can still expand in vitro in response to EBV antigen challenge. Importantly from a safety perspective, extensive PCR analysis of retroviral integration sites did not reveal any evidence for the emergence of dominant T-cell clones within these gene-marked populations.

Overall, this study clearly demonstrates the long-term safety and efficacy of EBV-CTL infusion in a post-HSCT setting. Nevertheless, questions as to its optimal role still remain, particularly since the advent of an effective alternative in the form of anti-CD20 monoclonal antibody treatment for PTLD.[6] Clearly EBV-CTLs can still fill an important niche, for example, in patients who do not respond to or relapse after antibody treatment. Methodological improvements have now shortened the time required to manufacture a CTL product from a specific donor, and the attractive concept of an "off-the-shelf" CTL product, from third-party HLA-compatible donors, has also been developed into an effective reality in the context of solid-organ transplantation.[7] Furthermore, the engineering of EBV-CTLs resistant to calcineurin inhibitors is an advance for those patients who require ongoing immunosuppressive therapy.[8]

However, the greater importance of this work currently lies in its heuristic value for the field of adoptive T-cell therapy, whether one is seeking to target other viral infections of the immunocompromised or other virus-associated malignancies. In this latter context, EBV-positive lymphomas such as Hodgkin and T/NK-cell lymphomas, where viral target antigen expression is much more limited, constitute the next battleground.[9] Looking further ahead, might T-cell preparations against such a common human virus be exploited for even greater therapeutic benefit? That is as recipients of additional reactivities, conferred by T cell–receptor transfer in vitro, where the second specificity is directed against a target antigen of choice while the native EBV specificity guarantees retention of the cells in the virus-carrying host.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Dragani et al, page 1054

The tale of two COXs

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In this issue of *Blood*, Dragani and colleagues provide evidence in ET for increased reticulated platelets and for a role of COX-2 in the enhanced thromboxane A_2 production. These findings highlight the need to rethink the optimum antithrombotic regimens in ET and other states with accelerated platelet generation.

A key feature of platelet biology in essential thrombocythemia (ET) patients is increased thromboxane production, as shown by enhanced excretion of the urinary metabolite of thromboxane A_2. These patients have an increased incidence of arterial thrombotic events. The most common therapy has been low-dose aspirin in addition to cytoreduction. The platelet mechanisms involved in the thrombotic events and the optimum therapeutic approaches remain unknown. The studies of Dragani et al shed new insights on these areas.[1]

Cyclooxygenase-1 (COX-1) and COX-2 isozymes catalyze the conversion of arachidonic acid to prostaglandin (PG)H_2, the initial step in prostanoid synthesis leading to the formation of several products including thromboxane A_2, PGE_2, and PGI_2. Aspirin acetylates Ser529 in human COX-1 and Ser516 in human COX-2. When given once daily at low doses, aspirin almost completely inhibits COX-1 in platelets, which lack the ability to replenish the irreversibly inactivated enzyme. Much higher doses are needed to inhibit COX-2, an inducible enzyme; this is influenced also by the ability of nucleated cells to rapidly regenerate the enzyme.

Under physiologic conditions, platelet thromboxane production is primarily driven by COX-1 and endogenous PGI_2 by COX-2. Less than 10% of normal platelets have COX-2.[2] Recent studies show that COX-2 is present in megakaryocytes, up-regulated during megakaryopoiesis, and expressed in young platelets.[2] These findings have drawn attention to the potential contribution of COX-2 to thromboxane production in diverse clinical conditions. Dragani et al show convincingly that younger thiazole-staining reticulated platelets are increased, indicating enhanced platelet generation, and that platelet COX-2 expression is up-regulated in ET patients.[1] This is also described in other states associated with increased platelet generation.[2-4] Dragani et al show that aspirin at doses that almost completely suppress thromboxane production in healthy subjects is not as effective in ET. In an open-label randomized study in ET patients who are taking daily aspirin, the authors show that the addition of a COX-2 inhibitor to aspirin decreases by approximately 30% both urinary excretion of a thromboxane metabolite (marker of endogenous production) and ex vivo thromboxane production in serum (measure of total synthetic capacity). These studies corroborate that COX-2 contributes to endogenous thromboxane production in ET. Of note, the addition of the COX-2 inhibitor did not abolish thromboxane production, suggesting the role of additional factors.

