enhances T-cell reconstitution after transplantation. Recently, the group of Zúñiga-Pflücker has shown that human CD34+ CD38neg or low subsets of CB could be induced similarly to CD34+ CD7+ progenitors in OP9-DL1 cocultures, and these progenitors engrafted the thymus of immunodeficient mice.

However, the use of a stromal cells to support early T-lineage differentiation is less evident for clinical use. In this respect, the pioneering work of Bernstein and coworkers wherein immobilized Delta1ext-IgG is used to trigger Notch receptors on HSCs is more promising. They succeeded by culturing BM LSK progenitors onto immobilized DLL1 ligand to increase the number of progenitors capable of repopulating the thymus with accelerated early T-cell reconstitution. Initially, they used this approach to try to expand human CD34+ CD38- CB progenitors with lymphoid and myeloid reconstituting ability. However, they showed that induction of Notch signaling not only enhanced the generation of NOD/SCID repopulating cells but also enhanced thymic engraftment. The same group more recently presented data wherein enhanced T-cell reconstitution was reported for human CD34+ CD38- CB HSCs in engraftment studies in sublethally irradiated NOD/SCID mice after culture on immobilized Delta1ext-G.9

So far, only Notch-primed CB HSCs have been analyzed in vivo, but their use is limited by the low number of CD34+ cells that is seldom sufficient for transplantation in adults. BM and PBSC CD34+ progenitors are routinely used in HSC transplantation, but they have reduced T-lineage potential in vitro. The work of Meek et al now demonstrates that Notch-activated CD34+ PBSCs can engraft the thymus of immune-deficient mice. Therefore, they cultured CD34+ progenitors on a monolayer of murine thymus-derived stromal TSt-4 cells expressing either of the Notch ligands DLL1 or DLIL4. The cells expanded and matured toward pre-T cells with an iCD3i CD45RA+ CD7+ CD35+ phenotype and were able to fully mature in the thymus in vivo upon transfer into newborn Rag2-/- mice.

This report is of interest because it shows that the widely used PBSCs can be triggered by Notch ligands to generate a progenitor population that displays T-cell reconstitution in vivo. However, some points need further clarification. Apparently, the progenitor cells are blocked at an early stage of T-cell differentiation and cannot further mature on TSt-4 stromal cells. Yet, it is unclear whether this is related to the nature of the stem cell, the properties of the stromal cell line, or the lack of appropriate growth factors. It is also unclear whether there was a net gain in T-lineage output, and the results further indicate that CB HSCs are still superior to PBSCs. Finally, these data do not imply that precommitted T cells exist in vivo that are a prerequisite to home and to repopulate the thymus. It is possible that CD34+ HSCs that are precommitted in vitro are miraculous artifacts that are particularly suited to repopulate a thymus whose architecture and function have been affected by irradiation.

It is clear that there are still many questions that need to be answered to determine the optimal protocol for the clinical application of Notch-primed HSCs. These include a detailed analysis of the different sources of SCs as their different T-lineage potentials are still poorly defined, a comparison between the different stromal cell layers and the concentration of immobilized Notch ligands in a cell-free system, an evaluation of the use and dosage of growth factors, and a comprehensive in vivo mouse model that recapitulates HSTC as closely as possible.

However, the experimental data that have been obtained in recent years (see table) encourage us to believe that we can obtain similar successful results in patients with human CB, BM, and PBSC progenitors as Van den Brink obtained in mice cultured in a safer stromal cell–free system. These exciting developments should sound like a “stairway to heaven” to the ears of hematologists and their transplant recipients.

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IMMUNOBIOLOGY
Comment on Funderburg et al, page 161

Monocytes tied to HIV-associated thrombosis

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In this issue of Blood, Funderburg and colleagues provide new evidence that may help explain why HIV infection is associated with an increased risk of thrombosis. These researchers elegantly show that increased risk for HIV-infected persons has increased proportions of monocytes expressing the procoagulant cell surface tissue factor and propose that this may contribute to increased clotting in vivo.

HIV infection has emerged as a well-recognized prothrombotic condition. Venous thrombotic events (VTE) occur more commonly among HIV-infected persons than in the general population, and they often occur in relatively young patients. HIV-infected
persons receiving antiretroviral treatment (ART) have also been shown to exhibit greater atherosclerotic disease compared with their HIV-negative counterparts. Cardiovascular disease is likely to increase with enhanced longevity in HIV-positive persons on highly active antiretroviral therapy.

