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THROMBOSIS AND HEMOSTASIS

Comment on Gerds et al, page 1646

REVEAL puts leukocytes into risk stratification

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In this issue of *Blood*, Gerds et al identified persistent leukocytosis above the upper normal value as a risk factor for thrombosis in polycythemia vera. Furthermore, this association has been demonstrated in both arterial and venous thrombosis, in high- and low-risk patients, and even in patients in whom hematocrit was adequately controlled.[1]

Thrombosis risk stratification is the basis for treatment selection in patients with polycythemia vera. Until now, the ECLAP (European Collaboration on Low-dose Aspirin in Polycythemia Vera) study, which included 1638 patients with a prospective follow-up of 2.7 years, provided the best evidence to guide treatment in this disease.[2] In that study, age and history of thrombosis were the main risk factors for thrombosis. Patients are usually stratified on the basis of this study into 2 risk categories: low risk (aged <60 years without a history of thrombosis) and high risk (aged >60 years or history of thrombosis). Since the publication of the ECLAP study in 2005, most clinical practice guidelines recommend treating low-risk patients with phlebotomies and high-risk patients with cytoreduction.[3,4]

The REVEAL (The Prospective Observational Study of Patients with Polycythemia Vera in US Clinical Practices) study included a total of 2510 patients with a median follow-up of 3.7 years, and it is the largest prospective study published to date in polycythemia vera.[1] In the 1768 high-risk patients, leukocytosis, thrombocytosis, and hematocrit >45% were associated with a higher probability of thrombosis, supporting that normalization of blood counts is associated with lower risk of thrombosis. This observation is in line with the results of a randomized clinical trial in patients with polycythemia vera resistant/intolerant to hydroxyurea, in which complete hematological response while on cytoreduction was associated with better event-free survival.[5] Regarding the type of thrombosis, the REVEAL study confirms that there are different risk factors for arterial and venous thrombosis, an observation that has also been reported in essential thrombocythemia.[6] Both leukocytosis and thrombocytosis, poor hematocrit control, and age were associated with a higher risk of arterial thrombosis, whereas female sex, history of previous thrombosis, and leukocytosis turned out to be the main risk factors for venous thrombosis. The fact that age was not associated with the risk of venous thrombosis could be explained by the excess burden of this type of thrombosis in patients with polycythemia vera of any age, which could cancel out the risk described for age in the general population. In this sense, venous thrombosis has been associated in polycythemia vera with a neutrophil-to-lymphocyte ratio >5 and a *JAK2*V617F variant allele frequency >50%, suggesting an important role for the biological characteristics of the mutated clone in this type of thrombosis.[7,8]

Some of the limitations of the REVEAL study include the fact that the median time elapsed from diagnosis to inclusion was 4 years. Thus, the proportion of newly diagnosed patients was small, which excludes from the analysis the period with higher thrombotic risk. On the other hand, cardiovascular risk factors have not been considered in the different analyses, precluding a precise evaluation of risk factors for arterial thrombosis. Finally, there was no information available on the mutational load of *JAK2* or the neutrophil-to-lymphocyte ratio that could help confirm previous observations in this regard, as well as exploring its interaction with leukocytosis.

Previous studies had postulated the possible role of leukocytosis as a risk factor for thrombosis and there is an important body of evidence on the role of leukocyte and platelet activation in the pathogenesis of thrombosis in patients with myeloproliferative neoplasms.[9] Despite this evidence, current clinical practice guidelines do not include leukocytosis in risk stratification.[3,4] Although a prospective clinical trial proving that normalization of leukocytosis reduces this risk could be of interest, low thrombotic rate and the need for a high number of patients with long follow-up make this option unfeasible. In the meanwhile, data from REVEAL support the inclusion of leukocytosis in the definition of high-risk polycythemia vera, and 2 aspects of clinical practice could be changed. First, classic low-risk patients with persistent leukocytosis could be elevated to the high-risk category and, therefore, be candidates for cytoreduction. Second, normalization of leukocytes should be included as an objective of treatment in patients receiving cytoreduction.

