T and NK cell immunity after hematopoietic stem cell transplantation

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Chapter 8

Summary

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Chapter 1 provides the reader with basic background information regarding hematopoietic stem cell transplantation and viral complications after HSCT, as well as the clinical importance and immunologic background of the reconstitution of T cell and NK cell immunity after HSCT.

In the **first part** (Chapter 2-4) of this thesis, the focus is on the interaction between viral reactivation and T cell reconstitution.

In **Chapter 2**, we provide evidence that early and transient CMV reactivation leaves a long-lasting, dynamic and specific signature on the composition of the T cell compartment in pediatric HSCT recipients. We evaluated the composition of the cellular immune system in 131 HSCT recipients, of which 46 experienced a CMV reactivation in the first months after transplantation. One year after HSCT, patients that had encountered (and cleared) early CMV reactivation showed a marked relative as well as absolute expansion of the late differentiated $CD8^+$ EM and EMRA T cell populations, followed by a contraction in the second year after HSCT. This typical pattern was not seen in patients with early EBV or HAdV reactivation. CMV reactivation did not have a significant impact on $CD4^+$ T cells and NK cell numbers at one year after HSCT. Importantly, early CMV reactivation did not compromise the reconstitution of the naive and central memory compartments required for a balanced immune system with a broad specificity.

In **Chapter 3**, we describe the development of a clinical grade method to select virus-specific T cells for the restoration of T cell immunity against CMV, EBV and HAdV in one single procedure. Because not all virus-specific T cells produce interferon-γ upon activation, we sought for alternatives for the interferon-γ capture assay. For this, we compared the upregulation of various activation markers upon stimulation with viral peptide pools and identified CD25 as a good candidate. The proportion of T cells that upregulated CD25 expression was 3-7 times higher than the proportion of T cells producing IFN-γ, indicating that a larger proportion of virus-specific T cells could be isolated using this approach. As CD25 is highly expressed on regulatory T cells (Treg), the cell-product generated using this approach was not only enriched for virus-specific cells and depleted of alloreactive cells, but was also capable of suppressing alloreactivity *in vitro*.

In **Chapter 4**, the effectiveness of pre-emptive cidofovir treatment for human adenovirus viremia was evaluated in 42 HAdV reactivations after HSCT. Rapid HAdV load reduction and HAdV clearance during cidofovir treatment were associated with concomitant T cell reconstitution (n=20). In the absence of T cell reconstitution (n=22), the HAdV viremia was controlled in 75% of cidofovir treatments. Reduction of the HAdV load in the absence of T cells always coincided with a ≥5-fold increase of NK cell numbers in the peripheral blood. In only 2 out of 6 cases with an absence of both NK and T cells, HAdV load stabilization was observed during a >3 weeks period of cidofovir treatment. These data underscore the importance of T cell reconstitution to control adenovirus infections and the need for more effective antiviral drugs. In addition, these

data are suggestive of a role for NK cells in the control of HAdV viremia when T cells are not present.

In the **second part** (Chapter 5-7) of this thesis, the focus is on NK cells.

Chapter 5 addresses one of the restrictions of working with cryopreserved material. In freshly analyzed blood from healthy donors and HSCT recipients, the NK cell compartment consists of two subsets ($CD56^{bright}CD16^{+/-}$ or $CD56^{dim}CD16^{+}$). However, after cryopreservation of PBMC from HSCT recipients obtained in the first month after HSCT, a third NK cell subset appeared, which had a $CD56^{dim}CD16^{-}$ phenotype. Because this artifact is only seen under specific conditions, these cells can be mistaken for an important and distinct NK cell population if flowcytometric results on biobanked material are not validated with freshly isolated PBMC.

In **Chapter 6**, the reconstitution of NK and T cells was evaluated in 93 pediatric allogeneic HSCT recipients treated for leukemia. Early after HSCT, an expansion of $CD56^{bright}$ NK cells (>300 cells/µl blood) occurred in a subgroup of 38 patients characterized by a delayed T cell reconstitution. Post-transplant $CD56^{bright}$ NK cells showed a high expression of inflammatory chemokine receptors and cutaneous lymphocyte antigen, but had a reduced expression of homeostatic chemokines. In contrast to healthy donor $CD56^{bright}$ NK cells, post-transplant $CD56^{bright}$ NK cells expressed granzyme B and were cytotoxic. Patients with $CD56^{bright}$ NK cell expansion during delayed T cell reconstitution and patients with rapid T cell reconstitution had a similar clinical outcome. These data indicate that $CD56^{bright}$ NK cells form a versatile cell population, which can expand and acquire additional effector functions when T cells are not present to exert their function.

In **Chapter 7**, we identified a novel NK cell population in human lymphoid tissues (ltNK) based on the co-expression of the tissue retention marker CD69 and the chemokine receptor CXCR6. This population represented 30-60% of NK cells in bone marrow (n=20), spleen (n=7) and lymph node (n=3), but was absent from peripheral blood. Their surface marker expression profile was distinct from the conventional $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets. Functionally, ltNK cells produced interferon-γ at levels comparable to $CD56^{dim}$ NK cells. They constitutively expressed perforin, but like $CD56^{bright}$ NK cells, they required pre-activation to express granzyme B and to exert cytotoxicity. After HSCT, $CD69^{+}CXCR6^{+}$ lymphoid tissue NK cells showed a delayed reconstitution in comparison to conventional NK cell populations. The identification of this NK cell population in lymphoid tissues provides tools to further evaluate the cellular interactions and role of NK cells in human immunity.