Platelets are the Achilles' heel of Wiskott-Aldrich syndrome

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Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive disorder caused by loss of function of the Wiskott-Aldrich syndrome protein (WASp), a major regulator of the actin cytoskeleton in hematopoietic cells. Virtually all hematopoietic lineages are affected, from T, B, and natural killer lymphocytes to mononuclear cells (monocytes and dendritic cells), polymorphonuclear cells, and platelets.[1] This panhematopoietic profile accounts for the breadth of the phenotypic spectrum in patients with WAS, the most severe cases of which feature a predisposition to infections, allergy, autoimmunity, cancer, and bleeding related to thrombocytopenia. Hence WAS is a life-threatening disease that can only be cured by allogeneic hematopoietic stem cell transplantation (HSCT)[2] or (as recently demonstrated) gene therapy.[3-5]

The mechanism underlying WAS-associated thrombocytopenia has long been subject to debate. It includes a central component related to defective thrombocytopoiesis and a peripheral component in which the autoantibody-mediated destruction of platelets has a key role.[6,7] In this issue of the *Journal*, Sereni et al[8] provide a thorough analysis of the platelet defects in patients with WAS. In addition to the profound thrombocytopenia, abnormally low platelet size, and impaired aggregation capacity, the researchers highlight the abnormal ultrastructure of WASp-deficient platelets, the platelets' hyperactivated phenotype, the abnormally high production of reactive oxygen species, and, most interestingly, a perturbed proteomic profile with low expression of cytoskeletal proteins and signal transduction molecules and low levels of metabolic activity. In fact, WASp-deficient platelets appear to be senescent. When WASp is defective, megakaryocytes are still able to release platelets in good numbers[7]; however, rapid destruction of defective platelets in the spleen is combined with the effect of anti-platelet autoantibodies in many cases.[1] The fact that splenectomy can be effective in correcting WAS-associated thrombocytopenia and low platelet size supports this pathophysiologic hypothesis for the platelet disease in patients with WAS.

In the modern era, both allogeneic HSCT and WASp gene therapy have been shown to correct the immunodeficiency, although mixed chimerism (probably related to WASp-deficient B cells) is associated with recurrence of autoimmunity in a few cases. Transplantation fully corrects the thrombocytopenia[2,3] in almost all cases.

Gene therapy is less effective than HSCT because platelet counts of between 20 and 100 $\times$ 10^9/L are found 1 to 8 years after transplantation. This is good enough to dramatically reduce bleeding episodes and to avoid the need for platelet transfusions. This partial correction nevertheless prevents the application of gene therapy to patients with moderate forms of WAS (ie, so-called X-linked thrombocytopenia). In their extensive study Sereni et al[8] show that more than 90% of the platelets circulating in treated patients more than 2 years after gene therapy have much the same phenotype as wild-type platelets. The recipients' platelets express near-normal levels of WASp, although the fact that this is not the case at 1 year after treatment remains to be explained.

Overall, one can conclude that lentivirus-mediated gene therapy of WAS (based on use of an endogenous WAS promoter to drive WASp expression) restores qualitatively functional platelets but that there is still a quantitative issue. Why is this so? An unlikely explanation might be a weak but persistent antibody-mediated autoimmune reaction against the platelets. No evidence of this has been reported to date, and it is hard to believe that this phenomenon could occur in all treated patients. Alternatively, today's gene transfer technology might not provide a sufficient number of transduced hematopoietic stem cells (HSCs; Fig 1). A subnormal pool might be sufficient to enable normal levels of lymphopoiesis because of the expansion of transduced lymphocyte precursor cells and the relatively long lifespan of lymphocytes (relative to platelets). In support of the latter hypothesis, the vector copy number of the infused transduced $CD34^+$ cells is somewhat correlated with the platelet count after gene therapy.[3,4] However, one cannot completely rule out the possibility that the HSCs that are committed to platelet development[9] are less well transduced than other HSCs. It would be useful to compare the lentiviral integration site pattern within megakaryocytes and in other hematopoietic cell lineages. Also, $WASp^+$ megakaryocytes might have no selective advantage over nontransduced ones, thus reducing the output of $WASp^+$ platelets.

