Circulating progenitor cells in chronic myeloproliferative diseases

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Knowledge in the field of Philadelphia chromosome-negative myeloproliferative diseases has increased greatly in last few years, thanks to new pathophysiological findings that have been quickly exploited in new diagnostic tools. At the turn of the new century, it was reported and then widely confirmed that an increased number of hematopoietic progenitor cells ($CD34^+$ cells, assessed according to the ISHAGE guidelines) circulate in the peripheral blood of patients with myelofibrosis with myeloid metaplasia (MMM) but not in patients affected by polycythemia vera (PV) or essential thrombocythemia (ET).[1-3] This finding has clinical relevance and is now routinely used for the differential diagnosis between MMM and PV or ET, although different reports have shown that 5% to 14% of patients with MMM have a number of circulating $CD34^+$ cells below the upper limit of the normal range (5×10^6/mL).[4,5] One year ago, it was reported that a substantial number of patients with MMM, PV, or ET harbor, in a variable proportion of their hematopoietic cells, the Val617Phe mutation of the tyrosine kinase JAK-2, which results in a gain of function of the gene.[6-9] The JAK-2 mutation has become not only an important marker of the malignant clone but also a useful diagnostic tool, although the presence of the mutation in all three types of disorders, which have their own distinctive clinical manifestations and prognosis, suggests that other genetic abnormalities may be involved in the pathogenesis of these myeloproliferative diseases. This year, it was reported that some patients with MMM and ET carry a mutation of the MPL gene, the receptor for thrombopoietin.[10,11] In a few cases mutations of both JAK-2 and MPL were detectable in the same patients.[11]

With respect to abnormal $CD34^+$ cell trafficking it should be remembered that endothelial progenitor cells (EPC), and not only hematopoietic progenitor cells (HPC), are mobilized in the peripheral blood of patients with MMM. In fact, by assessing the number of circulating cells co-expressing the CD34, the CD133, and the VEGFR2 antigens (at the moment the more rigorous phenotypic definition of EPC) it was shown that patients with MMM have significantly higher numbers of EPC in their peripheral blood than do patients with PV or ET or normal healthy subjects.[12] Interestingly, an inverse correlation was found between the number of $CD34^+$ HPC and the number of $CD34^+CD133^-VEGFR2^+$ EPC whereas there was a direct correlation between the frequency of EPC and both young age of the patients and a diagnosis of pre-fibrotic MMM. Thus, these data indicate that mobilization of EPC from the bone marrow takes place in an early phase of the disease, before the mobilization of HPC.[12]

The peculiar mechanisms underlying the mobilization of HPC and EPC are not yet known and neither are the reasons why mobilization seems to be prevalent in MMM and infrequent in PV or ET. Unfortunately, studies aimed at investigating the biological features of progenitor cells in MMM are hampered by the difficulty in aspirating bone marrow cells because of the fibrosis that characterizes the disease, and must mainly rely on circulating HPC and EPC. Nevertheless, some observations which could help in dissecting the mechanism of progenitor cell mobilization have been made. It has been shown that the plasma levels of metalloproteinase (MMP)-9 and neutrophil elastase are elevated in patients with MMM, although it was also found that these proteases were increased in the plasma of patients with PV as well.[13] In the same report, the soluble form of vascular cellular adhesion molecule-1 (VCAM-1) was found to be elevated in the plasma of MMM patients but not in that of PV patients or healthy subjects and, interestingly, the levels of soluble VCAM were correlated with the absolute number of circulating $CD34^+$ cells in the same cohort of patients. It was concluded that the elevation of neutrophil elastase associated with the elevation of soluble VCAM-1 observed in MMM patients could play a role in the progenitor cell mobilization characteristic of this disease. In the same study it was also shown that $CD34^+$ cells derived from the peripheral blood of MMM patients migrated through an *in vitro* reconstituted basement membrane more efficiently than did $CD34^+$ cells from normal subjects and that this accelerated migratory activity was reduced by incubating MMM $CD34^+$ cells with MMP-9 and MMP-13 inhibitors.

Although these studies do not incontestably prove the role of proteases in determining progenitor cell mobilization in MMM, they seem to suggest that the presence of an altered proteolytic extra- and intra-cellular microenvironment can play a role in abnormal progenitor cell trafficking in patients with MMM. Very recently, Passamonti *et al.* showed that patients with Philadelphia negative chronic myeloproliferative disease have granulocyte activation patterns similar to those observed in the granulocytes of healthy donors treated with granulocyte colony-stimulating factor for progenitor cell mobilization. Interestingly, they found a JAK-2(Val617Phe) gene dosage effect on granulocyte

