



Immune reconstitution following stem cell transplantation

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Learning Objectives

- Understanding immune reconstitution after hematopoietic stem cell transplantation
- Clinical implications of delays in post-transplant immune reconstitution
- Strategies to improve post-transplant immune reconstitution

Delayed immune reconstitution after hematopoietic stem cell transplantation (HSCT) has been associated with significant morbidity and mortality, especially after allogeneic HSCT (allo-HSCT), including infections and relapse.¹⁻³ In particular T-cell immunity is affected by the combined effects of the conditioning regimen, thymic involution in the host, donor age,⁴ type of graft, stem cell dose, ex vivo or in vivo T-cell depletion, donor-host disparity, graft-versus-host disease (GVHD) prophylaxis, and GVHD itself (both acute and chronic).

Innate immunity recovers in the first months after HSCT: first monocytes, followed by granulocytes and natural killer cells.⁵ In contrast, adaptive immunity, which consists of cellular (T lymphocytes) and humoral (B lymphocytes) immunity, takes 1-2 years to recover and a significant number of patients will incur even longer-lasting deficits.^{6,7} Post-transplant T-cell recovery can occur through 2 mechanisms: (1) survival and peripheral expansion of infused donor (memory) T cells, and (2) de novo generation of donor T cells in the thymus from donor hematopoietic precursors.^{6,7} The thymus is the primary site for the development of T cells. Lymphoid precursors traffic from the BM to the thymus and undergo a complex process, including proliferation, differentiation, and positive and negative selection resulting in the export of functional CD4 and CD8 T cells. Thymopoiesis occurs as a crosstalk between developing thymocytes and the stroma, which includes dendritic cells, macrophages, fibroblasts, endothelial cells, B cells, and thymic epithelial cells (TECs). In the first months after a HSCT peripheral expansion of the donor T cells is the dominant mechanism for T-cell recovery (except in the recipients of a T-cell depleted allograft) and results in particular in clonal expansion of CD8+ T cells with a limited repertoire.⁸⁻¹⁰

De novo T cell recovery: (1) gradually increases after a few months, (2) is dependent on a functional thymus, (3) is particularly important

for CD4+ T cell recovery, (4) provides a more diverse T cell repertoire, (5) is impaired in older patients due to age-associated thymic involution, and (6) can be measured by T-cell receptor rearrangement excision DNA circles (TRECs; see next section).¹¹ Similar to HIV patients the risk of post-transplant infections is associated with the CD4 count.¹²

B cell counts recover by 6 months after auto HSCT and by 9 months after allo-HSCT. Recovery of humoral immunity is: (1) initially impaired because of limited antibody repertoire, (2) dependent on T cell help, and (3) decreased due to GVHD prophylaxis and treatment, and GVHD itself.¹³

When comparing immune recovery across graft sources and transplant approaches, the available data suggest that immune recovery occurs most rapidly in recipients of autologous grafts. As noted above several factors influence immune recovery after allogeneic HSCT. In general, immune recovery occurs more rapidly after unmodified graft transplants than in the setting of in vivo or ex vivo T-cell depletion. Recovery after cord blood transplants is also dependent on the use of in vivo T-cell depletion as outlined below and can be on par with unmodified grafts in the absence of anti-thymocyte globulin (ATG). Finally, there are increasing numbers of haploidentical transplants being performed with post-transplant cyclophosphamide being used to abrogate alloreactive T cells. Unpublished data indicate that immune recovery after post-transplant cyclophosphamide is similar in haploidentical or matched unrelated donor transplants, but delayed compared to unmodified HSCT with standard GVHD prophylaxis (McCurdy and Luznik, personal communication, September 21, 2015).

Monitoring of post-transplant immune reconstitution

Several assays are used to assess post-transplant immune recovery, including tests that are performed routinely in clinical laboratories [absolute lymphocyte counts (ALCs), lymphocyte subsets (CD4+ and CD8+ T cells, NK cells, B cells), and antibody titers], as well as assays that are currently performed in the research setting (measures of thymic output and T cell and B cell repertoire). Studies have demonstrated an association between the ALC early after autologous,^{14,15} or allogeneic transplant,^{16,17} and progression-free survival (PFS) and overall survival (OS). For example, an ALC > 500 cells/mcl at day 15 after autologous HSCT was shown to be an

