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Review

Clinical strategies to enhance T cell reconstitution

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Abstract

Strategies to enhance T cell recovery are of increasing clinical importance to overcome long lasting T cell deficiencies, which occur in association with infections, autoimmunity and chemo/radiotherapy as well as aging of the immune system. In this review we discuss those strategies that are close to or in the clinic. Interleukin-7, sex steroid modulation, keratinocyte growth factor, growth hormone and cellular therapies using *ex vivo* generated T-cell precursors are currently being tested in recipients of a hematopoietic stem cell transplantation and patients with malignancies or HIV/AIDS.

Keywords: T cell reconstitution; Bone marrow transplantation; Interleukin-7; Keratinocyte growth factor; Growth Hormone; Sex steroid modulation; OP9-DL1

T cell deficiencies can occur through infection (such as HIV), autoimmune diseases, chemo/radiotherapy or as a consequence of aging of the immune system (especially the thymus). T cell deficiency has been associated with an increased risk of infection and malignancies and a failure to respond to vaccination. For example, much of the late morbidity and mortality following hematopoietic stem cell transplantation (HSCT) can be attributed to delayed T cell reconstitution, leading to increased opportunistic infection and malignant relapse. There is also a growing body of evidence suggesting that early lymphocyte reconstitution, following both allogeneic and autologous HSCT, is a good prognostic indicator of disease outcome [1–5].

Several strategies to enhance immune reconstitution have been developed in preclinical models (reviewed in [6]), but few have made it into clinical trials. We will focus on those strategies which are close to entering the clinic.

1. Interleukin-7

Interleukin 7 (IL-7) is a 25 kDa glycoprotein produced by stromal cells in the thymus and bone marrow [7], as well as by keratinocytes and enterocytes [8]. IL-7 binds the IL-7 receptor (IL-7R) which consists of the α-chain (also known as CD127) and the common cytokine receptor γ-chain (γc) [9]. IL-7R is expressed on many cells of the immune system including common lymphoid precursors, triple negative (CD3−CD4−CD8−) and single positive (CD3+CD4+CD8− or CD3+CD4−CD8+) thymocytes, CD4+ and CD8+ T cells, developing B cells, γδ T cells, thymic dendritic cells (DCs) and monocytes, as well as nonhematopoietic cells such as intestinal epithelial cells and keratinocytes (reviewed in [8]).

IL-7 is a non-redundant cytokine for both T and B cell development in mice. Mice treated with anti-IL-7 antibodies, and those deficient in IL-7Rα or IL-7 exhibit severely impaired lymphocyte development. Thymic cellularity is profoundly decreased in both types of knockout mice [10,11], and while a small number of αβ T cells develop, γδ T cells are absent [12]. In humans, a defect in the IL-7Rα results in a complete lack of T cells and severe combined immunodeficiency syndrome (SCID) [13]. Interestingly, in contrast to mice IL-7 does not appear to be a requirement for normal B cell development in humans [13].

IL-7 promotes both the survival and differentiation of immature triple negative and mature single positive thymocytes [14,15]. It is also essential for the production of γδ T cells [12] and thymic dendritic cells [16]. In the periphery, IL-7 has been identified as a key regulator of peripheral T cell survival and function. It has anti-apoptotic effects, possibly through the upregulation of Bcl-2 [17], and while it is not essential for the initiation of a T cell-mediated antigenic response, it is necessary for the generation of memory T cells [18–21]. IL-7 has also been

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shown to be a non-redundant regulator of homeostatic expansion of $CD4^+$ and $CD8^+$ naïve and memory T cells in settings of lymphopenia [17].

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 [22–28]. IL-7 treatment not only increased T cell numbers but also enhanced their function. However, IL-7 did not show any effect on the T cell repertoire [25].

One of the concerns about the use of IL-7 in the setting of an allogeneic HSCT has been whether IL-7 may lead to or exacerbate graft versus host disease (GVHD). As demonstrated in a number of studies, IL-7 does not lead to GVHD in the setting of a T cell-depleted (TCD)-BMT [25]. However, depending on the dose of T cells and the duration and dose of IL-7 administration, the cytokine may exacerbate GVHD when it is administered in a T cell-replete HSCT [27,28]. Finally, IL-7 administration preserved the graft-versus-leukemia (GVL) activity of a T cell-replete allograft [25].

