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Emerging strategies to boost thymic function

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Abstract

The thymus constitutes the primary lymphoid organ for the generation of T cells. Its function is particularly susceptible to various negative influences ranging from age-related involution to atrophy as a consequence of malnutrition, infection or harmful iatrogenic influences such as chemotherapy and radiation. The loss of regular thymus function significantly increases the risk for infections and cancer because of a restricted capacity for immune surveillance. In recent years, thymus-stimulatory, -regenerative and -protective strategies have been developed to enhance and repair thymus function in the elderly and in individuals undergoing hematopoietic stem cell transplantation. These strategies include the use of sex steroid ablation, the administration of growth and differentiation factors, the inhibition of p53, and the transfer of T cell progenitors to alleviate the effects of thymus dysfunction and consequent T cell deficiency.

Keywords

thymus

Introduction

The principal function of the immune system is to resist infectious agents and to eliminate malignantly transformed cells. This challenging task is effected by two closely interacting defense mechanisms known as the innate and the adaptive immune systems. The innate arm provides an immediate but generally non-specific protection that is activated by pattern recognition receptors identifying structural components well conserved among different pathogens. In contrast, the adaptive arm of the immune response is characterized by antigen-specificity and immunological memory and is achieved by two interdependent systems: Antibody-producing B-lymphocytes and the cellular defense of T lymphocytes.

Both lymphoid cell types arise from pluripotent hematopoietic stem cells (HSC) resident in the bone marrow. In contrast to B cell development that largely takes place in the bone marrow, T cell maturation requires the specialized microenvironment of the thymus as a site for lineage commitment and differentiation. The thymus is located in the upper anterior mediastinum, is structured into an outer cortex and an inner medulla and continuously generates T cells that are exported to the periphery. Intrathymic T-cell development constitutes an intricate process of cellular cross-talk where an array of interacting stromal

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cell types provide the molecular cues required for the appropriate maturation, expansion and selection of nascent T cell precursors (known as thymocytes). Combined, these stromal cells thus provide the thymic microenvironment required for normal T cell development.

Physiological and pathological deficits in thymic function arise in a variety of clinical situations. Defects in the thymus microenvironment compromise the adaptive immune system and may consequently cause life-threatening immunodeficiencies, autoimmunity and an increased risk for tumor relapse secondary to impaired immunological surveillance. In this review, we discuss recently emerging concepts to stimulate thymus function under various pre-clinical and clinical conditions associated with thymus atrophy. Special attention will be given to measures that protect, repair and enhance the function of thymic stromal cells.

The thymus as the primary lymphoid organ for T cell development

As the primary site for the life-long formation of new T lymphocytes, the thymus does not contain self-renewing hematopoietic precursor cells [1,2]. Hence there is a need for progenitor cells to be continuously recruited from the blood. Marked by a $CD3^{-}CD4^{-}CD8^{-}$ (i.e. triple negative, TN) phenotype, these cells enter the vascularized microenvironment of the thymus at the cortico-medullary junction. Once situated within the thymus, these cells are now designated early T lineage progenitors (ETPs). They begin to proliferate and commit to the T cell lineage after engagement of their Notch1 receptor with Delta-like 4 (DL4) ligand on cortical thymic epithelial cells (TEC). Following an ordered sequence of distinct developmental steps, these immature T cells express over time both CD4 and CD8 and the complete antigen-specific T cell receptor (TCR). At this stage of development, these cells are referred to as double positive (DP) thymocytes and become subject to a selection process aimed at testing the usefulness of the randomly selected TCR specificity. Thymocytes whose antigen receptors recognize with a sufficiently high affinity the self-peptide/MHC complexes expressed on cortical TEC are positively selected and, as a result, continue their intrathymic maturation. In contrast, DP cells expressing a TCR that fails to interact with self-peptide/MHC complexes or only binds these with a low affinity do not receive the necessary survival signals and are eliminated within 3 to 4 days by a process referred to as “death by neglect”. Positively selected thymocytes are exposed in the thymus medulla to a negative selection process, which results in the programmed cell death of thymocytes displaying a binding affinity above a critical threshold for self-peptide/MHC complexes. This step removes thymocytes with reactivity to self-peptides and purges the population of emerging T cells from cells that may elicit a harmful autoimmune response. Thus, thymic selection favors the survival of T cells that respond to foreign (i.e. non-self) antigens in the context of self-MHC molecules but fail to react productively against peptides derived from self-antigens. Selected thymocytes that express a TCR restricted to MHC class I molecules will attain a $CD4^{-}CD8^{+}$ phenotype whereas cells with a MHC class II-restricted TCR specificity will accomplish a $CD4^{+}CD8^{-}$ phenotype. Following a period of post-selectional maturation, these naïve single positive (SP) cells (i.e. $CD4^{-}CD8^{+}$ and $CD4^{+}CD8^{-}$ thymocytes) exit from the thymic medulla to the periphery employing a mechanism that involves signalling via the sphingosine 1-phosphate receptor type 1 (S1P1).

