Published in final edited form as:

J Immunotoxicol. 2010 March ; 7(1): 1–7. doi:10.3109/15476910903453296.

An Overview of IL-7 Biology and Its Use in Immunotherapy

Nahed ElKassar and Ronald E Gress

Experimental Immunology and Transplantation Branch, National Cancer Institute, Bethesda, MD

Abstract

Interleukin (IL)-7 is required for T-cell development as well as for the survival and homeostasis of mature T-cells. In the thymus, double negative (DN) CD4[−] CD8[−] thymocyte progenitor transition into double positive CD4⁺ CD8⁺ cells requires Notch and IL-7 signaling. Importantly, IL-7 seems to have a dose effect on T-cell development, and at high doses, DN progression is blocked. Naïve T-cells in the thymus, and after their exit to the periphery, are dependent on IL-7 and TCR signaling for survival. Upon antigen exposure, they proliferate and differentiate into memory T-cells. Because IL-7 intervenes at all stages of T-cell development and maintenance, it has been introduced recently into clinical trials as an immunotherapeutic agent for cancer patients (of particular note, those who had undergone T cell depleting therapy) in an attempt to increase their population sizes of CD4⁺ and CD8⁺ cells overall, and specifically of CD8⁺ (CD45RA⁺CCR7⁺ and/or CD27⁺), CD4⁺ (CD45RA⁺CD31⁺), and CD4⁺ central memory T-cells (CD45RA[−]CCR7⁺). Interestingly, IL-7 in humans induced a preferential expansion of naïve T-cells resulting in a broader T-cell repertoire than before the treatment, and this effect was independent of age. This suggests that IL-7 therapy could enhance immune responses in patients with limited naïve T-cell numbers as in aged patients or after disease-induced or iatrogenic T-cell depletion. This overview highlights the role of IL-7 on T-cells in mice and humans.

Keywords

IL-7; Immunotherapy; Aging

Introduction

Interleukin (IL)-7 is a non-redundant cytokine in T-cell development and function. It is required in early T-cell development as well as for T-cell homeostasis and is secreted by stromal cells in the thymic and bone marrow (BM) environment (Mazzucchelli and Durum, 2007). Recently, studies have shown that IL-7 is produced by fibroblastic reticular cells (FRC) in T-cell zones of lymph nodes (LN) (Link et al., 2007). The FRC produce IL-7 and chemokines CCL19 and CCL21 (the ligands for CCR7) to attract T-cells and to provide them with survival signals (Link et al., 2007).

IL-7 binds to its receptor which is composed of the two chains IL-7R α (CD127), shared with the thymic stromal lymphopoietin (TSLP) (Ziegler and Liu, 2006), and the common γ chain (CD132) for IL-2, IL-15, IL-9 and IL-21. Whereas γ c is expressed by most hematopoietic cells, IL-7R α is nearly exclusively expressed on lymphoid cells. After binding to its receptor, IL-7 signals through two different pathways: Jak-Stat (Janus kinase-Signal transducer and activator

Please address all correspondence to: Dr. Ronald E. Gress, Experimental Immunology and Transplantation Branch, National Cancer Institute, Building 10, CRC, Room 3-3330, Bethesda, Maryland 20892. (T) 301-496-1791 (F) 301-480-4354 gressr@mail.nih.gov.

Declaration of Interest

The Authors report no conflicts of interest. The Authors are alone responsible for the content and writing of the paper.

of transcription) and PI3K/Akt responsible for differentiation and survival, respectively. The absence of IL-7 signaling is responsible for a reduced thymic cellularity as observed in mice that have received an anti-IL-7 neutralizing monoclonal antibody (MAb); Grabstein et al., 1993), in IL-7^{-/-} (von Freeden-Jeffry et al., 1995), IL-7Rα^{-/-} (Peschon et al., 1994; Maki et al., 1996), γc^{-/-} (Malissen et al., 1997), and Jak3^{-/-} mice (Park et al., 1995). In the absence of IL-7 signaling, mice lack T-, B-, and NK-T cells. IL-7α^{-/-} mice (Peschon et al., 1994) have a similar but more severe phenotype than IL-7^{-/-} mice (von Freeden-Jeffry et al., 1995), possibly because TSLP signaling is also abrogated in IL-7α^{-/-} mice. IL-7 is required for the development of γδ cells (Maki et al., 1996) and NK-T cells (Boesteanu et al., 1997).

