Biol Blood Marrow Transplant xxx (2015) 1–12

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Immune Reconstitution after Allogeneic Hematopoietic Cell Transplantation in Children

Q7 Coco de Koning [1], Maud Plantinga [1], Paul Besseling [1], Jaap Jan Boelens [1,2], Stefan Nierkens [1,*]

[1] *Laboratory of Translational Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands*
[2] *Pediatric Blood and Marrow Transplantation Program, University Medical Centre Utrecht, Utrecht, The Netherlands*

Article history:
Received 2 June 2015
Accepted 25 August 2015

Key Words:
Allogeneic hematopoietic cell transplantation
Immune reconstitution
Secretome
Pediatric patients
Serotherapy
Biomarkers

A B S T R A C T

Allogeneic (allo) hematopoietic cell transplantation (HCT) has evolved into a potent curative treatment option for a variety of malignant and nonmalignant diseases. The occurrence of complications and mortality after allo-HCT is, however, still high and is strongly associated with immune reconstitution (IR). Therefore, detailed information on IR through immunomonitoring is crucial to improve survival chances after HCT. To date, information about the reconstituting immune system after allo-HCT in pediatric patients is mostly derived from routine standard-of-care measurements. More profound knowledge on IR may provide tools to better predict and modulate adverse reactions and, subsequently, improve survival chances. Here, we provide an overview of IR (eg, immune cell subsets and circulating chemokines/cytokines) after allo-HCT in children, taking into account different cell sources and serotherapy, and discuss strategies to enhance immunomonitoring. We conclude that available IR data after allo-HCT contain limited information on immune cell families (mostly only generic T, B, and NK cells), which would improve with more detailed information on reconstituting cell subsets or effector cell functionality at earlier time points (<1 month). In addition, secretome data (eg, multiplex cytokine/chemokine profiles) could add to the understanding of IR mechanisms and cell functionality and may even provide (early) biomarkers for individual disease outcome, such as viral reactivity, graft-versus-host disease, or graft-versus-leukemia. The present data and suggestions for more detailed, standardized, and harmonized immunomonitoring in future (pediatric) allo-HCT studies will pave the path to "precision transplantation:" an individualized HCT approach (including conditioning), based on detailed information on IR and biomarkers, aiming to reduce transplantation related mortality and relapse, and subsequently improve survival chances.

INTRODUCTION

Allogeneic (allo) hematopoietic cell transplantation (HCT) has evolved into a potent curative treatment option for a variety of malignant (eg, leukemia, lymphoma, multiple myeloma) and nonmalignant diseases (eg, primary immunodeficiencies, hemoglobinopathies, lysosomal storage diseases). Risk factors associated with this procedure may, however, lead to severe and life-threatening conditions. They involve transplantation-related mortality (TRM), infections, the occurrence of relapse (in case of malignancies), acute graft-versus-host disease (aGVHD) and chronic complications, such as chronic graft-versus-host disease (cGVHD) [1-3]. These complications are mainly due to a poor and prolonged lymphopenic state, as illustrated in Figure 1, and immune dysregulation as a result of cytokine fluctuations caused by conditioning [4-6].

Furthermore, strategies to reduce the probability of GVHD, such as in vivo T cell–depleting therapy with serotherapy, such as antithymocyte globulin (ATG) and Campath (alemtuzumab), have major impact on immune recovery [7-11] and, thus, affect the risk of relapse or infections. In particular, the unpredictability of the effects of conditioning and additional therapies on immune reconstitution (IR) in individual patients challenges survival outcome after allo-HCT therapy.

More profound knowledge on how this myriad of variables impacts IR after allo-HCT may provide tools to better predict and modulate adverse reactions and, subsequently, improve survival chances. Moreover, IR is dependent on age-related physiological aspects (eg, thymic function, hormones) and, therefore, demands separate study in adults and

Financial disclosure: See Acknowledgments on page 10.

* Correspondence and reprint requests: Stefan Nierkens, Laboratory of Translational Immunology, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands.
E-mail address: snierken@umcutrecht.nl (S. Nierkens).

http://dx.doi.org/10.1016/j.bbmt.2015.08.028
1083-8791/

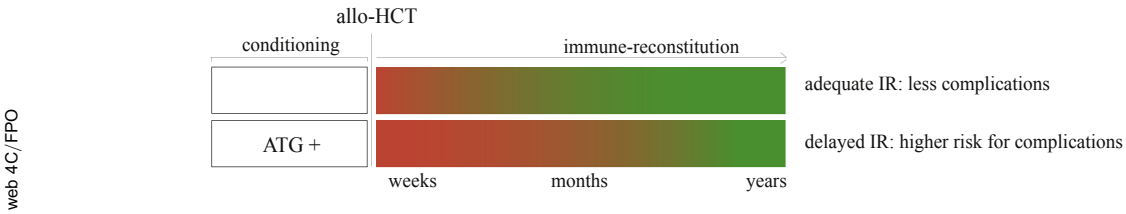

Figure 1. Delayed immune reconstitution after conditioning with ATG is an important factor in risk for allo-HCT related complications. After conditioning before to allo-HCT, and transplantation, immune cell numbers have to recover to normal/reference values of healthy controls. When ATG is added, immune reconstitution is delayed putting the patient at higher risk for transplantation-related complications and mortality.

children. In the present review, we focus on IR, regarding immune cell subsets and secretome after allo-HCT in children, related to the application of different cell sources (bone marrow [BM], peripheral blood [PB], and umbilical cord blood [UCB]) and serotherapy with ATG and discuss strategies to improve IR monitoring.