Thymocytes rely for their development on signals provided by different stromal cells such asTEC which control the homing, expansion, maturation and selection of T cell precursors [1,3]. Arranged in a three-dimensional network where single cells contact each other via dendrite-like cell processes, the population of TEC is both phenotypically and functionally heterogeneous with separate subpopulations present in the anatomically distinct compartments of cortex and medulla, respectively. The molecular programs that control TEC growth, maintenance and repair have recently attracted considerable interest, not least

because the number and function of these cells are frequently affected by injurious stimuli as diverse as the physiological aging process and iatrogenic interventions such as cytoablative conditioning in the course of cancer treatment.

Effect of age on thymic structure and function

Thymic function begins to decline as early as the second year of life. This physiological involution is marked by a gradual, albeit progressive decrease in the frequency and absolute number of mainly cortical but also medullary TEC. Consequently, epithelial free-areas appear, the perivascular space enlarges, and connective and adipose tissue components increase excessively [4]. The loss of a clear morphological demarcation between cortex and medulla and a decreased expression of MHC class II molecules constitute additional hallmarks of thymus senescence. Irrespective of the altered phenotype composition and organization of its stroma, the thymus of an old individual maintains a capacity for thymopoiesis. This potential is progressively restricted with age but nonetheless allows throughout life for an increasingly diminished export of naïve T cells that continue to express a diverse antigen receptor repertoire [5] [6].

Several intrinsic and extrinsic factors have been linked to the process of age-related thymic atrophy. Among the intrinsic factors that ostensibly play a significant role are leukemia inhibitory factor (LIF), oncostatin M (OSM), interleukin (IL)-6, and stem cell factor (SCF) because their thymic transcripts are increased with age and their administration to mice causes thymic atrophy [5]. In contrast, the thymic tissue concentrations of several cytokines and hormones such as growth hormone (GH) and insulin-like growth factor (IGF) decrease with senescence and thymic atrophy. Specifically, a decline in IL-7 transcripts is observed in aged mice and this change correlates both with a drop in the number of cortical TEC and with a partial block in early T cell development leading to decreased thymic T cell output [7]. Yet, an increased availability of IL-7 by means of transferring IL-7-secreting stromal cells to the thymic microenvironment does not succeed in reversing thymic senescence [7] and IL-7 transcripts do not change with age in unseparated human thymic tissue [5], arguing against a decisive role for IL-7 in the process of thymic involution.

The extrinsic factors identified to affect thymus senescence include changes in systemic levels of lutenizing hormone releasing hormone (LHRH), estrogens and androgens, but also the decrease in both number and function of ETP has been fundamentally linked to thymic atrophy [8–14]. While the ablation of androgen activity ameliorates the features of age-related thymic atrophy, a complete and prolonged restoration of thymic function and structure has so far not been achieved with physical or pharmacological castration [11–14]. Whether the blood-borne T cell precursors immigrate into an aged thymus at a reduced rate and hence further contribute to the phenomenon of attenuated thymopoiesis remains presently unknown. Nonetheless, ETP are present in the aged thymus at a reduced frequency and exhibit a diminished proliferative capacity and a higher apoptosis rate castration [14–16]. These features appear to be cell-autonomous as the capacity of the precursor cells to proliferate and differentiate is not “rescued” by the thymic microenvironment of a young mouse. Moreover, thymus cellularity and architecture remain unchanged in aged mice irrespective of the availability of adoptively transferred young ETP [17].

Effect of radio-chemotherapy and graft-versus-host disease on thymic function

In the context of allogeneic hematopoietic stem cell (HSC) transplantation (HSCT), injuries to the thymus are predominantly caused by cytoreductive radio/chemotherapy and by graft-versus-host disease (GVHD). Both of these transplant-related toxicities independently

restrict the T-cell reconstitution following HSCT [18]. The exposure to γ-radiation results in a rapid damage of both thymocytes and stromal cells involving the transforming growth factor (TGF)-β system [19]. Comparable to thymic injury following cytoreductive treatments, GVHD also impairs the composition and architecture of the thymus microenvironment. Preclinical models of allogeneic HSCT have recently begun to provide a mechanistic understanding of the complex events leading to thymus dysfunction in the context of acute GVHD. Alloantigen-specific recognition of host TECs by alloreactive donor T cells constitutes the principal mechanism of cellular injury [20].Consequently, thymocytes are depleted, faultily selected and exported to the periphery only at a reduced rate [21].