Biology of IL-7 Signaling

IL-7 and Jak/Stat

Janus Kinase-3 (Jak3) binds selectively to the γc chain (Suzuki et al., 2000) while Jak1 binds to IL-7Rα (Rodig et al., 1998). Mutations in γc (Cao et al., 1995; Disanto et al., 1995; Ohbo et al., 1996) or Jak3 (Baird et al., 2000; Nosaka et al., 1995; Park et al., 1995) are responsible for severe combined immunodeficiency in humans, characterized by the lack of T- and NK, but not B-cells, designated T⁻B⁺ SCID. When IL-7 binds to IL-7Rα, it recruits the γc chain bringing together their intracellular domains bearing Jak1 and Jak3, respectively. This induces their transphosphorylation that increases their intrinsic kinase activity and phosphorylation of the critical Y449 site on IL-7Rα (Jiang et al., 2005). Then, phosphorylated receptors become a docking site for Stat5 protein binding. Stat5 proteins bind to IL-7Rα via SH2-phosphotyrosine interaction, become phosphorylated by Jaks on a conserved residue, and dimerize through reciprocal phosphotyrosine/SH2 interactions. Stat5 dimers are then exported to the nucleus where they bind to DNA (Figure 1A) (Foxwell et al., 1995; Lin et al., 1995). The suppressor of cytokine signaling-1 (SOCS-1) protein inhibits signaling by multiple means: (1) binding and inhibiting Jaks; (2) binding cytokine receptors and blocking Stat recruitments; and, (3) promoting ubiquitination and degradation of the Jak/receptor complex (Jiang et al., 2005). SOCS-1 is constitutively-expressed in double positive (DP) thymocytes before positive selection and is down-regulated after T-cell receptor (TCR) signaling, thereby allowing IL-7R signaling in positively-selected cells.

PI3K Pathway

Activation by IL-7 results in phosphorylation of the Y449 residue on IL-7Rα (Jiang et al., 2005). The p85α subunit of the PI3Kinase binds directly to phosphorylated Y449 via an SH2 domain. This is followed by the allosteric activation of the catalytic subunit P110. The PI3K is recruited to the membrane where it produces the phosphatidyl-inositol PIP3 by phosphorylating PIP2. PIP3 activates downstream genes with a pleckstrin homology domain such as PDK1 and Akt (Shiroki et al., 2007). Akt, in turn, phosphorylates genes that regulate cell metabolism, cell cycle progression and survival, such as GSK3β, P27 and the death protein Bad. IL-7 regulates proliferation at different levels. By phosphorylating GSK3β, IL-7 prevents the phosphorylation of the cytoplasmic signaling molecule β-catenin, which impedes its degradation; hence, it is translocated to the nucleus where it combines with different transcription factors, like TCF/LEF-1, to induce the expression of several genes, such as cyclin D1 which induces cell cycle progression via regulation of RB hyper-phosphorylation and inactivation (Fresno Vara et al., 2004). IL-7 also decreases the transcription and increases the degradation of P27 via PI3K and Akt (Barata et al., 2004; Chan and Tsichlis, 2001; Jiang et al., 2005; Juntilla and Koretzky, 2008; McKenzie et al., 2006) (Figure 1B).

Pro and anti-apoptotic and other target genes of IL-7 signaling

IL-7 promotes T-cell survival by up-regulating the expression of anti-apoptotic genes; the anti-apoptotic genes Bcl-2 and Mcl-1 (myeloid-cell-leukemia sequence-1) are the major targets in

naïve T-cells. The protein products encoded by these genes inhibit the pro-apoptotic proteins Bax and Bak. Consequently, IL-7 signaling inhibits the release of cytochrome c and the consequent activation of caspases from the mitochondria that cause the death of the cell. Also, IL-7 inhibits the pro-apoptotic proteins Bim, Bid, and Bad (Mazzucchelli and Durum, 2007) (Figure 1B).

Thymic development and IL-7

Thymic development and IL-7R α expression

IL-7R α is expressed at many different stages of T- and B-cell development. The development of $\alpha\beta$ T-cells in the thymus proceeds through well-defined stages, i.e., double negative (DN) stages (DN1-4), immature single positive (ISP), DP, and SP CD4⁺ or CD8⁺ T-cells (Godfrey et al., 1993; Rothenberg et al., 1993). Within different progenitors with T- and B-cell potentials, the early thymocyte progenitors (ETPs) show the highest canonical T-cell capacity (Allman et al., 2003); these ETPs have a negative or low level of IL-7R α expression. They are defined as lineage marker negative (Lin⁻) CD44⁺CD25⁻ckit⁺ (Allman et al., 2003). During early T-cell development, the highest IL-7R α expression is on DN2 (Lin⁻CD44⁺CD25⁺c-kit⁺) progenitors. DN3 (Lin⁻CD44⁺CD25⁺c-kit⁻) cells express lower levels of IL-7R α , while the DN4 (Lin⁻CD44⁺CD25⁺c-kit⁻) cells express the lowest levels. Therefore, DN cell progression in the thymus requires IL-7R signaling. This signal is required for at least two reasons: protection from apoptosis (Akashi et al., 1997) and rearrangement of the gene encoding TCR γ (Durum et al., 1998). T-Cell development, but not TCR γ rearrangement, is restored by over-expressing Bcl-2 (Maraskovsky et al., 1997; Schlissel et al., 2000) or by the ablation of the pro-apoptotic Bax gene (Khaled et al., 2002). This suggests that IL-7 is required for TCR γ rearrangement and this effect is independent from inhibition of apoptosis. The rearrangement of the other TCR genes is not totally dependent on IL-7 signals (Candeias et al., 1997).

IL-7 signaling is absent in DP cells due the absence of IL-7R α expression and the presence of SOCS-1 protein (Yu et al., 2006). This allows the negative selection and death by neglect to occur in these cells. ISP and DP cells do not express IL-7R α unlike CD4SP and CD8SP thymocytes where IL-7 promotes survival and proliferation (Mazzucchelli and Durum, 2007). IL-7 also promotes differentiation into CD8⁺ cells (Brugnera et al., 2000). IL-7R α is expressed by the common lymphoid progenitors (CLPs) (Kondo et al., 1997), as well as by pro-B and pre-B-cells. It is not expressed in immature/mature B-cells (Hardy et al., 1991).