CYT 99 007 (Cytheris Inc.), a recombinant non-glycosylated form of human IL-7, has been studied in phase I clinical trials. As of 1 March 2007, 61 patients have been treated with subcutaneous CYT 99 007 in five different phase I dose-escalation trials conducted in various clinical settings, in a dose range varying from 3 to 60 mcg/(kg dose) (R. Buffet, personal communication). In general, administration of IL-7 was associated with little toxicity, and immunologic efficacy was demonstrated in these early studies. Repeated doses of CYT 99 007 induced a dose-dependent sustained expansion of $CD4^+$ and $CD8^+$ T cells with memory and naïve phenotypes, including recent thymic emigrants (RTEs) defined as $CD45^+CD31^+$ T cells [29]. As expected, expansion resulted from an increase in both T cell proliferation and survival, assessed through the expression of Ki-67 and Bcl-2 markers, which also appeared to be strongly linked to the IL-7 dose. Consistent with the homeostatic role of IL-7, the magnitude and duration of T cell expansion seemed to be more pronounced in patients with lower T cell counts at baseline. The same T cell expansion profile was observed in a patient treated with CYT 99 007 after an allogeneic HSCT (Perales, unpublished observation). Studies of a potentially less immunogenic glycosylated formulation of IL-7 (CYT107, Cytheris) are currently underway.

2. Sex steroid modulation

Apart from their conventional roles in sexual dimorphism, differentiation and development, sex steroids are known to be involved in many other biological systems, including the immune system. The effects of sex steroids on the immune system might be responsible for gender disparity in susceptibility to autoimmune disease, and decreased T cell immunity during pregnancy. However, sex steroids appear to affect most hematopoietic developmental stages, as well as the function of mature immune cells [30–45].

Most studies agree that thymic atrophy becomes most pronounced at the time of puberty, concomitant with an increase in circulating sex steroids [46–51]. The link between sex steroid ablation (via surgical or chemical castration) and reversal of age-related thymic atrophy is well established. When mice or rats are castrated before puberty, thymic atrophy is delayed and if they are castrated later in life thymic atrophy is reversed [52–57]. Castration and the subsequent reversal of thymic atrophy results in an increase in RTEs, resulting in an increase in peripheral naïve T cells [57]. Increased T cell numbers translate to an increase in peripheral T cell function [57].

Administration of estrogens, progesterone or testosterone leads to reversible thymic atrophy, which resembles that observed with age [55,58–63]. Furthermore, the thymic enlargement/regeneration observed following castration is inhibited or reversed in a dose-dependent manner by the administration of testosterone or estrogen [55,59,64].

LHRH agonist administration results in a reversible inhibition of testicular steroidogenesis and spermatogenesis in males (chemical castration) [54]. LHRH agonist administration leads to an increase in thymic weight and reversal of age-related thymic structural defects, including the appearance of a clear distinction between cortex and medulla and an obvious corticomedullary junction [54,65–67].

Many groups have studied the expression of classical intracellular androgen receptors (iARs) in the thymus. iARs have been shown to be present in both male and female thymi [68], in whole thymus homogenate [20,58,68–73], thymic stroma [68,74], purified thymocytes [20,75] and all thymocyte subsets (based on expression of CD4 and CD8) [76]. Thymic stroma and thymocytes express both estrogen receptor α (ERα) and estrogen receptor β (ERβ) [77–79].

Because ARs and ERs have been identified on both thymocytes and thymic stromal cells, sex steroids may either act directly on thymocytes to affect apoptosis, proliferation and/or differentiation, or act indirectly via the thymic stroma. The mechanisms by which sex steroids act on the thymus remain to be fully elucidated. Olsen et al. used Tfm mice that have a point mutation in the androgen receptor and significantly larger thymi than androgen-sensitive mice to study the affect of androgen receptor signalling in the thymus [80]. BMT experiments were used to show that the presence of a functional androgen receptor on the stromal components of the thymus but not the thymocytes is essential for normal age-related thymic atrophy and the regeneration seen following sex steroid ablation [80]. Similar experiments were carried out using ERα knockout mice (ERKOs), ERβ knockout mice (BERKOs) and double knockouts (DERKOs) [81,82]. Again, BMT experiments were used to demonstrate that it was the expression of ERα, or lack thereof, on the thymic stromal cell components that predominantly mediated the changes in thymic cellularity observed [81].

Studies from Boyd and co-workers [56,57] have identified very early effects on hematopoietic thymic precursors following surgical castration. Early thymic progenitors (ETPs) are decreased in number with age [56,83] and surgical castration restores ETP numbers [56]. Normalization of triple negative thymocyte proportions were also observed [56,57]. Medina et al.