The thymus as a target organ of malnutrition and infection

Thymic atrophy and signs of clinically significant cellular immune deficiency are also caused by protein calorie malnutrition and deficiencies in micronutrients such as zinc and antioxidant vitamins as well as to bacterial, viral, protozoal and fungal infections [22–24]. Increased levels of circulating glucocorticoids and decreased serum concentrations of leptin have been linked to thymic atrophy under conditions of malnutrition. Indeed, leptin protects thymocytes from dexamethasone-induced apoptosis, possibly via an increase in IL-7 expression by medullary TEC [25], and enhances thymopoiesis in the atrophic thymus of mice exposed to endotoxins [26,27]. Although the mechanisms by which infectious agents prompt thymic atrophy may vary considerably between different pathogens, the activation of the hypothalamus-pituitary-adrenal axis with a surge in glucocorticoid serum concentrations and the release of proinflammatory, thymotoxic cytokines (IL6, IFN-α, TNF-α) is critical for the characteristic manifestations of thymus atrophy. In addition, several pathogens also infect lymphoid and/or stromal cells in the thymus resulting in a direct or indirect increase of thymocyte apoptosis and a lowered export of mature T cells to the periphery [22].

Improving thymic function

The failure to maintain a functionally competent thymus microenvironment severely compromises the adaptive immune system. Improved understanding of how TEC lineage commitment, differentiation, maintenance and function are controlled has formed the rationale for novel strategies to correct the age-related loss of thymic function and to prevent or repair treatment- and infection-related TEC damage [8–11,28]. Increasing the number of TEC, and consequently, expanding the availability of developmental niches within the thymic microenvironment have successfully been applied in diverse experimental systems to achieve (almost) regular thymus function. In parallel, approaches that increase the number of lymphoid precursor cells and that improve their homing to the thymus may constitute alternative or additive strategies to enhance the production of naïve T cells. Here we summarize data from preclinical and clinical studies undertaken to improve thymic function.

Growth hormone and insulin-like factor 1

Thymus formation and maintenance are under the physiological control of several growth factors including GH and IGF-I. GH is typically produced by several different cell types including thymocytes and TEC, which also express the corresponding receptor, GHR. Hence, GH acts on these cells in an autocrine fashion influencing cell growth, cell proliferation and the cytoskeleton. GH also evokes the synthesis of IGF-I which acts as a primary mediator of the biological effects of GH. IGF-1 may indirectly affect the frequency of T cell precursors as IGF-1 has been implicated in the expansion of primitive multilineage hematopoietic progenitor cells [29], in their lineage decisions [30] and in the regulation of hematopoietic stem cell accumulation and differentiation by osteoblastic niche cells [31].

The role of GH as a growth factor influencing thymus size was initially suggested in anterior hypopituitary (i.e. GH-, thyroxine- and prolactin-deficient) Snell-Bagg mice under stress and in hypophysectomized rats since both conditions cause an early, progressive thymus involution [32–35]. The therapeutic benefits of this polypeptide as an immunostimulator was demonstrated in studies where an increased thymic cellularity, improved numbers of recent thymic emigrants and a broader TCR repertoire among peripheral T cells was noted in hypopituitary mice substituted with recombinant GH and in old, wild type animals treated with the GH secretagogue ghrelin [36] [37]. GH also accelerates thymocyte recovery and the reconstitution of the peripheral T cell compartment in lethally irradiated mice engrafted with allogeneic T cell-depleted bone marrow cells [38]. However, GH per se is not required for normal thymopoiesis because GH-deficient mice housed under stress-free conditions display a regular lymphoid development [39].

Studies in humans have recently established the clinical benefit of recombinant GH to enhance thymic function in adults with thymus atrophy. Treated with a highly active antiretroviral therapy (HAART) and daily subcutaneous GH injections, HIV infected adults reveal an increased thymic mass, an improved T cell output and higher numbers of circulating naïve and total $CD4^+$ T cells when compared to patients treated only with HAART [40]. Notably, the withdrawal of GH in adults with a GH deficiency mirrors these observations as the interruption of GH substitution swiftly decreases the frequency of recent thymic emigrants.