IL-7 over-expression effect on thymic development

We used IL-7 transgenic (Tg) mice under the control of the LCK proximal promoter in order to explore the effect of IL-7 on early T-cell development (ElKassar et al., 2004). We obtained different lines expressing different levels of IL-7 in the thymus. The founder line with the lowest level of IL7 over-expression (9-fold higher in newborn thymic tissue) showed increased DP, CD4SP and CD8SP thymocyte numbers. In contrast, in the founder line with the highest over-expression of IL-7 (39 fold), thymic cellularity was decreased with reduced numbers of all thymic subsets except for mature CD8SP cells (Figure 2A) (ElKassar et al., 2004). In addition, an increase of TCR $\gamma\delta$, NK1.1⁺ and B220⁺ cells was observed in the IL-7 Tg thymus (Figure 2B). We also noted a decreased proliferation and a block in the development of DN stages with high IL-7 expression by Ki67 staining and BrdU incorporation (ElKassar et al., 2004). These data suggested a role for IL-7 in T-cell development progression at the DN stage and before reaching the DP stage, similar to the observations described for Notch signaling.

T-Cell homeostasis and IL-7R α expression

T-Cell homeostasis, cytokines and TCR signal

Due to removal of thymocytes by positive and negative selection, only a few thymocytes with low affinity for self-peptide+MHC (pMHC) ligands are allowed to leave the thymus (Starr et al., 2003). These mature cells populate the periphery and respond to the presence of foreign antigens (Krogsgaard et al., 2007). When activated by foreign antigens, naïve T-cells undergo massive expansion and become effector T-cells (Sprent and Surh, 2002; Harty and Badovinac, 2008). A small fraction of effector cells survive as memory cells; the majority of effector cells die.

It is now known that IL-7 is an essential cytokine for both survival and homeostatic proliferation of naïve and memory T-cells (Surh and Sprent, 2008). Experimentally, the typical situation of homeostatic proliferation occurs when T-cells are injected into a lymphopenic recipient. The cells undergo a unique type of proliferation called lymphopenia-induced proliferation (LIP) (Rocha et al., 1989). In this situation, both cytokines and pMHC are not rate-limiting. Blood IL-7 concentration increases with lymphopenia (Fry and Mackall, 2001) and is associated with an increased T-cell proliferation rate, likely by enhanced TCR stimulation by self-antigen/MHC (Goldrath and Bevan, 1999). This LIP is severely diminished in the absence of either IL-7 (Goldrath and Bevan, 1999; Schluns et al., 2000; Tan et al., 2001) or MHC (Lee et al., 1999). The propensity of naïve T-cells to undergo homeostatic proliferation depends on the strength of the TCR signal and IL-7R as shown by TCR transgenic cells of high affinity such as OT-1, compared to lower affinity transgenic cells such as P14 (Kieper et al., 2004). Furthermore, in presence of a mutated Y449 residue on IL-7R α , T-cells cannot undergo homeostatic proliferation (Osborne et al., 2007).

In addition to IL-7, IL-15 plays an important role in supporting homeostasis of naïve CD8 T-cells as shown by the reduced number of these cells in IL-15-deficient mice (Berard, 2003). Interestingly, SOCS-1 deficient CD8 T-cells undergo homeostatic proliferation in IL-7-deficient hosts but not in IL-15 deficient hosts, showing that SOCS-1 regulates sensitivity to IL-15 (Davey et al., 2005).

Regulation of IL-7R α expression

Stromal cells produce IL-7 at relatively constant amounts and independent of external stimuli (Mazzucchelli and Durum, 2007). T-cell responses to IL-7 are modulated by IL-7R α expression. Consequently, IL-7R α transcription is under control of IL-7 in order to maximize the utilization of the limited cytokine store and maintain T-cell homeostasis in the periphery (Park et al., 2004). Briefly, IL-7 signaling triggers negative feedback to IL-7R α transcription in order to prevent depletion by activated expanding cells, and ensure that IL-7 is available to other IL-7 dependent lymphocytes. The amounts of IL-7 available vary also depending on the clearance by the receptors present on lymphoid cells (Fluur et al., 2007). Therefore, in lymphopenic patients like that found early after bone marrow transplantation (BMT), blood levels of IL-7 are high. This promotes remaining cells to proliferate and achieve a homeostatic proliferation. In contrast, excess T-cells will not survive because the cells will compete for the limited amount of IL-7.

An interesting characteristic of IL-7 is that it acts to repress its own receptor in CD8 and CD4 cells. This mechanism is Gfi-1-dependent in CD8 T-cells, but is Gfi-1-independent in CD4 T-cells (Park et al., 2004). Gfi-1 binds to introns 2 and 4 of the IL-7R α gene and down-regulates its expression after stimulation by IL-7. Recently, IL-7R α expression on activated CD8 T-cells during lymphocytic choriomeningitis virus infection has been explored (Chandele et al., 2008). In IL-7R α ^{high} memory precursor effector CD8 cells, GA binding protein- α (GABP α)

was required for hyperacetylation of the IL-7R α promoter. In contrast, Gfi-1 was responsible for the stable repression in memory IL-7R α^{low} CD8 cells by antagonizing GABP α binding (Chandele et al., 2008).