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TRANSPLANTATION

Comment on Takahashi et al, page 1656

Treg depletion supercharges ASCT power

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In this issue of *Blood*, Takahashi et al[1] reveal that during stem cell mobilization (SCM), T cells originating from the bone marrow migrate to the blood and exhibit a preference for returning to the marrow after adoptive transfer. Additionally, the study demonstrates that depleting regulatory T cells (Tregs) during SCM results in the expansion of polyfunctional effector T cells within mobilized grafts, ultimately enhancing antimyeloma immunity following autologous stem cell transplantation (ASCT) in a preclinical model (see figure).

For the >3 decades since its inception, ASCT remains the gold standard for the treatment of young patients with multiple myeloma (MM). Even the advent of groundbreaking agents, such as immunomodulatory drugs (IMiDs), proteasome inhibitors, and monoclonal antibodies, has not supplanted ASCT, but rather underscored its pivotal role in standard care.[2] The therapeutic success of myeloablative chemotherapy and ASCT was long assumed to be predicated on cytoreduction. However, there is now clear evidence that establishment of antimyeloma immunity is a key factor for the therapeutic success of ASCT. Along those lines, Geoffrey Hill's working group has developed a preclinical model demonstrating that the therapeutic benefit of ASCT lies with the generation of antigen-specific CD8 T cells that are able to control myeloma progression.[3] Despite these advances in our understanding and the clinical application of ASCT, a significant proportion of patients inevitably experience disease progression following treatment. This is intricately linked to the emergence of immune escape features of MM cells, underscoring the critical need for novel strategies to fortify antimyeloma immunity and address the limitations associated with disease relapse after ASCT.

In the present study, Takahashi et al explored factors influencing antimyeloma immunity during ASCT. To elucidate this topic, the authors analyzed the T-cell composition of bone marrow (BM) and peripheral blood stem cells (PBSCs) from patients with myeloma. Interestingly, mobilized stem cell grafts included a high number of effector CD8 T cells compared with the BM, while also containing a significant amount of immunosuppressive Tregs. The authors determined that these cells were mobilized from the BM into the peripheral blood during SCM. This is particularly relevant as the myeloma-experienced CD8 T cells from the donor transplant are crucial for tumor-specific immunity after ASCT.

However, their data indicated that unique immunosuppressive BM Tregs are also mobilized into PBSC grafts, which would reduce the beneficial effect of CD8 T cells.[4] Accordingly, the genetic depletion of Tregs during SCM resulted in a higher frequency of polyfunctional CD8 T cells in the graft and led to tumor-specific long-term control of myeloma after ASCT. Conversely, antibody-mediated depletion led to a reduction in the number of Tregs, but not to an increase in CD8 T cells, consistent with a pilot study following anti-CD25 (basiliximab) treatment.[5] Interestingly, the authors discovered a new strategy and used a synthetic interleukin 2 (IL-2)/IL-15 receptor agonist (NL-201) to expand the polyfunctional CD8 T cells in the mobilized graft (without triggering Tregs), which led to protection against myeloma progression in recipients of ASCT in an animal model.

Two particular aspects of this study should be emphasized. First, the study adds further support to the hypothesis that long-term tumor control can be mediated by antigen-specific T cells in myeloma. This leads to the interesting research avenue of characterizing these T cells more closely, particularly focusing on the clonality and the targeted antigen landscape. This information may have high translational value, as there could be myeloma-specific neoantigens or defined antigens that are not engaged due to Treg suppression. The murine Vk*MYC model used by the authors displays a high mutation load and chromothripsis,[6] as in humans, which supports the development of neoantigens and neoantigen-specific T-cell activation in myeloma.[7] The identification of such antigens or associated T-cell receptors may hold the key for a therapeutic intervention to better control myeloma with antigen-based immunotherapy.

Second, the study strongly suggests that selective enhancement of antimyeloma activity during ASCT by the novel and