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independent predictor of improved PFS and OS in patients with multiple myeloma, non-Hodgkin lymphoma, Hodgkin Lymphoma, acute myelogenous leukemia, primary systemic amyloidosis, and metastatic breast cancer.¹⁴ Similarly, in recipients of allografts, a higher ALC at days 21 or 30 was associated with improved OS and disease-free survival (DFS), as well as lower relapse rates.¹⁶⁻¹⁸ More recent studies have shown similar results in recipients of cord blood transplant (UCBT). Lymphocyte populations including T, B, and NK cells are also routinely measured on a clinical basis. Early recovery of CD4⁺ T cells correlated with OS,¹⁹ non-relapse mortality,¹⁹ as well as the risk of opportunistic infections.¹⁹⁻²¹ Higher levels of CD3⁺ and CD8⁺ T cells also correlated with improved PFS.²²

The use of multiparameter flow cytometry enables identification of additional subsets of T, B and NK cells, as well as myeloid subsets such as dendritic cells (DC). T cell subsets include naïve (CD45RA⁺CCR7⁺), central memory (CD45RA⁻CCR7⁺), effector memory (CD45RA⁻CCR7⁻) and effector (CD45RA⁺CCR7⁻) T cells, regulatory T cells (CD4⁺CD25^{hi}FoxP3⁺), and T helper 17 cells. A recent study showed that effector memory CD4⁺ and CD8⁺ T cells were the predominant T-cell subset early after T-cell depleted allogeneic HSCT.²³ Flow cytometry can also assess thymic output by detection of recent thymic emigrants (RTEs) identified by the CD4⁺CD45RA⁺CD31⁺CD62L^{bright}CD95^{dim} and CD8⁺CD103⁺CD62L^{bright}CD95^{dim} phenotypes. B-cell subsets include CD27⁻IgD⁺ naïve B cells, CD27⁺IgD⁺ “able to class switch” memory cells, and CD27⁺IgD⁻ “isotype switched” memory cells.²⁴ NK populations include NK and TCR-V-alpha-24⁺NKT cells, and dendritic cells include myeloid DCs (CD123^{low/+}CD11c⁺) and plasmacytoid DCs (CD123^{bright}CD11c^{neg}). Flow cytometry can also identify antigen-specific responses using either intracellular cytokine detection or tetramers.

Functional assays can provide important additional information on post-transplant immune recovery. Although T-cell proliferative responses (measured by ³HTdR incorporation) to mitogens (PHA, OKT3), recall (candida, tetanus), viral or allogeneic antigens are still used in clinical laboratories,²⁵ more quantitative functional assays are now used in the research setting. These tests include the ELISPOT, intracellular cytokine secretion detected by flow cytometry, and tetramers. Cytokine secretion can be elicited by incubation with cells, lysates, proteins, or peptides. The use of protein-spanning pools of overlapping peptides has been used to detect both CD4⁺ and CD8⁺ responses without being limited by the patient's HLA.²⁶ These assays can evaluate viral-specific responses including those to cytomegalovirus (CMV), and Epstein-Barr virus (EBV), as well responses to tumor antigens, such as WT1, and track cells after adoptive transfer. Polyfunctional T cells that secrete multiple cytokines can also be detected by intracellular cytokine secretion. Studies in infectious diseases have shown that the ability to generate polyfunctional T-cell responses correlates with improved control of viral replication. More recently, tumor-specific polyfunctional CD8⁺ T cells have been demonstrated in patients with advanced melanoma immunized against gp100 and tyrosinase.²⁷

Molecular tests can be used to assess thymic output,^{22,28,29} as well as newly derived functional bone marrow B cells.^{30,31} TRECs are markers of thymopoiesis, and have more rapid recovery in younger patients and in recipients of conventional grafts compared to T-cell depleted grafts.²⁹ Low TREC values correlate strongly with severe opportunistic infections.²⁹ Production of B cells is assessed by

detection of kappa-deleting recombination excision circles (KRECs).^{30,31}

Finally, molecular techniques can also be used to assess T-cell receptor (TCR) repertoire and B-cell receptor (BCR) gene rearrangement diversity.^{23,32} With the development of next generation sequencing, an increasingly detailed analysis of T-cell and B-cell diversity is emerging.^{22,32-34} We recently reported on the TCR diversity in allogeneic HSCT and found significantly higher diversity in CD4⁺ T cells than CD8⁺ T cells, demonstrating the need to study subsets separately.³³ Furthermore, we showed that the most rapid recovery in TCR diversity was seen in cord blood recipients, followed by conventional grafts and T-cell depleted grafts. It should be noted that recipients of cord blood transplant in this study did not receive ATG as part of the conditioning regimen. This likely explains improved immune recovery, but also higher rates of GVHD, than in other series of cord blood transplant where the use of ATG in combination with the graft's naïve immune system has resulted in delayed immune recovery. Next generation sequencing can also be used to identify and monitor individual clonotypes, including known clonotypes specific for viral epitopes.^{32,33}