Other mechanisms are involved in the regulation of IL-7 receptor expression. PU.1 has been shown to bind to the IL-7R α promoter and is required for the development of lymphoid progenitors lacking in PU knockout mice (Dakic et al., 2005). In addition to IL-7, multiple signals have been shown to affect IL-7R α transcription in T-cells. Other cytokines, such as IL-2, IL-4, IL-6, and IL-15, as well as TCR signaling, repress IL-7R α expression (Chandele et al., 2008).

IL-7 and recent thymic emigrants

Recent thymic emigrants (RTEs) correspond to newly-formed mature T-cells in the thymus that have exited to the periphery. Due to the lack of phenotypic markers distinguishing RTEs, measurement of T-cell receptor excision circles (TRECs) was developed for quantifying RTEs in humans (Chandele et al., 2008). We used this method to explore the effect of exogenous IL-7 administration in mice (Chu et al., 2004). After continuous injection of IL-7 by sub-continuous pump for 7–14 days, a significant increase in TRECs numbers, and naïve CD4 $^{+}$ and CD8 $^{+}$ peripheral cells was observed. A significant increase also occurred in thymectomized mice. Thymocyte cell numbers were unchanged after IL-7 treatment. The increase in peripheral naïve T-cell number was due to increased proliferation induced by IL-7 as measured by Ki67 and BrdU incorporation experiments. More interestingly, the increased TREC number was the result of preferential accumulation of RTEs in the lymph nodes. These results indicated that short-term exogenous IL-7 administration effect on TREC levels does not result from enhanced thymic function (Chu et al., 2004).

Clinical application of IL-7

IL-7 is an attractive cytokine for immunotherapy as it has distinct actions on different subsets of T-cells (Overwijk and Schluns, 2009). Specifically, because of its actions on naïve T-cells, IL-7 represents a potential treatment to enhance T-cell reconstitution and vaccine efficacy. Alternatively, because of its broad role in T-cell homeostasis and its effects on B-cells, toxicities, including autoimmunity, might also result from its administration. Two Phase I reports have identified no clinically significant toxicity, but have noted efficacy of recombinant human IL-7 (rIL-7) treatment in improving CD4 $^{+}$ and CD8 $^{+}$ counts (Rosenberg et al., 2006; Sportes et al., 2008). In the study reported in 2006, four groups of patients with metastatic cancer received four different doses of rIL-7 (3, 10, 30, and 60 $\mu\text{g/kg}$) every 3 days, for eight doses. Absolute numbers of CD4 $^{+}$ and CD8 $^{+}$ T-cells increased in a dose-dependent manner over the 21-day period. There was no change in B-cell, NK cell, or CD4 $^{+}$ Foxp3 $^{+}$ (T $_{\text{reg}}$) numbers. At 60 $\mu\text{g/kg}$, both CD4 $^{+}$ and CD8 $^{+}$ counts increased 3 to 7-fold by Day 21. By Day 28, lymphocyte counts were decreasing in the same group. The bone marrow (BM) was analyzed in patients who received 30 and 60 $\mu\text{g/kg}$. An increase of B-cell progenitors was observed, but this did not translate into increased numbers of peripheral B-cells. The rIL-7 administered was produced by *Escherichia coli* and did not contain the normal glycosylation seen in eukaryotic IL-7. Some patients developed low titer anti-rIL-7 antibody detected by enzyme-linked immunosorbent assay by Day 28. These antibodies did not have a significant neutralizing potential when tested by specific bioassay and no patient developed lymphopenia related to these antibodies during the follow-up period (Rosenberg et al., 2006).

In the study reported in 2008, 16 patients with refractory cancer of various types were enrolled on a Phase I dose escalation trial. The CD4 $^{+}$ and CD8 $^{+}$ counts increased similarly in a dose dependent manner. The increase of T-cell proliferation was confirmed by Ki67 expression at Day 7 of the treatment. After Day 7, Ki67 and IL-7R α declined as expected after signaling by

rIL-7. Bcl-2 expression induced by rIL-7 was sustained for several weeks after cessation of the treatment. Perhaps due to their low baseline IL-7R α expression, CD4⁺Fop3⁺ cells were not increased by rIL-7 treatment. The TCR repertoire diversity in CD4⁺ and CD8⁺ cells was increased one week after the treatment. rIL-7 treatment increased selectively naïve CD4⁺ and CD8⁺ (CD45RA⁺CCR7⁺ and/or CD27⁺), CD4⁺ RTEs (CD45RA⁺CD31⁺), and CD4⁺ central memory T-cells (CD45RA⁻CCR7⁺). The T-cells were functional as their capacity to proliferate *in vitro* was increased 10-fold. Absolute numbers of circulating TRECs/millimeter³ between Days 7 and 21 were also significantly higher in rIL-7-treated patients in CD4⁺ and CD8⁺ cells (Figure 3). The TREC frequencies were decreased in sorted naïve CD4⁺ and CD8⁺ cells, which confirmed their augmented proliferation by rIL-7. No increase of thymus size was observed on CT evaluation. The rIL-7 was well tolerated in this trial and its T-cell effects were independent of age (Figure 4). Five out of 12 patients showed the same low-level of anti rIL-7 antibodies as in the previous study.

