Published in final edited form as:

Blood Cells Mol Dis. 2008 ; 40(1): 44–47. doi:10.1016/j.bcmd.2007.07.015.

Enhancing T cell reconstitution after hematopoietic stem cell transplantation: a brief update of the latest trends

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Abstract

Hematopoietic stem cell transplantation (HSCT) is associated with a period of immune incompetence that particularly affects the T cell lineage. Strategies to enhance T cell reconstitution could significantly improve the survival of HSCT recipients by decreasing the incidence of fatal infectious complications and by enhancing graft-versus-tumor activity. In recent years, a variety of promising strategies have been established in preclinical models to improve T cell recovery in particular after allogeneic T cell-depleted HSCT, without aggravating graft-versus-host disease while preserving or even improving graft-versus-tumor activity. These therapies include treatment with keratinocyte growth factor (KGF), growth hormone (GH), LHRH agonists, interleukin 7 (IL-7) and interleukin 15 (IL-15). Thanks to the establishment of Notch-based culture systems, adoptive cellular therapies with T lineage-committed precursor cells have become feasible, since early T cell progenitors can now easily be generated *in vitro* in large quantities and have been proven to be very effective in enhancing T cell reconstitution and anti-tumor activity after allogeneic T cell-depleted HSCT. The translation of most of these strategies into clinical trials is likely and in some cases Phase I/II studies are already underway.

Keywords

hematopoietic stem cell transplantation; T cell deficiency; KGF; cytokine therapy; adoptive cell therapy

Introduction

In contrast to the relatively early recovery of innate immunity (myeloid and NK cells), all, but especially adult, recipients of an allogeneic hematopoietic stem cell transplantation (HSCT) experience post-transplant deficiencies in their B and T cell reconstitution, which can exceed the period of lymphopenia, due to a delay in recovery of function, and can persist for more than one year [1;2;3]. This prolonged post-transplant lymphoid deficiency is associated with an increased risk of infections [1;2;3;4], relapse of malignancy [5], the development of secondary malignancies [6], graft failure, and impairs the application of immunotherapeutic strategies, such as vaccination against microbes or tumors. The risk of opportunistic infections

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in the post-transplant period is directly correlated with the recovery of T cells (especially CD4⁺ T cells) [1;2;3;4]. Strategies to enhance post-transplant T cell reconstitution could therefore significantly improve the overall survival after HSCT. The most promising approaches are based on the administration of hormones, growth factors, cytokines, or committed precursor cells, and have been reviewed in depth elsewhere [7;8;9].

Hormones, growth factors and cytokines

Previous studies have indicated that Growth Hormone (GH) is involved in immune reconstitution after allogeneic HSCT by enhancing thymopoiesis and B and T cell recovery in peripheral blood, without increasing GVHD [10]. GH is known to stimulate immune responses indirectly by regulating production of insulin-like growth factor (IGF-1) but may have a direct effect as well. Receptors for both GH and IGF-1 are found on all hematopoietic cells in the bone marrow and on B and T cell subsets in secondary lymphoid tissues [11]. IGF-1 treatment enhances T cell reconstitution after syngeneic HSCT [12]; when administered after allogeneic HSCT, it produces an increased number of thymic precursors, peripheral T cells, myeloid populations, and B cells and does not exacerbate GVHD morbidity and mortality [13].

Sex steroids are known to have a profound negative influence on lymphopoiesis. Several studies have linked age-related thymic atrophy with pubertal increases in circulating sex steroids and conversely, thymic atrophy is both delayed and reversed with the blockade of sex steroids [14;15;16;17;18;19]. B cell development is also negatively regulated by sex steroids and again removal of sex steroids enhances B cell development and reverses age-related decreases in B cell production [20;21]. Sex steroid ablation results in a significant increase in B and T cell reconstitution after both autologous [22] and allogeneic [23] HSCT and in humans who underwent reversible chemical castration using an LHRH agonist [24].

Keratinocyte Growth Factor (KGF) is a member of the fibroblast growth factor (FGF) family that promotes epithelial cell proliferation and differentiation. It recognizes a splice variant of one of the FGF receptors (FGFR) expressed by epithelial cells in a variety of tissues, including the thymus [25;26]. Systemic administration of recombinant human KGF provides significant cytoprotection to epithelial tissues in animal models for chemo/radiation-induced injury to the gut, lung, urinary bladder, and hair follicles [25;27]. The cytoprotective effect of KGF on the thymus is most likely mediated by increased production of intrathymic interleukin 7 (IL-7) by thymic epithelial cells. Several studies evaluated if the cytoprotective effects of KGF on epithelial cells could protect or enhance the regeneration of the thymic stroma in HSCT recipients. Indeed, KGF treatment of recipients prior to syngeneic [28] and allogeneic [28; 29] HSCT lead to enhanced thymopoiesis as well as peripheral T cell reconstitution [28;29].

IL-7 is an essential cytokine for T cell development and survival and is secreted by stromal cells of various tissues, including bone marrow and thymus. It signals through a receptor complex containing the IL-7R α and the common cytokine receptor γ chains. IL-7 administration has several stimulatory effects on T cell development, including increased thymopoiesis in mice (*in vitro* and *in vivo*) [30] and humans (in thymic organ culture [30]). Preclinical studies in mouse HSCT models have demonstrated that post-transplant IL-7 administration can enhance T cell reconstitution in recipients of a syngeneic or allogeneic HSCT through increased thymopoiesis, increased homeostatic proliferation of transferred and *de novo* generated mature T cells, and decreased peripheral T cell apoptosis [30;31;32]. IL-7 can safely be administered to recipients of a T cell-depleted allogeneic HSCT to enhance lymphoid reconstitution without aggravating GVHD.