The mechanisms by which HIV infection causes thrombosis are multifactorial and complex. HIV-associated factors, as opposed to traditional risk factors (eg, smoking, immobility, family history, hospitalization), are believed to be central to the pathogenesis of thrombosis. Several coagulation abnormalities have been reported among HIV-infected patients including the presence of antiphospholipid antibodies, increased levels of von Willebrand factor, elevated homocysteine, and deficiencies of protein C, protein S, antithrombin III, and heparin cofactor II. Previous studies suggest that ART, in particular highly active antiretroviral therapy (HAART), negatively impacts the cardiovascular system. However, there are also several publications in which patients reported to manifest with VTE did not receive ART. Advanced HIV disease may be another risk factor for the development of thromboses, perhaps due to an increased inflammatory state or the presence of concurrent comorbidities, such as infections.

In the Strategies for Management of Antiretroviral Therapy (SMART) trial, mortality from non-AIDS events such as cardiovascular disease was found to be higher for participants randomized to interrupted, CD4-guided ART (drug conservation arm) than to continuous ART (viral suppression arm). This resulted in researchers stopping the trial prematurely. An increased risk of death was associated with higher levels of low-sensitivity C-reactive protein (hsCRP), interleukin 6 (IL-6), and D-dimers. IL-6 and D-dimer increased at 1 month by 30% and 16%, respectively, in the drug conservation arm but by only 0% and 5%, respectively, in the viral suppression arm (P < .0001). Moreover, the increases in these inflammatory/thrombotic markers in the drug conservation arm were related to HIV RNA levels at 1 month (P < .0001). These findings led the investigators to suggest that HIV-induced activation of inflammation and a hypercoagulable state increases the risk of death among HIV-positive patients, and that interrupting ART further increases this risk.

Funderburg et al suggest that the increased risk for coagulation in HIV-infected persons may be related to increased expression of the procoagulant tissue factor (TF, thromboplastin). They further demonstrate that monocyte expression of TF correlates with HIV RNA and D-dimer levels in plasma. A total of 60 HIV-infected patients in this study were analyzed, whom the investigators subdivided into viremic (28 patients) and aviremic (32 patients) groups, depending on whether their HIV viremia was above or below 400 copies/mL of HIV RNA. Although they mention that 13 of their patients (46%) in the viremic group were on highly active antiretroviral therapy, their paper failed to report whether ART in this subgroup in any way influenced their findings.

Recent findings suggest that, in addition to HIV viral replication, other factors (eg, microbial translocation) could drive immune activation. Accordingly, it is of great interest that Funderburg et al demonstrate that the bacterial Toll-like receptor, ligands lipopolysaccharide from *Escherichia coli* and flagellin from *Salmonella typhimurium*, induced monocyte TF expression in vitro. On the basis of their findings, these investigators propose that direct activation of monocytes by microbial products (eg, via bacterial translocation from the gut) may be a key player in promoting thrombosis in HIV infection.

The findings of Funderburg et al bring us ever closer to elucidating the mechanism by which HIV infection promotes thrombosis. More work in this field is needed, including the use of TF+ monocytes as a potential thrombotic marker (eg, for DVT prophylaxis) among HIV-infected patients, and the justification for using immunomodulators as adjuvant therapy to ART.

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**PLATELETS & THROMBOPOIESIS**

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Comment on Mumford et al, page 363

**A bleeding disorder is born**

**David Varon**

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In this issue of *Blood*, Mumford and colleagues report a D304N variant of the thromboxane A2 receptor (TxA2 R) in a patient with a bleeding diathesis. In their report, they describe the exciting path of a research journey initiated by a clinical observation, and leading to a discovery of a novel mechanism of a bleeding disorder: a mutation in the TxA2 R gene, leading to a D304N substitution. This mutation leads to a loss of function of the receptor due to reduced ligand binding.

Searching for the cause of a bleeding disorder may become quite frustrating in patients who have normal screening tests of coagulation and platelet function. Many such cases are left with the presumptive diagnosis of an “undefined bleeding disorder,” with general hemostatic support as the only therapeutic modality.

Our current screening tools for investigating primary hemostasis-related bleeding disorders are limited to platelet aggregometry (usually induced by ADP, epinephrine, collagen, ristocetin, and arachidonic acid), as well as testing von Willebrand factor antigen and activity. This limitation explains the quite frequent event of a “final”
Monocytes tied to HIV-associated thrombosis

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