The effect of rIL-7 was attributed to a combination of increased cell cycling via TCR triggering to cross reactive self-antigens and diminished programmed cell death (Sportes et al., 2008). An augmented trafficking from the lymphoid tissues to the bloodstream was also possible in this study because of the spleen and LN enlargement seen in these patients by computerized tomography (CT), and their increased metabolic activity on positron emission tomography (PET). These two studies imply that rIL-7 in humans induces a dramatic and prolonged naïve, polyclonal, and diverse repertoire of CD4⁺ and CD8⁺ without any increase in T_{regs}. This suggests that rIL-7 would be effective in improving immune function in patients with impaired immunity.

Conclusion

IL-7 functions at all stages of T-cell differentiation and homeostasis. IL-7 treatment has been shown to increase the peripheral naïve and central memory T-cell pool making it a potential treatment for patients with impaired T-cell populations. The IL-7 effect on thymus function is unclear. In both mice and human studies there was no evidence of improvement of thymopoiesis by excess IL-7 (Chu et al., 2004; Sportes et al., 2008). Because of our *in vivo* animal model showing a negative effect of high IL-7 levels on thymus development (ElKassar et al., 2004), one would suspect that it would be unlikely by IL-7 treatment alone to improve thymus function while optimally increasing peripheral expansion of T-cells.

Acknowledgments

The Authors thank Francis A Flomerfelt for expert review of the manuscript.

References

- Akashi K, Kondo M, vonFreedenJeffrey U, Murray R, Weissman IL. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. *Cell* 1997;89:1033–1041. [PubMed: 9215626]
- Allman D, Sambandam A, Kim S, Miller JP, Pagan A, Well D, Meraz A, Bhandoola A. Thymopoiesis independent of common lymphoid progenitors. *Nat Immunol* 2003;4:168–174. [PubMed: 12514733]
- Baird AM, Lucas JA, Berg LJ. A profound deficiency in thymic progenitor cells in mice lacking Jak3. *J Immunol* 2000;165:3680–3688. [PubMed: 11034372]
- Barata JT, Silva A, Brandao JG, Nadler LM, Cardoso AA, Boussiotis VA. Activation of PI3K is indispensable for IL-7-mediated viability, proliferation, glucose use, and growth of T-cell acute lymphoblastic leukemia cells. *J Exp Med* 2004;200:659–669. [PubMed: 15353558]
- Boesteanu A, Silva AD, Nakajima H, Leonard WJ, Peschon JJ, Joyce S. Distinct roles for signals relayed through the common cytokine receptor- γ chain and IL-7 receptor- α chain in natural T-cell development. *J Exp Med* 1997;186:331–336. [PubMed: 9221763]

