation of mature blood cells is found in a not negligible portion of patients who do not carry the *JAK2* (V617F) mutation. From a clinical point of view, simple reliable approaches for evaluating cell activation may be useful for diagnostic purposes in these patients. As regards pathophysiology, the above observation clearly indicates that molecular mechanisms other than *JAK2* (V617F) can be responsible for the pathogenesis of myeloproliferative disorders. For instance, constitutive

mobilization of CD34<sup>+</sup> cells is found in most patients with idiopathic myelofibrosis who are *JAK2* (V617F)-negative. In addition, it is found in those who are positive but have low percentages of mutant alleles that would not *per se* involve significant granulocyte activation.<sup>19</sup> Interestingly, these additional pathophysiological mechanisms appear to operate, at least in part, through the same pathways activated by *JAK2* (V617F). This will hopefully lead to the identification of the currently unknown gene(s) whose mutations result in myeloproliferative phenotypes.<sup>34</sup>

*Dr. Cazzola's studies on myeloproliferative disorders are supported by AIRC (Milan), Fondazione Cariplo (Milan) and IRCCS Policlinico San Matteo (Pavia, Italy). The authors reported no potential conflict of interest relevant to this editorial comment.*

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