inside blood

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HEMATOPOIESIS & STEM CELLS

Comment on Meek et al, page 261

A Notch stairway to thymus?

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In this issue of *Blood*, Meek and colleagues report that DLL-mediated predifferentiation of human mobilized CD34$^+$ cells in vitro results in a population that selectively homes to the murine thymus and displays faster T-lineage differentiation when injected.[1]

Allogeneic hematopoietic stem cell transplantation (HSCT) is a life-saving treatment in a variety of diseases but is accompanied by delayed T-cell restoration. This prolonged immune-incompetence is associated with an increased risk for severe life-threatening infections. Therefore, there is continued interest in strategies to improve T-cell reconstitution after conditioning and HSCT.

Radtke and colleagues discovered that Notch signaling is necessary for T-cell development.[2] The laboratory of Zúñiga-Pflücker elegantly applied this significant finding by engineering the murine bone marrow (BM)–derived OP9 stromal cell line to express the mouse DLL1 Notch ligand (OP9-DL1).[3] This strategy enables the induction of a T-cell differentiation path in mouse and human hematopoietic stem cells (HSCs) in the absence of a thymus microenvironment and differentiation toward the late stages of T-cell differentiation in human cord blood (CB)[4] and BM[5] HSCs. To date, this has not been reported for granulocyte-colony stimulating factor–mobilized peripheral blood HSCs (PBSCs).

Although of great scientific interest, the low number of T cells that reach the end stage of differentiation in this coculture system and the lack of appropriate signals for HLA-restricted education preclude the use of these in vitro–generated cells for transfusion in patients to enhance T-cell reconstitution after HSCT.[6] Therefore, several investigators have used the Notch trigger solely to induce HSC differentiation toward the first stages of T-cell development to speed up T-cell recovery in the thymus. The most encouraging data have been reported by the group of Van den Brink.[7] They obtained a 2000- to 5000-fold expansion of murine bone marrow–derived LSK (Lin$^-$Sca-1^{hi}c-kit^{hi}) cells with OP9-DL1 coculture. These expanded cells contained predominantly T-cell precursors of the DN2 (CD44$^+$CD25$^+$) or DN3 (CD44$^-$CD25$^+$) phenotype. Only the DN2 population contributed to thymus reconstitution after intravenous injection into lethally irradiated recipients. Importantly, the OP9-DL1–derived T-cell precursors substantially improved donor T-cell chimerism and gave rise to host-tolerant CD4$^+$ and CD8$^+$ populations with normal T-cell antigen receptor repertoires, cytokine secretion, and proliferative responses to antigen. The resistance to infection with *Listeria monocytogenes* was increased, and a significant graft-versus-tumor activity was present without graft-versus-host disease. This demonstrated the proof of principle that adoptive transfer of OP9-DL1–derived T-cell precursors markedly

Cell source	Species	Phenotype	Notch trigger	Cytokine culture	Read-out	Ref
BM	mouse	LSK	OP9-DL1	IL-7 + FLT3L (5ng/ml)	300 cGy TBI, reconstituted i.v.	Zakrzewski[7]
BM	mouse	LSK	Immobilized Delta1 $^{ext-myc}$	SCF, FLT3L, IL-6 (100ng/ml) IL-11	10 Gy, TBI reconstituted i.v.	Dallas[10]
CB	man	CD34$^+$CD38$^-$	Immobilized Delta1 $^{ext-myc}$	SCF, FLT3L(300ng/ml), TPO, IL-6,IL7 (100ng/ml), IL-3, GM-CSF, G-CSF (10ng/ml)	NOD/SCID or NOD/SCID $\beta2m^{-/-}$ 300-350 cGy reconstituted i.v.	Ohishi[9]
CB	man	CD34$^+$CD38$^-$ /lo	OP9-DL1	IL-7, SCF, FLT3L (30ng/ml)	Balb/c Rag2$^{-/-}$ $\gamma c^{-/-}$ or NOD/SCID/γc^{null} Nonirradiated neonates injected intra hepatically	Awong[8]
CB	man	CD34$^+$CD38$^-$	Immobilized Delta1 $^{ext-myc}$	SCF, FLT3L(300ng/ml), TPO, IL-6, (100ng/ml), IL-3, GM-CSF, G-CSF(10ng/ml)	NOD/SCID/$\gamma c^{-/-}$ 275 cGy i.v.	Dallas [11]
PBSC	man	CD34$^+$	TSt-4 –DLL1 or 4	SCF, FLT3L(100ng/ml), TPO (50ng/ml), IL-7 (10ng/ml), first week, then IL-7 (20ng/ml)	Balb/c Rag2$^{-/-}$ $\gamma c^{-/-}$ or NOD/SCID/γc^{null} 2×1.25 Gy Neonates injected intra hepatically	Meek[1]

Protocols of expansion of HSC progenitors that enhance T-cell reconstitution in vivo.

enhances T-cell reconstitution after transplantation. Recently, the group of Zúñiga-Pflücker[8] has shown that human $CD34^+CD38^{neg\ or\ low}$ 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 $Delta1^{ext\text{-}IgG}$ is used to trigger Notch receptors on HSCs is more promising.[9] 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.[9] 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 NOS/SCID/$\gamma c^{-/-}$ mice after culture on immobilized $Delta1^{ext\text{-}IgG}$.[10,11]

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-triggered $CD34^+$ 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 DLL4. The cells expanded and matured toward pro-T cells with an $iCD3^+CD45RA^+CD7^+CD5^+$ phenotype and were able to fully mature in the thymus in vivo upon transfer into newborn $Rag2^{-/-}\ \gamma_c{}^{-/-}$ 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 to and 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 HSCT 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.

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

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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.[1] These researchers elegantly show that increased risk for HIV-infected persons have 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.[2] HIV-infected