- Brugnera E, Bhandoola A, Cibotti R, Yu Q, Guinter TI, Yamashita Y, Sharrow SO, Singer A. Co-receptor reversal in the thymus: Signaled CD4⁺8⁺ thymocytes initially terminate CD8 transcription even when differentiating into CD8⁺ T-cells. *Immunity* 2000;13:59–71. [PubMed: 10933395]
- Candeias S, Muegge K, Durum SK. IL-7 receptor and VDJ recombination: Trophic versus mechanistic actions. *Immunity* 1997;6:501–508. [PubMed: 9175828]
- Cao X, Shores EW, Huli J, Anver MR, Kelsall BL, Russell SM, Drago J, Noguchi M, Grinberg A, Bloom ET, Paul WE, Kayz SI, Love PE, Leonard WJ. Defective lymphoid development in mice lacking expression of the common cytokine receptor- γ chain. *Immunity* 1995;2:223–238. [PubMed: 7697543]
- Chan TO, Tsichlis PN. PDK2: A complex tail in one Akt. *Sci STKE* 2001 2001:PE1.
- Chandele A, Joshi NS, Zhu J, Paul WE, Leonard WJ, Kaech SM. Formation of IL-7R α^{high} and IL-7R α^{low} CD8 T-cells during infection is regulated by the opposing functions of GABP α and Gfi-1. *J Immunol* 2008;180:5309–5319. [PubMed: 18390712]
- Chu YW, Memon SA, Sharrow SO, Hakim FT, Eckhaus M, Lucas PJ, Gress RE. Exogenous IL-7 increases recent thymic emigrants in peripheral lymphoid tissue without enhanced thymic function. *Blood* 2004;104:1110–1119. [PubMed: 15130942]
- Dakic A, Metcalf D, Di Rago L, Mifsud S, Wu L, Nutt SL. PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. *J Exp Med* 2005;201:1487–1502. [PubMed: 15867096]
- Davey GM, Starr R, Cornish AL, Burghardt JT, Alexander WS, Carbone FR, Surh CD, Heath WR. SOCS-1 regulates IL-15-driven homeostatic proliferation of antigen-naïve CD8 T-cells, limiting their autoimmune potential. *J Exp Med* 2005;202:1099–1108. [PubMed: 16216888]
- Disanto JP, Muller W, Guygrand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin-2 receptor- γ chain. *Proc Natl Acad Sci USA* 1995;92:377–381. [PubMed: 7831294]
- Durum SK, Candeias S, Nakajima H, Leonard WJ, Baird AM, Berg LJ, Muegge K. Interleukin-7 receptor control of T-cell receptor- γ gene rearrangement: Role of receptor-associated chains and locus accessibility. *J Exp Med* 1998;188:2233–2241. [PubMed: 9858510]
- ElKassar N, Lucas PJ, Klug DB, Zamisch M, Merchant M, Bare CV, Choudhury B, Sharrow SO, Richie E, Mackall CL, Gress RE. A dose effect of IL-7 on thymocyte development. *Blood* 2004;104:1419–1427. [PubMed: 15155461]
- Fluur C, De Milito A, Fry TJ, Vivar N, Eidsmo L, Atlas A, Federici C, Matarrese P, Logozzi M, Rajnavolgyi E, Mackall CL, Fais S, Chiodi F, Rethi B. Potential role for IL-7 in Fas-mediated T-cell apoptosis during HIV infection. *J Immunol* 2007;178:5340–5350. [PubMed: 17404319]
- Foxwell BMJ, Beadling C, Guschin D, Kerr I, Cantrell D. Interleukin-7 can induce the activation of Jak-1, Jak-3, and Stat-5 proteins in murine T-cells. *Eur J Immunol* 1995;25:3041–3046. [PubMed: 7489741]
- Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 2004;30:193–204. [PubMed: 15023437]
- Fry TJ, Mackall CL. Interleukin-7: Master regulator of peripheral T-cell homeostasis? *Trends Immunol* 2001;22:564–571. [PubMed: 11574281]
- Godfrey DI, Kennedy J, Suda T, Zlotnik A. A developmental pathway involving four phenotypically and functionally distinct subsets of CD3⁺CD4⁺CD8⁺ triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. *J Immunol* 1993;150:4244–4252. [PubMed: 8387091]
- Goldrath AW, Bevan MJ. Low-affinity ligands for the TCR drive proliferation of mature CD8⁺ T-cells in lymphopenic hosts. *Immunity* 1999;11:183–190. [PubMed: 10485653]
- Grabstein KH, Waldschmidt TJ, Finkelman FD, Hess BW, Alpert AR, Boiani NE, Namen AE, Morrissey PJ. Inhibition of murine B-lymphopoiesis and T-lymphopoiesis *in vivo* by an anti-interleukin 7 monoclonal antibody. *J Exp Med* 1993;178:257–264. [PubMed: 8315381]
- Hardy RR, Carmack CE, Shinton SA, Kemp JD, Hayakawa K. Resolution and characterization of pro-B and pre-pro-B-cell stages in normal mouse bone marrow. *J Exp Med* 1991;173:1213–1225. [PubMed: 1827140]
- Harty JT, Badovinac VP. Shaping and reshaping CD8⁺ T-cell memory. *Nat Rev Immunol* 2008;8:107–119. [PubMed: 18219309]
- Jiang Q, Li WQ, Aiello FB, Mazzucchelli R, Asefa B, Khaled AR, Durum SK. Cell biology of IL-7, a key lymphotrophin. *Cytokine Growth Factor Rev* 2005;16:513–533. [PubMed: 15996891]