activation, at least in PV patients, with abnormal patterns of activation being more frequently associated with patients with more than 50% of mutated alleles, suggesting that CD34+ cell mobilization had occurred in these patients through granulocyte activation.[14] An alternative but not exclusive hypothesis on the mechanism underlying progenitor cell trafficking in chronic myeloproliferative diseases stems from studies performed in both murine models and healthy human subjects showing that the interactions between SDF-1α and its unique receptor CXCR4 are involved in the regulation of both the retention and the mobilization of HPC (reviewed in Dar *et al*).[15] In this regard, it has been reported that reduced CXCR4 expression on circulating CD34+ cells from patients with MMM is associated with HPC mobilization.[16] It was found that the percentage of CD34+CXCR4+ cells was significantly lower in MMM patients than in patients with PV or ET or in healthy subjects, and that CXCR4 mean florescence intensity of MMM-derived CD34+ cells was also lower than that of controls. Accordingly, the mRNA levels of CXCR4 in purified CD34+ cells from patients with MMM were lower in those from healthy subjects. Moreover, there was an inverse correlation between the percentage of CXCR4+CD34+ cells and the number of circulating HPC.[16] These observations suggest that an altered expression and, in turn, a potential signaling defect of CXCR4 could be involved in the mobilization of progenitor cells in MMM.

Finally, it has been suggested that the alteration of the bone marrow microenvironment occurring as a consequence of the extensive fibrosis that characterizes this disease could favor the progenitor cell mobilization in MMM.[17] This hypothesis has the value of relying on the distinctive feature of fibrosis that distinguishes MMM from the other Philadelphia-negative chronic myeloproliferative diseases. However, the rate of failure of engraftment of allogeneic hematopoietic stem cell transplants in MMM patients is not higher than in other diseases.[18] This observation seems to make it unlikely that the disruption of bone marrow structure by progressive fibrosis could be responsible for progenitor cell mobilization in MMM.

In this issue of Haematologica, Oppliger Leibundgut *et al*.[19] report that patients with MMM have increased numbers of circulating HPC and EPC, thus confirming and extending previously published data on the same subject.[1-4,12] Interestingly, they also found that a small proportion of patients with PV and ET had increased percentages of circulating HPC and EPC, although they do not report whether there is a significant difference between the frequency of this phenomenon in these patients and in patients with MMM. More interestingly, taking advantage of the presence of the Val617Phe mutation of JAK-2, and of a trisomy 8 in one MMM patient, they show that both hematopoietic and endothelial progenitor cells carry the same genetic defect. This observation has important implications but also raises some concerns. First, the presence of the JAK-2 mutation in the two lineages implies that both belong to the same malignant clone that sustains the disease. This, in turn, raises the question of the role, if any, of circulating EPC in the pathogenesis of the disease. It is well known that MMM is characterized by increased bone marrow and spleen microvessel density.[20,21] The formation of new vessels into regenerating organs of the adult as well as in tumors, a phenomenon called neoangiogenesis, is thought to be due to the release of angiogenic growth factors by malignant cells and could also require the presence of (increased) numbers of circulating EPC.[22] The finding by Oppliger Leibundgut *et al*. that EPC in MMM are part of the malignant clone, together with the previously reported increased of VEGF plasma levels in patients with MMM,[23] supports the concept that neoangiogenesis is involved in the pathogenesis of the disease, and establishes a rationale for the use of anti-neoangiogenetic drugs in the treatment of MMM.[5]

Second, it indirectly proves that there is an immature progenitor cell common to both the hematopoietic and the endothelial lineages. The existence of this progenitor, commonly referred to as a hemangioblast, has been undoubtedly shown during embryogenesis but has been matter of debate in the adult individual.[24] The presence of the JAK-2 mutation in both hematopoietic and endothelial cells seems to indicate that such a cell is detectable, at least in patients with MMM. However, there are no reports so far of the presence of the JAK-2 mutation in T-cells of patients with Philadelphia-negative chronic myeloproliferative disease, suggesting that the mutated hemangioblast of patients with MMM derives from a progenitor cell more mature than the multipotent hematopoietic progenitor cell giving rise to the lymphoid lineage. Alternatively, normal activity of JAK-2 may be essential for T-cell differentiation/proliferation to occur. Finally, it cannot be ruled out that, since the JAK-2 mutation is an acquired event, most of the circulating T cells derive from pre-existing wild-type stem cells.

This exciting finding does, however, need to be accompanied by some words of caution. In fact, the endothelial cultures performed by the authors allow the growth of early endothelial colonies which make their appearance one week after seeding.[25] The real nature of this type of endothelial colony has been debated in the last years, because it was shown that they can derive from an *in vitro* differentiation of monocytes.[26,27] In fact, the authors themselves found in their sorting experiments that both monocytes and CD14-CD146+ cells (the latter being a marker of the endothelial lineage) were able to give rise to early

endothelial colonies *in vitro*.

Moreover, no data are reported on the genetic status of JAK-2 in the so-called late outgrowing endothelial cells which perhaps represent the progeny of a more immature type of endothelial progenitor, able to give rise to large endothelial colonies *in vitro* after 2-4 weeks of culture.[28,29]

Despite these concerns, which need to be resolved by further and more extensive investigations, the paper by Oppliger Leibundgut *et al*. paves the way for a new and wider interpretation of the pathogenesis of MMM based on the novel concept that increased mobilization of HPC and EPC as well as an increase of bone marrow and spleen neoangiogenetic processes are essential elements of the pathogenesis of the disease.

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