Which strategies could be used to increase platelet counts in patients with WAS treated with gene therapy? All the alternatives are associated with risks, which should be carefully calculated and compared. Increasing the concentration of viral particles (ie, "multiplicity of infection") during the transduction phase might augment the number of transduced cells but would also increase the vector copy number per cell, which might be dangerous. Using a stronger promoter would usefully increase WASp expression by platelets immediately after gene therapy but might not translate into greater

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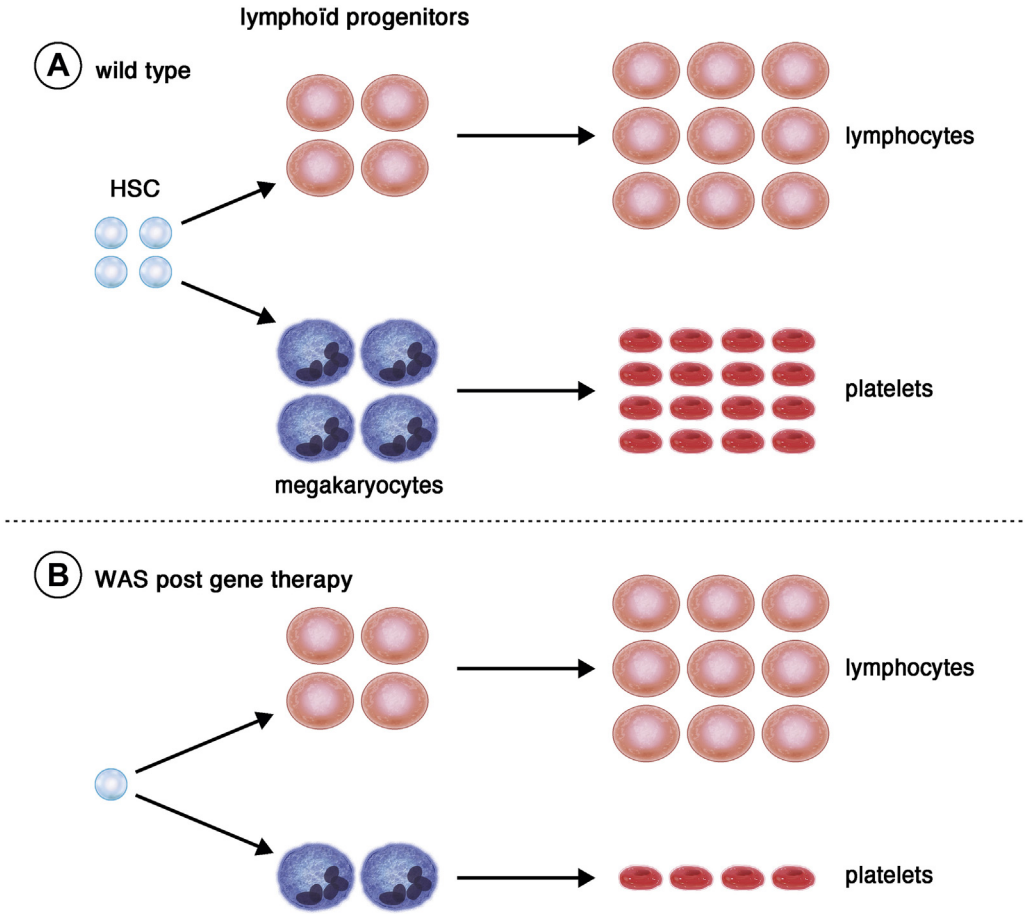


FIG 1. Lymphocytopoiesis and thrombocytopoiesis in healthy control subjects and patients with WAS after gene therapy. The size of the *circles* provides an estimation of the cell pool. For clarity, only the lymphocyte and platelet differentiation pathways are shown. $WASp^{+}$ lymphocyte precursor cells can expand and develop into long-lived lymphocytes. It is not as clear whether megakaryocytes can expand in a similar matter, whereas platelets have a much shorter lifespan than lymphocytes. Thus, after WASp gene transfer into HSCs, correction of platelet deficiency requires many more transformed cells than correction of lymphocyte deficiency.

numbers of transduced platelets. Using more aggressive myeloablative chemotherapy to clear the bone marrow niches for hosting greater numbers of transduced HSCs is obviously associated with a risk of myelotoxicity. However, the myeloablative chemotherapy regimens currently given to patients with WAS before allogeneic HSCT appear to be well supported.[10] Intraosseous administration, use of engineered stroma, transient inhibition of p53 or of the interferon type 1 pathway, and subtle improvements in virus-cell interaction, virus cell entry, or cell preactivation might also have a small but significant effect. It is important to note that deployment of today's gene editing technology would probably not do any better, given the low frequency of faithful recombination in noncycling HSCs.

Today, clinicians must choose between allogeneic HSCT with a risk of myelotoxicity and a safe gene therapy procedure that clearly corrects WAS immunodeficiency in the long term but also results in a persistent (although rarely symptomatic) mild thrombocytopenia. However, recent data suggest that the success rate for HSCT is now close to 90%, even when (pediatric) patients undergoing transplantation with hematopoietic cells from haploidentical donors are included.[10]

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