- Juntilla MM, Koretzky GA. Critical roles of the PI3K/Akt signaling pathway in T-cell development. *Immunol Lett* 2008;116:104–110. [PubMed: 18243340]
- Khaled AR, Li WQ, Huang J, Fry TJ, Khaled AS, Mackall CL, Muegge K, Young HA, Durum SK. Bax deficiency partially corrects IL-7 receptor- α deficiency. *Immunity* 2002;17:561–573. [PubMed: 12433363]
- Kieper WC, Burghardt JT, Surh CD. A role for TCR affinity in regulating naive T-cell homeostasis. *J Immunol* 2004;172:40–44. [PubMed: 14688307]
- Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 1997;91:661–672. [PubMed: 9393859]
- Krogsgaard M, Juang J, Davis MM. A role for “self” in T-cell activation. *Semin Immunol* 2007;19:236–244. [PubMed: 17548210]
- Lee DS, Ahn C, Ernst B, Sprent J, Surh CD. Thymic selection by a single MHC/peptide ligand: Autoreactive T-cells are low-affinity cells. *Immunity* 1999;10:83–92. [PubMed: 10023773]
- Lin JX, Migone TS, Tsang M, Friedmann M, Weatherbee JA, Zhou L, Yamauchi A, Bloom ET, Mietz J, John S, Leonard WJ. The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity* 1995;2:331–339. [PubMed: 7719938]
- Link A, Vogt TK, Favre S, Britschgi MR, Acha-Orbea H, Hinz B, Cyster JG, Luther SA. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T-cells. *Nat Immunol* 2007;8:1255–1265. [PubMed: 17893676]
- Maki K, Sunaga S, Komagata Y, Kodaira Y, Mabuchi A, Karasuyama H, Yokomuro K, Miyazaki J, Ikuta K. Interleukin 7 receptor-deficient mice lack $\gamma\delta$ T-cells. *Proc Natl Acad Sci USA* 1996;93:7172–7177. [PubMed: 8692964]
- Malissen M, Pereira P, Gerber DJ, Malissen B, DiSanto JP. The common cytokine receptor- γ chain controls survival of $\gamma\delta$ T-cells. *J Exp Med* 1997;186:1277–1285. [PubMed: 9334367]
- Maraskovsky E, O'Reilly LA, Teepe M, Corcoran LM, Peschon JJ, Strasser A. Bcl-2 can rescue T-lymphocyte development in IL-7 receptor-deficient mice but not in mutant rag-1^{-/-} mice. *Cell* 1997;89:1011–1019. [PubMed: 9215624]
- Mazzucchelli R, Durum SK. Interleukin-7 receptor expression: Intelligent design. *Nat Rev Immunol* 2007;7:144–154. [PubMed: 17259970]
- McKenzie G, Ward G, Stallwood Y, Briend E, Papadia S, Lennard A, Turner M, Champion B, Hardingham GE. Cellular Notch responsiveness is defined by phosphoinositide 3-kinase-dependent signals. *BMC Cell Biol* 2006;7:10. [PubMed: 16507111]
- Nosaka T, Vandeursen JMA, Tripp RA, Thierfelder WE, Witthuhn BA, McMickle AP, Doherty PC, Grosveld GC, Ihle JN. Defective lymphoid development in mice lacking Jak3. *Science* 1995;270:800–802. [PubMed: 7481769]
- Ohbo K, Suda T, Hashiyama M, Mantani A, Ikebe M, Miyakawa K, Moriyama M, Nakamura M, Katsuki M, Takahashi K, Yamamura K, Sugamura K. Modulation of hematopoiesis in mice with a truncated mutant of the IL-2 receptor- γ chain. *Blood* 1996;87:956–967. [PubMed: 8562967]
- Osborne LC, Dhanji S, Snow JW, Priatel JJ, Ma MC, Miners MJ, Teh HS, Goldsmith MA, Abraham N. Impaired CD8 T-cell memory and CD4 T-cell primary responses in IL-7R α mutant mice. *J Exp Med* 2007;204:619–631. [PubMed: 17325202]
- Overwijk WW, Schluns KS. Functions of γ C cytokines in immune homeostasis: Current and potential clinical applications. *Clin Immunol* 2009;132:153–165. [PubMed: 19428306]
- Park JH, Yu Q, Erman B, Appelbaum JS, Montoya-Durango D, Grimes HL, Singer A. Suppression of IL7R α transcription by IL-7 and other pro-survival cytokines: A novel mechanism for maximizing IL-7-dependent T-cell survival. *Immunity* 2004;21:289–302. [PubMed: 15308108]
- Park SY, Saijo K, Takahashi T, Osawa M, Arase H, Hirayama N, Miyake K, Nakauchi H, Shirasawa T, Saito T. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity* 1995;3:771–782. [PubMed: 8777722]
- Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, Ziegler SF, Williams DE, Ware CB, Meyer JD, Davison BL. Early lymphocyte expansion is severely impaired in IL-7 receptor-deficient mice. *J Exp Med* 1994;180:1955–1960. [PubMed: 7964471]

