

Interleukin-2 at the Crossroads of Effector Responses, Tolerance, and Immunotherapy

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Interleukin-2 (IL-2) is a pleiotropic cytokine produced after antigen activation that plays pivotal roles in the immune response. Discovered as a T cell growth factor, IL-2 additionally promotes CD8⁺ T cell and natural killer cell cytolytic activity and modulates T cell differentiation programs in response to antigen, promoting naïve CD4⁺ T cell differentiation into T helper 1 (Th1) and T helper 2 (Th2) cells while inhibiting T helper 17 (Th17) and T follicular helper (Tfh) cell differentiation. Moreover, IL-2 is essential for the development and maintenance of T regulatory cells and for activation-induced cell death, thereby mediating tolerance and limiting inappropriate immune reactions. In this review, we focus on the molecular mechanisms and complex cellular actions of IL-2, its cooperative and opposing effects with other cytokines, and how both promoting and blocking the actions of IL-2 are being utilized in clinical medicine.

Introduction

Interleukin-2 (IL-2) was first discovered over 35 years ago as an activity present in supernatants of activated human T cells that mediates T cell growth and proliferation (Morgan et al., 1976; previously reviewed in Boyman and Sprent, 2012; Kim et al., 2006; Lin and Leonard, 2000; Malek and Castro, 2010). This four α -helix bundle type 1 cytokine (Bazan, 1990) was the first type 1 cytokine cloned (Taniguchi et al., 1983) and the first type 1 cytokine for which a receptor component was cloned (Leonard et al., 1984; Nikaido et al., 1984) and has served as a paradigm for other cytokines, particularly because it is one of two cytokines to share the IL-2 receptor β chain (IL-2R β) and one of six cytokines to share the common cytokine receptor γ chain, γ_c (Figure 1), with both of IL-2R β and γ_c having been discovered as components of the IL-2 receptor (Leonard, 2001).

Besides its potent T cell growth factor activity, IL-2 induces proliferation of natural killer (NK) cells and augments their cytolytic activity as well as that of lymphokine-activated killer cells (Siegel et al., 1987), promotes antibody production and proliferation by B cells (Mingari et al., 1984), and is essential for activation-induced cell death (AICD), which is important for homeostasis and the elimination of potentially harmful autoreactive cells, at least in part by a Fas and FasL-dependent mechanism (Leonard et al., 1999). IL-2 also drives the development of CD4⁺FOXP3⁺ regulatory T cells (Treg cells) (Malek and Castro, 2010), which have suppressor function and mediate tolerance (Littman and Rudensky, 2010; Sakaguchi et al., 2008; Shevach, 2009). More recently, the range of recognized actions of IL-2 has expanded, with roles in promoting the differentiation of T helper 1 (Th1) (Liao et al., 2011; Shi et al., 2008) and Th2 cells (Cote-Sierra et al., 2004; Liao et al., 2008), while inhibiting Th17 (Laurence et al., 2007) and T follicular helper (Tfh) cell (Ballesteros-Tato et al., 2012) development, but nevertheless promoting Th17 cell expansion once cells develop (Amadi-Obi et al., 2007). IL-2 also is critical for production of IL-9 (Schmitt et al., 1994). Thus, IL-2 has broad essential biological actions, not only driving T cell proliferation and modulating effector cell

differentiation, but also limiting potentially dangerous autoimmune reactions. Herein, we discuss the molecular and cellular biology of IL-2, its signaling mechanism, and its actions, as well as its relationship with the five other cytokines (IL-4, IL-7, IL-9, IL-15, and IL-21) that share components of the IL-2 receptor. Finally, we discuss the use of IL-2 as a therapeutic agent and the utility of blocking the action of IL-2 and related cytokines using Janus kinase (JAK) inhibitors, an exciting new class of immunosuppressive drugs.