Vaccines

As noted above, HSCT results in T-cell and B-cell deficiencies. In particular GVHD and rituximab use have a profound effect on B-cell recovery, irrespective of the stem cell source (double cord blood, conventional or T-cell depleted peripheral blood or bone marrow).³⁵ B-cell counts typically recover by 3-12 months post-HSCT, except in patients who received rituximab. CD4⁺ T cell recovery, which is impacted by factors, such as patient age, GVHD, and the use of T-cell depletion, usually occurs by 6-9 months after HSCT in pediatric patients, and up to twice as long in adult recipients. These delays in immune recovery result in decreased response to vaccines.³⁶ In the absence of revaccination, antibody titers to vaccine-preventable diseases decline during the first decade after autologous or allogeneic HSCT.³⁷⁻³⁹ HSCT recipients are therefore at increased risk for infections, particularly with certain organisms such as pneumococcal infection, Hemophilus influenza type b (Hib) infection, measles, varicella, and influenza. Furthermore, due to recent reductions in vaccination rates, there has been a decrease in herd immunity and resultant outbreaks of measles and mumps. Current guidelines recommend that HSCT recipients undergo revaccination after HSCT.⁴⁰

Although there is limited clinical data on vaccine efficacy in HSCT recipients, it is accepted that there has to be at least partial recovery of T and B cells. Although the timing of recovery differs between autologous and allogeneic HSCT and also based on graft source and manipulation, most guidelines on immunization are based on timing from HSCT. Vaccination with inactivated or toxoid containing vaccines is recommended as early as 3-6 months following HSCT, whereas administration of live-attenuated vaccines is recommended at 24 months post-HSCT.⁴⁰ The delayed use of live-attenuated vaccines is based on concerns about transmission of vaccine-mediated disease and the limited data on the safety and immunogenicity of earlier vaccination.⁴¹

Inactivated vaccines should be preferred over live vaccines for patients receiving immunosuppressants because of their reduced ability of mounting sufficient immune responses and the risks of uncontrolled virus replications. The question remains whether patients with ongoing GVHD should be vaccinated. Guidelines suggest that live vaccines should be avoided in these patients, but

there is no conclusive evidence showing that inactivated vaccines exacerbate GVHD.⁴²

Recent data from our center has shown the safety and immunogenicity of the live attenuated varicella vaccine when given according to pre-set immune milestones (CD4 cells >200/ μ L, normal PHA, IgG >500 mg/dL at least 6 weeks post-IVIG).⁴³ In contrast, we have also shown that despite acquisition of minimal milestones of immune reconstitution, only 15% of patients respond to a single conjugated meningococcal vaccine and 35% of patients did not respond to any of the 4 serotypes. This data suggests that a series of two MCV4 as currently recommended for patients with asplenia, complement deficiency, or HIV should be evaluated in this patient population.⁴⁴ Additional research is needed to ascertain the optimal timing of post-HSCT vaccines and immunization based on immune recovery parameters rather than time from HSCT.

Donor vaccination. Pre-HSCT donor vaccination may represent a potentially attractive strategy to boost immunity and prevent infections to pathogens that cause significant morbidity and mortality. However, despite an extensive effort, studies are still inconclusive and have not shown any beneficial effect in preventing infections.⁴⁵ In addition, there are ethical issues related to donor immunization. As a result, the 2013 Infectious Diseases Society of America guidelines recommend against immunizing the donor solely for the benefit of the recipient.

Virus-specific T-cell clones. An alternative approach to provide anti-viral immunity for transplanted recipients is through the isolation of donor derived virus-specific T cells or through the ex vivo amplification and expansion of virus-specific T cells stimulated with antigen-presenting cells expressing the viral antigens. Virus-specific T cells for common post-transplant pathogens (including EBV, CMV, and adenovirus) have been successfully generated, showing the safety and efficacy of these strategies in improving immune recovery after HSCT. Furthermore, recent studies have demonstrated that 3rd-party viral-specific T cells against EBV or CMV can be safely administered to allo-HSCT as well as solid organ transplant recipients with encouraging clinical results. Although, several limitations in this approach have to be addressed (such as the costs, the complexity of the manufacturing and the time to produce clinical grade T cells), adoptive transfer of virus-specific T cells still represents an attractive strategy to prevent post-transplant viral infections⁴⁶

Novel strategies

At present there is no “standard-of-care” approach to enhance post-transplant immune reconstitution, however, several strategies are being developed in preclinical models as well as early clinical trials. The following strategies are currently in clinical development.

Interleukin-7 (IL-7). IL-7 has many lymphopoietic effects on both T and B cells through: (1) supporting lymphoid precursors, (2) promotion of T-cell development in the thymus, and (3) anti-apoptotic effects during T-cell development.