The resumption of GH replacement in these patients is then also followed by a significant rebound in the thymic export of new T cells [41]. Both of these studies demonstrate in addition a positive correlation between the frequency of recent thymic emigrants and plasma IGF-I concentrations. As TEC express both IGF-I and IGF-I receptor in response to GH [42], the stimulatory effect of GH on these cells is likely accomplished by engagement of an IGF-I/IGF-I receptor-mediated circuit.

The potential of IGF-I as a therapeutic agent to stimulate thymopoiesis was suggested by several independent observations: young mice transgenic for IGF expression have a hypercellular thymus [43]; thymus organ cultures in which IGF-I or its receptor are neutralized demonstrate an early block in thymocyte maturation [44]; and the administration of recombinant IGF-I to naïve mice stimulates the proliferative expansion of thymocytes, which in turn results in an increased number of recent $CD4^+$ and $CD8^+$ thymic emigrants [45]. IGF-I also increases the number of circulating T cell precursors (defined as lineage negative, c-kit- and Sca-1-positive cells, i.e. LSK cells) following their exit from the bone marrow. This activity likely contributes to the thymus stimulatory effect of IGF-I. In parallel, IGF-I treatment enhances the number of cortical and medullary TEC possibly generating a bigger stromal scaffold to accommodate thymopoiesis. However, IGF administration alone does not fully restore thymic involution [46].

The role of IGF-mediated signaling in averting thymus atrophy is clearly more complex than previously acknowledged since a deficiency in the pregnancy-associated plasma protein A (PAPPA) also mitigates thymus involution [47]. PAPPA acts as a metalloproteinase catalyzing the release of IGF-I from its binding protein, IGF-BP4, and an engineered deficiency of this enzyme results in a slower delivery and a lower tissue availability of IGF-I. It is therefore surprising that PAPPA-null animals resist age-dependent thymic atrophy. Moreover, these mice have an increased frequency of bone marrow resident T cell progenitors [47], which may increase the availability of these cells for thymopoiesis although their developmental potential and survival still need to be determined. The molecular mechanisms by which a limited bioavailability and hence attenuated IGF-I

signaling in PAPPA-deficient mice increases the resistance to age-related thymus atrophy is however not yet known.

Fms-like tyrosine kinase receptor III ligand

ETPs that express the Fms-like tyrosine kinase receptor 3 (Flt3; CD135) are believed to represent the earliest intrathymic T cell progenitors [48]. The physiological ligand for Flt3 (designated Flt3 ligand, FL) is expressed under steady-state conditions by multiple cell types including bone marrow stromal fibroblasts that stimulate hematopoiesis [49] and at low levels by perivascular fibroblasts that surround the proposed thymic entry site of Flt3 positive T cell progenitors [50]. FL expression by the thymic microenvironment is very likely regulated by immature thymic T-cell precursors as lymphopenia correlates with an in situ upregulation of this cytokine [51]. Experimental evidence for an important role of Flt3 in thymopoiesis was suggested by in vitro observations demonstrating that the addition of Flt3L to thymus organ cultures provides a proliferative signal to the most immature T-cell progenitors [52]. In keeping with this result, the loss of Flt3 expression reduces the number of the earliest thymic progenitors in the fetal, postnatal, and adult thymus [53,54]. Conversely, the intrathymic expression of FL enhances the proliferation of T cell precursors and/or their commitment to the T cell lineage [50]. This critical role of FL for thymopoiesis is particularly evident in the absence of interleukin-7 signaling because double FL and IL-7 receptor alpha chain deficient mice show an extensive reduction of their T cell progenitors [54].

FL serum concentrations and perivascular FL expression are greatly increased after myeloablative conditioning, which strongly suggests that FL may contribute to the enhanced recovery of thymic cellularity following HSCT [49,50]. Indeed, FL given to irradiated HSCT recipients enhances total thymus cellularity, increases the number of thymus-dependent T cell progeny and stimulates the intrathymic differentiation of these cells to mature T cells that will repopulate the periphery [55–57].