IMMUNE RECONSTITUTION AFTER HCT

Allo hematopoietic cells can be derived from BM, PB, or UCB. Although recovery of innate immune cells generally takes weeks to a few months, complete reconstitution of adaptive immunity is often delayed, typically from months to even years. Detailed information on IR in pediatric patients is largely lacking, especially during the first month(s) after allo-HCT (Tables 1 and 2, detailed information on studies reviewed in Table 3).

Reconstitution of the Innate Immune System

Neutrophils

Neutrophils are the first cells that recover after HCT (Table 1) and are used as early validation of engraftment. In children, neutrophils recover around 20 days after allo-HCT (defined as the first day with more than 500 cells/μL for 3 subsequent days) for all cell sources. Neutrophil function (eg, chemotaxis, phagocytosis, superoxide production, and killing of bacteria) lags behind and only reaches normal levels after 2 months [42,43].

Natural killer (NK) cells

NK cells are lymphocytes of the innate immune system, of which different subsets can broadly be characterized by the expression of CD56 and CD16 in CD3-negative cells. However, most studies characterize NK cells by the expression of CD56, with or without CD16, whereas only some studies exclude $CD3^+$ cells [44]. NK cells trigger an immune response when major histocompatibility complex (MHC class I) is absent or low expressed, eg, on stressed cells, while maintaining self-tolerance to healthy, MHC-expressing tissues. In humans, a relatively large family of NK cell receptors for MHC is represented by killer immunoglobulin-like receptors (KIRs).

After allo-HCT, NK cells are generally the first lymphocytes to reconstitute to normal numbers in 1 to 4 months, independent of cell source (Table 1 and [36]). However, no information is available on earlier time points or NK cell subsets and functionality. Low numbers of total NK cells in the first weeks after HCT are associated with low overall survival and higher risk of infection [15,21], underscoring an important role for NK cells in outcome after HCT.

Monocytes

Monocytes are myeloid cells arising from the BM that travel in the bloodstream and differentiate mainly into macrophages in tissues. Their primary functions are phagocytosis and release of pro- and anti-inflammatory chemokines and cytokines in the innate immune response and during the onset of acquired immunity [45,46]. Expression of CD14 and CD16 classifies subpopulations into "classical" $CD14^{++}CD16^-$, "intermediate" $CD14^{++}CD16^+$, and "non-classical" $CD14^+CD16^{++}$ monocytes [47,48]. Although reconstitution data over time after allo-HCT are unavailable, levels of CD16+ monocyte subsets are increased during aGVHD and restored during aGVHD therapy [49]. Moreover, HLA-DR expression on monocytes is decreased before and during sepsis or bacterial infections and in hepatic veno-occlusive disease [49,50]. In contrast, HLA-DR levels are elevated before and during viral reactivation and aGVHD [50]. These findings suggest that monocyte activation reflects acute inflammatory mechanisms, which may help identify HCT patients at risk for complications.

Dendritic cells

Dendritic cells (DCs) are antigen-presenting cells (APCs) that process and present antigen peptides on their cell surface in MHC-I and MHC-II. After internalization of antigen they migrate to the lymph nodes where they stimulate T and B cells to incite an adaptive immune response specific for phagocytosed antigens. Several DC subtypes can be found within the bloodstream: conventional DCs (cDCs), also referred to as myeloid DCs, and plasmacytoid DCs (pDCs). cDCs and pDCs lack lineage defining surface markers, such as CD2, CD3, CD5, CD19, CD20. cDCs are categorized in two subtypes expressing $CD11c^+CD123^-$ with either CD1b/c or CD141 expression, and pDCs with a phenotype of $CD11c^-CD123^+CD303^+$ or $CD304^+$ [51,52].

Data on cDC and pDC reconstitution after allo-HCT in children are only available for BM transplantation (BMT) and levels seem to strongly depend on age and sex [24-26,38,53] (Table 1). These studies show an initial increase in both pDC and cDC early after transplantation. During the first month, cell counts reach levels similar to, or even higher than, healthy control values for pDCs and cDCs, respectively. Thereafter, a significant decline is observed and the DC counts remain low for a considerable amount of time. Although cDCs stabilize to control reference levels within 1 year, pDCs remain significantly lower and approach normal levels toward the end of a follow-up period of 7 years [25].

Associations between pDC reconstitution and the occurrence of GVHD show some conflicting data [38]. Relatively low pDC levels at days 0 to 60 after HCT are associated with moderate to severe aGVHD in children, which is in line with observations in adults [54-56]. On the other hand, high pDC peak levels at days 0 to 60 are associated with higher risk of relapse, whereas high overall levels of both cDCs and pDCs during the first 200 days after