IL-2

IL-2 is a 15.5 kDa type 1 four α -helical bundle cytokine produced primarily by CD4⁺ T cells following antigen stimulation (Leonard, 2001) but also produced to a lesser extent by CD8⁺ cells (Pallard et al., 1988), NK T cells (Yui et al., 2004), activated dendritic cells (DCs) (Granucci et al., 2001), and mast cells (Hershko et al., 2011). In T cells, induction of IL-2 transcription requires two signals, mediated by calcium and protein kinase C. IL-2 transcription is mediated by multiple transcription factors (Figure 2A), including nuclear factor of activated T cells (NFAT) family proteins (Müller and Rao, 2010), activator protein-1 (AP-1, FOS-JUN family dimers), nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B), and the octamer transcription factor, OCT-1 (Kim et al., 2006). Of five NFAT proteins (NFATC1, NFATC2, NFATC3, NFATC4, and NFAT5), all but NFAT5 are expressed in the cytosol, with NFATC1 and NFATC2 highly expressed in lymphocytes (Müller and Rao, 2010), and mice lacking both of these proteins express essentially no T cell receptor (TCR)-induced IL-2 (Peng et al., 2001). NFAT translocation to the nucleus is blocked by cyclosporine A and FK-506 (Flanagan et al., 1991). TCR engagement activates AP-1, which with NFAT, binds to composite sites in the IL-2 promoter (Figure 2A) (Müller and Rao, 2010). In anergic cells, the distal AP-1 site binds the transcription factors CREB and/or CREM instead of AP-1; CREB activates expression of the transcription factors cJUN, cFOS, FRA2, and FOSB, and a dominant-negative CREB greatly decreases TCR-induced IL-2 expression (Kim et al., 2006).



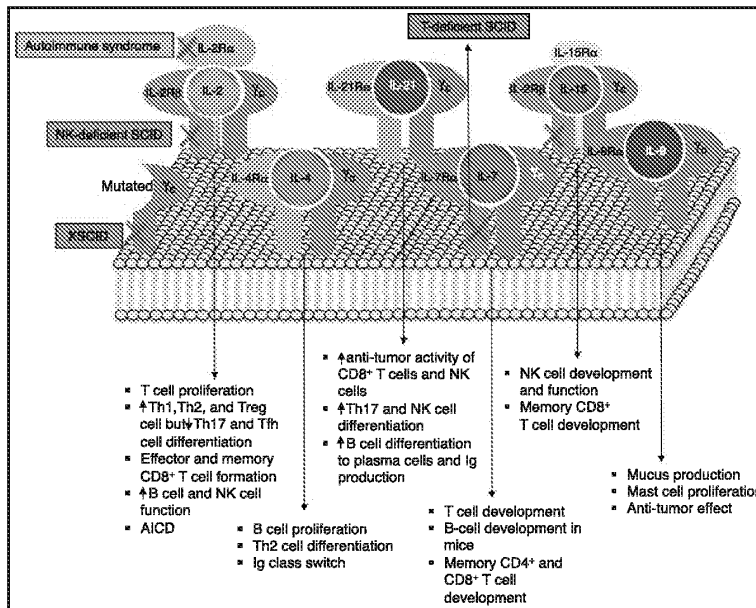


Figure 1. The γ_c Family of Cytokines
Shown are the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, as well as major actions for these cytokines. Crosses in red indicate that mutation of *IL2RG* gene, which encodes γ_c , results in X-linked severe combined immunodeficiency in humans (XSCID, where both T cells and NK cells are greatly diminished [T⁻B⁺NK⁻ SCID]), mutation of *IL2RA* results in an autoimmune syndrome, defective expression of *IL2RB* results in NK-deficient SCID (where T and B cells remain [T⁺B⁺NK⁻ SCID]), and mutation of *IL7R* causes T cell selective form of SCID, where B and NK cell numbers are normal (T⁺B⁺NK⁺ SCID). JAK3 is not shown as it interacts with the cytoplasmic domain of γ_c ; however, mutations in JAK3, as noted in the text, cause a T⁺B⁺NK⁻ for SCID, like XSCID.

not Th1 or Th0 cells, suppressing *IL2* expression in Th17 cells (Quintana et al., 2012). Interestingly, microRNAs (miRNAs) also play a role in regulation of IL-2 expression. For example, miR146a, which is induced by TCR stimulation in primary T cells, can impair AP-1 production and IL-2 expression (Curtale et al., 2010), whereas miR9 induced by TCR stimulation enhances IL-2 expression, at

least in part, by suppressing *PRDM1* expression (Thiele et al., 2012), and miR184 represses expression of IL-2 in umbilical cord CD4⁺ T cells (Weitzel et al., 2009). Thus, IL-2 production is both positively and negatively regulated.