In addition to its potent thymorestorative activity, FL also improves the homeostatic expansion of peripheral T cells in HSCT recipients and hence contributes via a second mechanism to the recovery of the peripheral T cell pool [55,56]. Although dispensable for the maintenance and post-transplant expansion of mouse hematopoietic stem cells [58], bone marrow cells from Flt3-deficient mice display, however, a diminished capacity to competitively replenish the T cell compartment in irradiated recipients [59]. Taken together, these observations provide an understanding of the functional relevance of Flt3 expression on intrathymic T cell progenitors and underscore the importance of the thymic stromal cell–mediated FL–Flt3 receptor interactions for the reconstitution of thymopoiesis early after lethal irradiation and HSCT.

c-kit

The receptor tyrosine kinase c-kit is expressed on different cells types and plays pivotal roles in cell proliferation, differentiation and survival. The stimulatory ligand for c-kit is stem cell factor (SCF) which is also widely expressed during embryogenesis and post-natal life. In the hematopoietic system, SCF mediated signaling within niches of the thymus microenvironment is important for early T cell development and c-kit expression constitutes a hallmark of a subpopulation of ETP. Interestingly, the pharmacological inhibition of thymic c-kit signaling also favors donor-derived thymopoiesis in conditioned HSCT recipients as the accessibility of T cell progenitor niches is improved [60]. In this single preclinical study, the pre-transplant treatment of HSCT recipient mice with Sunitinib, a tyrosine kinase inhibitor blocking primarily SCF signaling, preferentially facilitates thymic engraftment with donor-derived precursors as these cells have a competitive advantage over

host ETP in which c-kit signaling has been inhibited. Consequently, treated mice demonstrate an enhanced chimerism, increased thymus cellularity and a higher frequency of donor-derived T cells seeding to the periphery [60]. Despite these favorable effects, Sunitinib is likely to be used in allogeneic HSCT in combination with measures to prevent graft rejection under non-ablative conditions.

Fibroblast growth factor 7

The fibroblast growth factor (Fgf) 7, (a.k.a. keratinocyte growth factor, KGF) belongs to the family of the structurally related Fgfs (reviewed in [61]. As a secreted single-chain protein of 28 kDa, Fgf-7 acts in a paracrine fashion as a mediator of mesenchymal/epithelial interactions, potentially stimulating epithelial cell proliferation and repair. Within the thymic microenvironment Fgf-7 is produced under physiological conditions by mesenchymal stromal cells and by mature thymocytes of the αβTCR lineage. Fgf-7 binds to and activates the IIIb splice variant of the Fgf receptor 2 (FgfR2IIIb), which is expressed within the thymus exclusively on TECs [62]. Other ligands of FgfR2IIIb include Fgf10 which is also expressed by thymic mesenchymal cells [63–65].

The specific importance of Fgf:FgfR signaling for thymus organogenesis was initially established in experiments with gene targeted and transgenic mice. The targeted disruption of either FgfR2IIIb or Fgf-10 and the transgenic overexpression of a soluble, dominant-negative form of the receptor results in a hypoplastic thymus that lacks a regular cortico-medullary distinction but still supports an apparently regular T cell maturation [66,67]. Because the thymic cellularity of Fgf-10 deficient mice is larger when compared to that of FgfR2IIIb knock-out animals, thymic Fgf7 may provide signals that compensate in part for the loss of Fgf-10. Fgf-7 is in contrast dispensable for TEC differentiation and thymopoietic function as Fgf-7 deficient mice display a regular thymic structure and cellularity [68].

Fgf-7 plays also significant roles in the correction of thymus senescence [69], and the repair of thymus injury following irradiation [68] or as a consequence of GvHD. Aged mice (>15 months) treated with Fgf-7 increase their thymopoiesis by 4-fold in comparison to placebo-treated animals, which results in a thymic cellularity equivalent to that of 5–6 week old mice [70]. Moreover, enhanced T cell development is maintained for about 2 months after a single course of Fgf-7, and can be sustained for 3 months following monthly injections. This effect is paralleled by an increased number and regular organization of IL-7–producing TECs. However, Fgf-7 treatment of young mice only increases thymocyte cellularity by 2–2.5 fold. Thus, Fgf-7 preferentially acts in aged mice as a protective and trophic factor that mediates microenvironmental changes which in turn favor the generation and/or survival of immature thymocytes. Fgf-7 administered to mice prior to HSCT conditioning protects IL-7–expressing TECs from cytotoxic therapy-induced damage [71]. As a consequence of enhanced TEC recovery or TEC resistance to apoptosis, the number of donor-derived thymocytes increases in these treated mice. In addition, Fgf-7 protects the thymic microenvironment of mice with acute GVHD. Mice treated with Fgf-7 prior to lethal irradiation, HSCT and the induction of acute GVHD preserve a regular distribution of the different thymocyte subpopulations, a normal cell cycle progression of immature thymocytes and a normal architectural organization and viability of the different TEC subpopulations in the cortex and medulla. The positive effect of Fgf-7 on thymic function is also observed in an autologous non-human primate transplantation model, where repetitive administration of Fgf-7 preserves regular thymic architecture and, in parallel, increases the thymus-dependent reconstitution of the T cell compartment [72]. Unfortunately and contrary to these animal experiments, preliminary studies in human HSCT recipients of matched sibling donor grafts fail to demonstrate a beneficial effect of IL-7 treatment as an incidence of GVHD, donor cell engraftment, rate of CMV and invasive fungal infections, or over-all