Interleukin 15 (IL-15) is a member of the interleukin 2 (IL-2) family of cytokines secreted by cell types such as fibroblasts, epithelial cells, monocytes, lymphocytes, and mast cells. It stimulates proliferation of activated T cells, NK cells and B cells through a receptor complex

containing the IL-15R α , IL-2R β , and the common cytokine receptor γ chains. Studies in pre-clinical models demonstrated that post-transplant IL-15 administration specifically enhances peripheral expansion of CD8⁺ T cells but has the potential to aggravate GVHD [8;33;34]. Importantly, IL-15 was found to be less toxic than IL-2 [34]. In addition it has been suggested that IL-15 can be effectively utilized as an adjuvant for immunotherapy and tumor vaccination strategies [35;36;37].

Committed precursor cells generated by Notch-based culture

Notch signaling is required for various fetal and adult cellular differentiation processes and lineage decisions during the development of a multi-cellular organism [38]. Four Notch receptors (Notch1–Notch4) and five ligands (Jagged-1, Jagged-2, Delta-like 1 (DL1), DL3 and DL4) have been described in mammals. The inhibition of Notch1 results in a partial blockade of thymocyte differentiation at the DN1 to DN2 transition [38;39], as well as an accumulation of B cells [40] and TCR β -negative T cell precursors [41] in the thymus. These and other studies indicate that Notch 1 is essential for instructing bone marrow-derived lymphoid precursors to differentiate into T instead of B cells, as well as promoting T cell differentiation (reviewed in [38;39]). In particular, Notch signaling has been implicated in VDJ β rearrangement [41], commitment to $\alpha\beta$ versus $\gamma\delta$ T cell lineage [42;43;44], Th1 versus Th2 cell differentiation [45;46;47], and development of regulatory T cells [48;49;50].

Notch-based culture systems such as the OP9-DL1 (a mouse bone marrow stromal cell line transduced to express DL1) system [51] can be utilized to generate large numbers of early T cell precursors from hematopoietic stem cells [52]. Transfer of these T cell precursors, along with the allograft, to lethally irradiated allogeneic HSCT recipients resulted in increased thymic cellularity and chimerism, as well as enhanced peripheral T and NK cell reconstitution [52]. Combination of T cell precursor administration and treatment with KGF had additive effects on thymic reconstitution. In thymectomized recipients, adoptively transferred T cell precursors enhanced extrathymic T cell development. OP9-DL1 derived T cell precursors gave rise to host-tolerant CD4 and CD8 populations with normal T cell receptor repertoires, cytokine secretion, and proliferative responses to antigen. Administration of OP9-DL1 derived T cell precursors increased resistance to infection with *Listeria monocytogenes* and mediated significant GVT activity in the absence of GVHD. Adoptive transfer of *in vitro* generated T cell precursors therefore represents a promising novel strategy to enhance T cell reconstitution and GVT activity after T cell-depleted allogeneic HSCT. Moreover, recent advances in genetic engineering of hematopoietic stem cells [53;54] and T cells [55;56;57;58] suggest that gene transfer technology could be integrated into T cell precursor expansion protocols in order to develop functionally enhanced designer T cell precursors for gene therapy.

Clinical Perspectives

Phase I trials with IL-7 in cancer patients and a Phase I trial to assess safety and toxicity of IL-7 administration to HSCT recipients are currently underway. KGF has been shown to be a protective factor for oral mucositis in randomized clinical trial in patients with autologous HSCT [59]. It is currently in clinical trials to assess its effects on GVHD, mucositis and T cell reconstitution in HSCT recipients. Chemical sex steroid ablation by LHRH agonist treatment is also currently under investigation in clinical studies evaluating its effect on T cell reconstitution after HSCT. IL-15 has not been used clinically to date. However, a Phase I study to evaluate the safety and immunogenicity of an experimental HIV DNA vaccine with or without an IL-15 DNA adjuvant is currently being initiated. GH and IGF-1 are FDA-approved agents for the treatment of GH non-responsiveness in the general population of children with GH hyporesponsiveness or primary IGF-1 deficiencies. Recently, a double-blind clinical study in patients receiving intensive chemotherapy for various leukemias and lymphomas revealed

that administration of GH caused significantly faster platelet recovery [60]. Notch-based culture systems have been used to generate T cells from human cord blood and human adult BM derived CD34⁺ progenitor cells, indicating that this technique can be converted to a clinically applicable therapeutic method [61;62;63]. Bernstein and colleagues have established a fully humanized Notch-based culture system [64] and are currently using it to expand cord blood-derived CD34⁺ cells from a single cord blood unit before double cord blood unit transplantation.

In conclusion, novel strategies have been developed that can significantly improve T cell reconstitution after allogeneic HSCT (particularly in the T cell-depleted HSCT setting), and these are either already in clinical studies or have real potential to be translated into clinical studies in the near future.

Acknowledgements

This paper is based upon a presentation given at a Focused Workshop on Haploidentical Stem Cell Transplantation sponsored by The Leukemia & Lymphoma Society held in Catania, Italy from 4th-6th October 2007.

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