- Rocha B, Dautigny N, Pereira P. Peripheral T-lymphocytes: Expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios *in vivo*. *Eur J Immunol* 1989;19:905–911. [PubMed: 2500349]
- Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, King KL, Sheehan KCF, Yin L, Pennica D, Johnson EM Jr, Schreiber RD. Disruption of the Jak1 gene demonstrates obligatory and non-redundant roles of the Jaks in cytokine-induced biologic responses. *Cell* 1998;93:373–383. [PubMed: 9590172]
- Rosenberg SA, Sportes C, Ahmadzadeh M, Fry TJ, Ngo LT, Schwarz SL, Stetler-Stevenson M, Morton KE, Mavroukakis SA, Morre M, Buffet R, Mackall CL, Gress RE. IL-7 administration to humans leads to expansion of CD8⁺ and CD4⁺ cells, but a relative decrease of CD4⁺ T-regulatory cells. *J Immunother* 2006;29:313–319. [PubMed: 16699374]
- Rothenberg EV, Chen D, Diamond RA. Functional and phenotypic analysis of thymocytes in SCID mice. Evidence for functional response transitions before and after the SCID arrest point. *J Immunol* 1993;151:3530–3546. [PubMed: 8376791]
- Schlissel MS, Durum SD, Muegge K. The interleukin 7 receptor is required for T-cell receptor- γ locus accessibility to the VDJ recombinase. *J Exp Med* 2000;191:1045–1050. [PubMed: 10727465]
- Shiroki F, Matsuda S, Doi T, Fujiwara M, Mochizuki Y, Kadowaki T, Suzuki H, Koyasu S. The p85 α regulatory subunit of class IA phosphoinositide 3-kinase regulates beta-selection in thymocyte development. *J Immunol* 2007;178:1349–1356. [PubMed: 17237381]
- Sportes C, Hakim FT, Memon SA, Zhang H, Chua KS, Brown MR, Fleisher TA, Krumlauf MC, Babb RR, Chow CK, Fry TJ, Engels J, Buffet R, Morre M, Amato RJ, Venzon DJ, Korngold R, Pecora A, Gress RE, Mackall CL. Administration of rhIL-7 in humans increases *in vivo* TCR repertoire diversity by preferential expansion of naive T-cell subsets. *J Exp Med* 2008;205:1701–1714. [PubMed: 18573906]
- Sprent J, Surh CD. T-cell memory. *Annu Rev Immunol* 2002;20:551–579. [PubMed: 11861612]
- Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T-cells. *Annu Rev Immunol* 2003;21:139–176. [PubMed: 12414722]
- Surh CD, Sprent J. Homeostasis of naive and memory T-cells. *Immunity* 2008;29:848–862. [PubMed: 19100699]
- Suzuki K, Nakajima H, Watanabe N, Kagami S, Suto A, Saito Y, Saito T, Iwamoto I. Role of common cytokine receptor- γ chain- and Jak3-dependent signaling in the proliferation and survival of murine mast cells. *Blood* 2000;96:2172–2180. [PubMed: 10979963]
- Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, Surh CD. IL-7 is critical for homeostatic proliferation and survival of naive T-cells. *Proc Natl Acad Sci USA* 2001;98:8732–8737. [PubMed: 11447288]
- von Freeden-Jeffrey U, Vieira P, Lucian LA, McNeil T, Burdach SEG, Murray R. Lymphopenia in IL-7 gene-deleted mice identifies IL-7 as a non-redundant cytokine. *J Exp Med* 1995;181:1519–1526. [PubMed: 7699333]
- Yu Q, Park JH, Doan LL, Erman B, Feigenbaum L, Singer A. Cytokine signal transduction is suppressed in pre-selection double-positive thymocytes and restored by positive selection. *J Exp Med* 2006;203:165–175. [PubMed: 16390939]
- Ziegler SF, Liu YJ. Thymic stromal lymphopoietin in normal and pathogenic T-cell development and function. *Nat Immunol* 2006;7:709–714. [PubMed: 16785889]

Figure 1. IL-7 Signaling

(A) IL-7 signaling is through the Jak-Stat pathway. IL-7 binds to its receptor composed of two chains: IL-7R α and the γ_c chain. This induces the transphosphorylation of Jak1 and Jak3 followed by the phosphorylation of the tyrosine Y449 on the IL-7R α chain. Y449 becomes the docking site for Stat5. Stat5 becomes phosphorylated, dimerizes, and is translocated to the nucleus where it regulates gene expression implicated in differentiation. Socs-1 negatively regulates the responsiveness of T-cells to IL-7 by neutralizing the activity of Jaks.

(B) Activation by IL-7 induces the phosphorylation on tyrosine residues of IL7R α . PI3K is recruited to the membrane and binds to these tyrosine residues. PI3K becomes phosphorylated resulting in the phosphorylation of the phosphatidylinositol PIP2 into PIP3.

PIP3 recruits signaling proteins with plekstrin domain (PH) to the membrane, such as PDK-1 and Akt. PIP3 is turned again into PIP2 by the phosphatase PTEN (phosphatase and tensin homolog on chromosome ten). Akt becomes active by phosphorylation and activates many downstream genes important for cell cycle and inhibition of apoptosis. After activation by Stat5 and Akt pathways through IL-7, and together with signaling through TCR, the synthesis of various proteins including Bcl-2 and Mcl-1 is initiated. These two proteins prevent mitochondrial death by blocking Bim and Bid from activating Bax and Bad.

Figure 2. Effect of IL-7 over-expression on T-cell development

Absolute counts from 6–12 week-old mice are shown. The * symbol corresponds to statistical significance. **(A)** Absolute thymocyte and DP counts are increased in the IL-7 transgenic mice with the lowest transgene expression and decreased in mice with the highest transgene expression. **(B)** Significant increase of TCR $\gamma\delta$, NK1.1, and B220 cells in IL-7 transgenic (Tg) mice at both high and low IL-7 over-expression.

Figure 3. rIL-7 therapy increases the absolute numbers of T-cell receptor excision circles (TRECs) in the peripheral blood

TREC numbers were measured in a total thirteen individual patients (◆) at 0, Day 7, Day 14, and Day 21 in CD4⁺ (left) and CD8⁺ cells and normalized per 10⁵ cells. Mean TREC numbers were calculated for cohorts that were treated with different doses of IL-7: ● 3 µg/kg; △ 10 µg/kg; □ 30 µg/kg; or, ○ 60 µg/kg. The decrease of TREC numbers at Day 7 is consistent with the decrease of naïve T-cells observed in these patients. TREC numbers were increased at Day 14 and Day 21.

Figure 4. Preferential increase of naïve and central memory CD4⁺ cells with rIL-7

A model of the relative representations of different CD4 subsets in the total T-cell pool from the three patients are shown before and after treatment with at 30 µg/kg of rIL-7. These model representations are intended primarily to convey concepts and as such are approximations of experimental data. The changes observed were independent of age. The blue color corresponds to naïve CD4 cells, the red to central memory, the yellow to effector memory, and the green to effector RA cells.