patient survival remain the same when compared to untreated HSCT recipients [73]. The biological effect of exogenous Fgf-7 on the immune reconstitution in the human setting is thus not yet established and further studies (especially in recipients of mismatched T-cell depleted donor grafts) are required to reach a definitive understanding of the therapeutic value of Fgf-7 in HSCT.

Interleukin 7

IL-7 is required for intrathymic T cell development early during maturational progression of TN cells to DP cells as well as later at the SP stage where this cytokine promotes survival, proliferation and differentiation (reviewed in [74]). IL-7 is produced in the thymus by cortical and to a lesser extent medullary TEC [75,76]. The receptor for IL-7 (IL-7R) is almost exclusively expressed on lymphoid cells and has been demonstrated to mediate signals for cell differentiation via the Jak-Stat and for cell protection from apoptosis through engagement of the PIK3/Akt pathway. In addition, IL-7 is absolutely required for the rearrangement of the γ-chain of the TCR. In mice lacking IL-7 expression, the thymus cellularity is reduced by 20-fold whereas the composition of the different thymocyte subpopulations of the αβTCR lineage is almost unaffected and the organ architecture is maintained[77]. Mice deficient for the expression of the α-chain of the IL-7R demonstrate also a reduced thymic cellularity but, in contrast to IL-7 knock-out animals, display a profound block in thymocyte expansion that occurs before the onset of T cell receptor gene rearrangement and the expression of CD4 and CD8[78]. This difference in phenotype is explained by the fact that the IL-7Rα chain is an integral component of two distinct cytokine receptors, namely that for IL-7 and for thymic stromal lymphopoietin (TSLP). A loss of IL-7Rα expression will therefore abrogate signaling by both cytokines, with TSLP providing normally a co-mitogenic activity that is less potent than that of IL-7 [79].

IL-7 is an attractive cytokine for immunotherapy, because of its action on distinct subsets of T lymphoid cells during thymic differentiation and peripheral homeostasis [80]. The potential to use IL-7 to enhance thymic reconstitution was initially demonstrated in a syngeneic HSCT model where a 2-week course of IL-7 results in a significant but not sustained increase in the proliferation of TN thymocytes. These cells subsequently undergo normal thymic maturation and are efficiently exported to the periphery [81,82]. This stimulatory effect of IL-7 is, however, not consistently observed in other transplantation models where IL-7 has mainly been implicated in the preferential and selective expansion of recent thymic emigrants or mature T cells admixed to the bone marrow inocculum [83,84]. The biological role of IL-7 as an immunomodulatory agent has also recently been investigated in two published phase I clinical trials [85,86]. The results of these studies suggest that IL-7 may augment thymopoiesis only to a limited extent but preferentially exerts its effects on peripheral T cells as IL-7 mobilizes recent thymic emigrants (RTE, I.e. naïve T cells) from lymphoid tissues into the circulation. The absence of a significant effect on thymopoiesis is further underscored by the observation that the thymic size (determined by Computer Tomography and used as a surrogate measure for thymopoiesis) remains unaffected in IL-7 treated individuals [86].

Androgen blockade

The accelerated decline of thymic function at the time of and after puberty has been functionally linked to increasing sex steroid hormone levels [87]. This view is consistent with observations that both thymocytes and thymus stromal cells express sex steroid receptors and that the exogenous administration of androgens and estrogens causes the apoptosis of immature thymocytes leading to an almost complete collapse of thymopoiesis [88]. Indeed, TEC in particular but also all thymocyte subpopulations express the classical