Mouse models of allogeneic allo-HSCT have shown that IL-7 enhances thymopoiesis, stimulates T-cell proliferation, increases T-cell numbers, and enhances T-cell diversity.^{47,48} Initial clinical trials with recombinant human IL-7 (rhIL-7; CYT99-007, Cytheris) demonstrated a dose-dependent expansion of CD4+ and CD8+ T cells in patients with solid tumors or HIV infection^{49,50} We recently completed a phase I trial of rhIL-7 (CYT107, Cytheris) in patients

with myeloid hematologic malignancies who underwent a T-cell depleted allogeneic HSCT.²³ Patients were treated with escalating doses of rhIL-7 (3 at 10 mcg/kg, 6 at 20 mcg/kg, 3 at 30 mcg/kg) administered SQ weekly for 3 weeks starting at a median of 103 days post-transplant (range, 60-244 days). IL-7 was well tolerated and no patients have developed GVHD, anti-IL-7 antibodies or neutralizing antibodies. In most patients, we observed an increase in CD4+ and CD8+ T cells with evidence of recent thymic emigrants and TRECs, as well as increased TCR repertoire diversity and functional T-cell responses to viral antigens. A phase I clinical trial with IL-7 in HSCT recipients of a CD34+ selected allograft demonstrated low toxicity and no GVHD at doses, which could increase T-cell recovery and T-cell repertoire diversity.²³

Keratinocyte growth factor (KGF). KGF has been approved for the prophylaxis of mucositis in patients receiving chemo- and/or radiation therapy, however, preclinical studies have indicated that KGF administration can also enhance thymopoiesis through the induction of proliferation of TECs.⁵¹ A clinical trial to test the effect of KGF in combination with leuprolide (see below) on post-transplant T-cell reconstitution is underway.

Sex steroid ablation (SSA). Both estrogen and testosterone have inhibitory effects on early lymphoid precursors, thymopoiesis and B lymphopoiesis.⁵²⁻⁵⁸ The mechanisms through which this occurs are largely unknown, but studies are underway. For example, sex steroids in the thymus seem to decrease the expression of Notch ligand, which is an important driver of T-cell development.⁵⁹ Studies both in man and mouse demonstrated that SSA using castration (in mice) or the luteinizing hormone releasing hormone (LHRH) agonist Leuprolide after auto- and allo-HSCT or cytoablative therapy per se results in: (1) increased numbers of lymphoid precursors and import of thymic precursors into the thymus, (2) improved thymopoiesis, (3) enhanced B lymphopoiesis, and (4) improved recovery of functional immunity.⁵¹ As mentioned above, a clinical trial combining SSA with leuprolide and KGF in HSCT recipients is underway.

Growth hormone (GH). Preclinical studies have shown that GH administration can enhance thymopoiesis in old animals and improve HSC function. Clinical studies in HIV+ patients demonstrated enhanced thymopoiesis and antiviral immunity.^{60,61} In addition, several strategies are being developed in preclinical models, including: (1) Flt3L: administration of Flt3L enhances thymic dependent and independent T-cell recovery and increases Flt3L+ precursors in the BM, but decreases B lymphopoiesis.^{62,63} (2) IL-22: upon thymic injury innate lymphoid cells type 3 in the thymus secrete IL-22 to promote endogenous regeneration of TECs⁶⁴; IL-22 administration can promote thymic, as well as intestinal regeneration after injury⁶⁵ and a phase I study in patients with GVHD is planned. Other cytokines and growth factors, which can enhance immune reconstitution in animal models include: IGF-1, IL-2, IL-12, IL-15, parathyroid hormone, and retinoic acid.¹¹

T-cell precursors. Preclinical studies have shown that T-cell precursors can be generated and expanded from HSCs in an ex vivo culture system using Notch-1 stimulation, as well as IL-7 and Flt3L. Adoptive transfer of these cells with the allograft can be done across MHC barriers and results in enhanced thymopoiesis, chimerism, development of host-tolerant and fully functional T cells, and enhanced NK reconstitution.^{66,67}

Thymic tissue transplant has been used for the treatment of children with DiGeorge syndrome (congenital hypoplastic thymus).⁶⁸ A

number of groups are employing tissue engineering techniques to create an artificial thymus, using various biomaterials, thymic epithelial precursor cells, and/or mesenchymal cells.

Several studies have shown that the regulatory T cell (Treg) content in the allograft is associated with improved immune reconstitution and less GVHD and CMV infection.⁶⁹⁻⁷² Initial studies regarding adoptive cell therapy with donor-derived regulatory T cells have also shown improved T-cell reconstitution and less GVHD.^{73,74}

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