Three Classes of IL-2 Receptors

IL-2 signals via specific receptors (Robb et al., 1981), with three classes of cell surface receptors formed by various combinations of three IL-2R subunits (Figure 3), IL-2R α , IL-2R β , and IL-2R γ (Kim et al., 2006; Lin and Leonard, 2000; Malek and Castro, 2010). IL-2R α (CD25) was originally called Tac antigen based on the demonstration that anti-Tac monoclonal antibody (mAb) (Uchiyama et al., 1981) blocked the binding of IL-2 (Leonard et al., 1982). IL-2R β (CD122) (Sharon et al., 1986; Teshigawara et al., 1987; Tsudo et al., 1986) is also part of the IL-15R complex (Giri et al., 1994), and IL-2R γ (CD132) (Takeshita et al., 1992) was renamed as the common cytokine receptor γ chain, γ_c (Leonard et al., 1995; Noguchi et al., 1993a; Russell et al., 1993); γ_c is now known to be shared by IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (Leonard, 2001; Rochman et al., 2009).

IL-2R α is absent or minimally expressed on resting T and NK cells, but its transcription is potently induced on T cells stimulated via the TCR or IL-2 (Depper et al., 1984, 1985; Leonard et al., 1985) or on NK cells stimulated with IL-2 (Siegel et al., 1987); the expression level of the IL-2R β chain is low on T cells but is induced by certain stimuli, including IL-2 (Siegel et al., 1987); γ_c is also expressed on these cells but is less inducible than IL-2R α or IL-2R β (Cao et al., 1993). IL-2R α is the "low-affinity" IL-2 receptor ($K_d \sim 10^{-8}$ M); IL-2R β binds poorly ($K_d \sim 1 \mu$ M) by itself, but the combination of IL-2R β with γ_c forms the intermediate-affinity IL-2 receptor ($K_d \sim 10^{-9}$ M); and all three subunits together form the high-affinity IL-2 receptor ($K_d \sim 10^{-11}$ M) (Takeshita et al., 1992), the structure for which has been solved

FOXP2 also cooperates with NFAT to drive IL-2 expression, whereas FOXP3 can replace FOXP2 to inhibit IL-2 expression while inducing the Treg cell markers IL-2R α and CTLA4 (Wu et al., 2006). NF- κ B binds to two sites in the IL-2 promoter, including one in the CD28 response element, and expression of IL-2 is elevated in *Nfkb1*^{-/-} mice (Kim et al., 2006), supporting the model that p50 homodimers repress IL-2 expression, whereas complexes containing p65 or c-Rel activate its expression. Oct1 and Oct2 bind to octamer binding sites and cooperate with AP-1 for IL-2 gene induction by phorbol myristate acetate (PMA) + ionomycin; The transcription factors SP1, Egr1, and GABP also act as positive regulators (Kim et al., 2006). TRIM28, a component of heterochromatin, is phosphorylated after TCR stimulation and promotes IL-2 expression, with diminished IL-2 production in mice in which the *Trim28* gene was conditionally deleted as well as in *Trim28* small interfering RNA-treated human Jurkat T cells (Chikuma et al., 2012).

IL-2 transcription also is negatively regulated. *NIL2A* (TCF-8) is encoded by ZEB1, a zinc finger E box binding protein that binds ~105 bp 5' of the transcription start site and suppresses transcription (Williams et al., 1991). Prior to activation, the T-lineage factor SATB1 binds to the human IL-2 promoter and in a phosphorylation-dependent manner recruits histone deacetylase-1 (HDAC1) and silences *IL2* expression, whereas after HIV-1 infection, binding of HIV Tat as well as CBP and/or p300 to HDAC1 induces IL-2 (Pavan Kumar et al., 2006). BLIMP-1 and Aiolos (encoded by *Prdm1* and *Ikzf3*, respectively) also repress IL-2 expression. IL-2 induces BLIMP-1, which binds IL-2 and cFos promoter regions and inhibits their expression (Martins et al., 2008). In Th17 cells, Aiolos is induced in a signal transducer and activator of transcription-3 (STAT3)-dependent and aryl hydrocarbon receptor (Ahr)-dependent fashion and binds a CCTCCATGC motif in the *IL2* promoter in Th17 but