intracellular androgen receptor whereas the membrane androgen receptor is expressed within the thymus only on epithelia [89]. Thymic regeneration with a rapid and synchronous expansion of the different thymocyte subsets is observed in animals that have been either surgically or chemically castrated [13]. Specifically, the rate of thymocyte apoptosis decreases as a consequence of sex steroid ablation (SSA) and within five days the frequency of TN and DP thymocytes and by ten days that of SP thymocytes reach values that are typically observed in young animals. In parallel with this thymocyte expansion, TEC demonstrate an increased proliferation in the absence of sex steroids which may either be a direct consequence of SSA or, alternatively, the result of increased thymic crosstalk between the lymphoid and stromal compartments. In support of the former assertion is the finding that androgen receptor defective thymus stroma fails to undergo sex steroid induced atrophy [90]. SSA improves also the number and function of hematopoietic stem cells consequently increasing T cell precursor immigration into the thymus [91]. Twenty-eight days after SSA, the thymic microenvironment of old mice displays a frequency of TEC and dendritic cells that is comparable to the rate observed in young animals [13]. These significant changes in thymus function eventually translate into an enlarged pool of naïve, peripheral T cell with a normal CD4:CD8 ratio.

Whereas post-menopausal women have a significantly increased plasma level of biologically active testosterone due in part to ineffective inactivation of this hormone by low levels of testosterone-binding protein, an iatrogenic though temporary reduction in testosterone or estrogen serum levels is observed in HSCT recipients conditioned with chemoradiotherapy [92]. Clinically relevant, reversible SSA can be achieved by transiently disrupting the hypothalamus-pituitary-adrenal/gonadal axis where pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) control the production of gonadal sex steroids [93]. A suprasaturating and constant exposure of a potent agonist of LHRH will however desensitizes the pituitary to endogenous LHRH and consequently cease the production and secretion of LH and FSH subsequently preventing the formation of gonadal sex hormones [94]. Clinical proof-of-principle for chemical SSA to enhance thymus function has been obtained in male patients with prostate cancer where the treatment with a potent agonist variant of LHRH improves thymopoiesis, as measured by an enhanced peripheral appearance of recent thymic emigrants. Importantly, this effect persists for several months after the discontinuation of treatment.

SSA ablation together with other stimulatory strategies have also been investigated to restore thymic function in mouse HSCT recipients. Compared to animals without treatment, mice administered with a combination of Fgf-7 and a potent LHRH agonist (LHRH-A) display an additively increased recovery of both thymocytes and peripheral T-cells [95]. In comparison to other treatment groups, the thymic morphology and stromal composition is faster restored in mice treated with this combination which eventually also improves peripheral donor-derived T-cell reconstitution and function when challenged with neo-antigens or pathogens. As both Fgf-7 and leuoprolide acetate as a LHRH-A have been approved for clinical use, a combination therapy with both agents represents a viable approach to accelerate the immune recovery in human HSCT recipients conditioned with myeloablative radiotherapy.

p53 inhibitor

The tumor suppressor p53 is typically expressed only at low concentrations and regulates the transcription of genes involved in cell cycle arrest, DNA repair, senescence, and apoptosis in response to genotoxic stress [96]. Hence, p53 activity is the determinant of radiation and drug sensitivity *in vivo* and its loss of function causes genomic instability, tumor progression, resistance to chemotherapy, and increased angiogenesis [97–100]. The thymus

of p53-deficient mice is in contrast to wild type mice highly radio-resistant which further underscores the complete coincidence between the p53 status of tissues and their radiation-induced degree of apoptosis [97]. Transient inhibition of p53 using small molecules has therefore been investigated to protect normal tissues from chemo- and radiotherapy and to treat other pathologies associated with stress-mediated activation of p53. One such p53 inhibitor, designated pifithrin (PFT), increases not only in vitro the proportion of G2-arrested cell lines by attenuating the p53-dependent block of DNA replication but also in vivo improves the survival of mice exposed to gamma irradiation [101]. Importantly, the transient inhibition of p53 is also expected to prevent the development of secondary malignancies that occur as a consequence of conditioning-related cytotoxicity to normal tissue [102,103].