The presence of increased numbers of reticulated platelets in ET draws attention to another major mechanism—namely, COX-1 activity in newly released platelets not inhibited by the once-daily low-dose aspirin. This is because of the relatively short half-life of aspirin in plasma. In states with rapid platelet generation, one daily dose of low-dose aspirin may be insufficient because of the immense capacity of bone marrow to accelerate platelet production (even 10-fold of basal rate). Dragani et al go on to show that addition of aspirin (50μM) in vitro abolishes thromboxane production in patients already receiving aspirin, consistent with an effect on COX-1 not acetylated by the single dose of aspirin administered 24 hours earlier. In a study of 60 healthy subjects, Guthikonda et al showed increased thromboxane production in healthy subjects with higher reticulated platelets, while ex vivo addition of COX-1 or COX-2 inhibitor attenuated the thromboxane production; the effect was greater with a COX-1 inhibitor.[4] They also showed decreased inhibitory effects of aspirin and clopidogrel in patients with coronary artery disease with higher numbers of reticulated platelets.[3]

The findings of Dragani et al raise important issues regarding optimum regimen to suppress thromboxane production. What is clear is that low-dose aspirin administered once daily is inadequate to fully suppress thromboxane production in ET and likely in other disorders with increased platelet production. The addition of a COX-2 inhibitor may not be the answer, because of the persistent thromboxane production following a 7-day dual drug therapy with a COX-2 antagonist and aspirin. The short plasma half-life of aspirin argues against added benefits of higher doses at the same dosing interval. The studies of Dragani et al advance strong support for a dosing interval shorter than the traditional once-daily aspirin—and this remains to be tested with clinical endpoints of thrombotic events.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● RED CELLS & IRON

Comment on Chen et al, page 1070

Getting personal with B19 parvovirus

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In this issue of *Blood,* Chen and colleagues [1] identify the 11-kDa nonstructural protein of the parvovirus B19 as the main culprit for induction of apoptosis in primary human erythroblasts through activation of caspase 10.

Infection with B19 parvovirus, usually spread by the respiratory route, is common in human populations and induces a mild transient and usually benign anemia.[2] In immunosuppressed patients (patients undergoing chemotherapy, undergoing bone marrow or solid organ transplantation, or infected with HIV) and in patients with congenital anemia associated with shorter red cell survival (sickle cell anemia and enzyme deficiencies), the anemia is severe and persistent and may be life-threatening.[3] In some patients, B19 parvovirus infection may even trigger or aggravate autoimmune diseases.[4] In pregnancy, B19 infection may cause severe anemia nonimmune hydrops and fetal loss.[2] The infection may also be transmitted through transfusion,[5] leading transfusion medicine researchers to evaluate pathogen inactivation/purging methods for some blood components and derivatives and to consider whether screening for this virus should be included in routine donor testing, at least in spring when outbreaks appear more likely.[5] Therapy includes intravenous supportive red cell transfusion and immunoglobulin administration (commercial immunoglobulin preparations contain anti-B19 antibodies) and/or reduction in the intensity/frequency of immunosuppressive treatments. However, if autoimmunity develops, immunosuppressive therapy may be indicated. The lack of specific therapies is mostly due to poor knowledge of the pathogenesis of the virus. B19 parvovirus shows a high tropism for the erythroid lineage because the virus enters the cells through the epitope corresponding to the P blood group antigen on the erythroid-specific receptor globoside. The virus kills erythroid cells by inducing apoptosis through a mechanism largely unknown up to now, due to a lack of suitable in vitro models (human erythroid cell lines are resistant to B19 infection).[6] Recently, it was demonstrated that primary $CD36^+$ erythroblasts expanded in vitro from purified human $CD34^+$ cells are permissive to B19 infection,[7] providing the much-needed experimental model to explore in vitro the mechanism of apoptosis induced by this virus.

In this issue of *Blood*, Chen et al,[1] by using this experimental model, finally disclose the mechanism by which B19 parvovirus induces apoptosis of erythroid cells. The authors identify the 11 kDa (1 of 3 nonstructural proteins encoded by the virus) as the protein that triggers the caspase-dependent apoptotic pathway, activating specifically caspase 10. The identification of the 11 kDa–caspase 10 pathway opens the way to structural modeling studies that may identify therapeutic drugs. Competing/inhibiting the 11 kDa–mediated caspase 10 activation will specifically prevent anemia induced by B19 infection. It is predicted that the identification of these drugs will be greatly facilitated by the use of ex vivo expanded human $CD36^+$ erythroblasts as a readout assay. In fact, $CD36^+$ erythroblasts can be expanded in vitro in great numbers from progenitor cells present in both adult blood and cord blood.[8,9] These numbers are similar to those required for transfusion (2×10^{12} erythroid cells). However, cost and logistic considerations suggest that the first clinical application of these cells will not be for transfusion but for drug discovery or delivery.[10] The current article implies that erythroblasts expanded ex vivo from adult or neonatal hematopoietic progenitor cells, as well as from