Given the observations that TEC and in particular thymocytes are greatly depleted during conditioning in HSCT recipients and that that p53 regulates apoptosis in epithelial tissues, pre-treatment with PFT was investigated in mice to determine the extent of thymus protection following radiotherapy and congenic HSCT [95]. Though PFT treatment does not alter thymus cellularity in naïve mice, it partially protects TEC cellularity and maintains a regular thymocyte subset distribution in lethally irradiated and bone marrow reconstituted animals in comparison to untreated recipients. The export of donor-derived mature thymocytes to the periphery is also enhanced in PFT treated mice to the effect that these animals when challenged with *Listeria monocytogenes* significantly reduce their bacterial burden in the spleen when compared to controls. However, the PFT effect on thymocyte cellularity in treated recipients of congenic HSCT is only apparent as long as the reconstitution of thymopoiesis has not yet reached the steady-state kinetics typically observed in untreated but transplanted but untreated mice. In contrast, the treatment of allogeneic HSCT recipients with a combination of Fgf-7 and PFT results in a thymocyte cellularity that is higher than that observed in naïve, sex-and age-matched controls or in animals that have been administered only Fgf-7 or PFT [95]. This positive effect of a combined treatment persists for at least 12 weeks after treatment and also results in a higher frequency of donor-derived peripheral T cells, an improved restoration of T-cell zone fibroblastic reticular cells (FRCs) and a better capacity to express the chemokine CCL21 in lymphoid stroma. These peripheral findings correlate with an enhanced immune response against *Listeria monocytogenes* infections. Thus, transient p53 inhibition combined with Fgf-7 treatment represents a novel and potentially translatable approach to promote T-cell recovery after clinical HSCT [95].

T cell progenitors

Several factors have been described that promote the *ex vivo* survival, expansion and/or differentiation of hematopoietic stem cells [104]. Insight into the mechanisms regulating early hematopoiesis have now also been employed to generate ex vivo lineage-committed progenitor pools that when adoptively transferred into conditioned HSCT recipients are expected to overcome the shortage of early T cell progenitors as these cells are only present at a reduced frequency in the thymus shortly after HSCT. A proof of principle for this approach had earlier been provided by murine studies in which the co-transfer of freshly isolated common lymphoid precursors (CLP) with HSC accelerated the immune reconstitution by T, NK, and B cells during the first two weeks after transplantation and improved in treated mice the resistance to murine cytomegalovirus infections [105]. As Delta-like 1 (DL1)- and DL4-mediated Notch1 signaling is essential for T cell lineage commitment and differentiation, culture systems have been developed in which hematopoietic stem cells can be efficiently differentiated into T cell progenitors cells following their exposure to DL1 expressing OP9 bone marrow stromal cells [106,107] or to an immobilized DL1-containing fusion protein (DL1–hIgG fusion protein, $DL1^{ext\text{-}IgG}$; ref.

[108]. The adoptive transfer of *ex vivo* generated T cell progenitors together with T-cell-depleted mouse bone marrow or purified HSC into lethally irradiated allogeneic recipients results in an increased thymic cellularity, substantially improves donor T-cell chimerism, and favours normal T-cell selection and function [109]. Moreover, in vivo Fgf-7 treatment combined with the adoptive transfer of ex vivo generated T cell progenitors is additive in stimulating thymopoiesis. The transfer of human CD34+ cord blood progenitors expanded ex vivo with $DL1^{ext\text{-}IgG}$ and subsequently infused into a patient conditioned with a myeloablative preparative regimen for stem cell transplantation has now recently been reported [110]. Although a substantially shortened neutrophil recovery is observed in this patient, a sustained recovery of graft-derived T cells does not occur. Hence, further studies are needed to examine thymic output and to optimize thymopoiesis following HSCT combined with the adoptive transfer of high numbers of ex vivo expanded T cell progenitors.

Conclusion

The carefully orchestrated transcriptional and cellular events that control normal thymus function are susceptible to perturbations by senescence, malnutrition, infections and several iatrogenic influences such as chemoradiotherapy and cytotoxicity in the context of GVHD. Although an initial focus has been placed on the direct stimulation or replacement of thymocytes and its precursors, more recent attempts aim at enhancing and repairing the thymic microenvironment as this compartment constitutes the rate limiting factor in the restoration of effective thymopoiesis. Hormonal approaches such as the use of positive regulators such as GH or the removal of negative regulators via androgen blockade have shown clear benefits in accelerating thymopoiesis initially in pre-clinical investigations and have now been translated to clinical practice. Similarly, replacement of TEC products such as Fgf-7 or IL-7 are now tested in clinical trials designed to hasten thymopoiesis and peripheral T cell recovery after HSCT. Additional attention has been given to pharmacological measures that protect the thymic microenvironment from damage by radiochemotherapy. Finally, novel and potentially promising preclinical approaches suggest that the adaptive transfer of T cell progenitors expanded ex vivo will enhance T cell recovery in conditioned patients as these cells are expected to accelerate thymus seeding and T cell development following HSCT. Due to the complex cascade of signals and cellular communications between thymocytes and the microenvironment, a combination of adaptive transfer of in vitro expanded progenitors with agents that exert a thymopoietic and/or thymoprotective activity is likely to provide additive benefits to reconstitute T cell development.

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