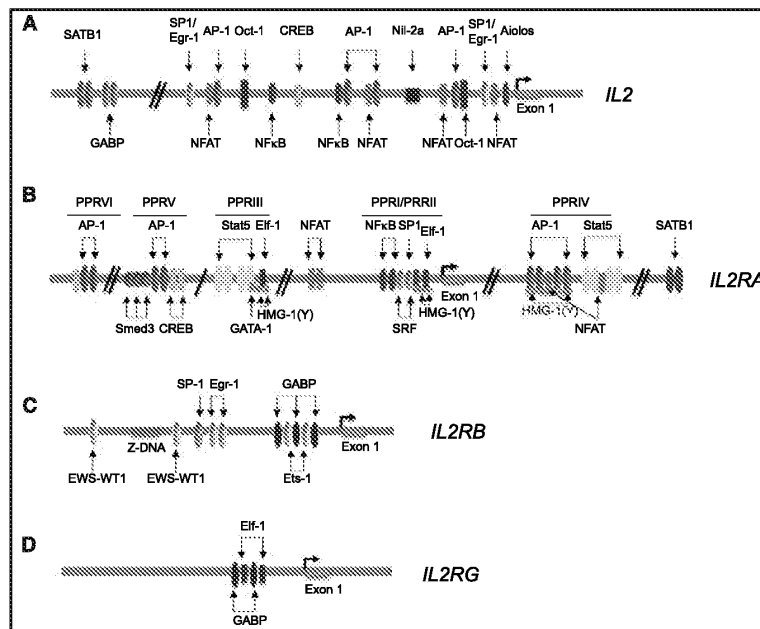


Figure 2. Factors Regulating the *IL2*, *IL2RA*, *IL2RB*, and *IL2RG* Genes

For each gene, the binding locations of transcription factors are shown. For some of these factors, there are only in vitro data that indicate their importance, whereas for others such as STAT5A and STAT5B, extensive in vivo data have established their importance (e.g., of STAT5A and STAT5B for regulation of IL-2R α expression [Nakajima et al., 1997; Imada et al., 1998]).

(A) Multiple factors, including for example NFAT, AP-1, and NF- κ B, bind to and regulate the *IL2* gene. (B) In the *IL2RA* gene, PPR1 binds SP1, SRF, and NF- κ B; PPRII binds Ets-1, as well as HMG-I and/or HMG-Y; PPRIII binds STAT5A, STAT5B, Ets-1, and GABP, as well as HMG-I and/or HMG-Y; PPRIV binds NFAT, AP-1, STAT5A, and STAT5B; PPRV binds SMAD3, AP-1, and CREB-ATF factors; and PPRVI binds AP-1 and CREB-ATF factors.

(C) Factors including Ets-1 bind to and regulate the *IL2RB* gene.

(D) Only limited information is available regarding the factors regulating *IL2RG*.

(Stauber et al., 2006; Wang et al., 2005). High affinity IL-2 binding results from IL-2's rapid on and off rates to IL-2R α coupled to slower on and off rates to intermediate affinity receptors (Lowenthal and Greene, 1987; Wang and Smith, 1987). When IL-2R α and IL-2R β are coexpressed without γ_c , "pseudo-high affinity" binding ($K_d \sim 10^{-10}$ M) (Arima et al., 1992) is achieved but no signaling occurs. The intermediate and high affinity receptors are functional, corresponding to the requirement for heterodimerization of IL-2R β and γ_c cytoplasmic domains for signaling (Nakamura et al., 1994; Nelson et al., 1994). Intermediate affinity receptors are present on resting T (Zhang et al., 1998) and NK cells (Siegel et al., 1987), whereas high-affinity receptors are expressed by activated lymphocytes (Robb et al., 1981). Binding of IL-2 to intermediate affinity receptors induces cell growth and cytolytic activity (Siegel et al., 1987) and IL-2R α transcription (Depper et al., 1985). After T cell activation, IL-2R α is rapidly induced and high-affinity receptors form, increasing responsiveness to IL-2. Although IL-2 primarily acts as a soluble factor via intermediate and high-affinity receptors, like IL-15, IL-2 can be presented in *trans*, where IL-2 bound to IL-2R α on one cell stimulates another cell that expresses IL-2R β and γ_c (Wuest et al., 2011) (Figure 3). However, unlike the relatively high-affinity binding of IL-15 to IL-15R α (Waldmann, 2006), IL-2 binds with relatively low affinity to IL-2R α with rapid on and off rates, as noted above. Accordingly, cis-based IL-2 signaling is likely favored whenever a cell expresses all three chains, and trans signaling presumably requires relatively high local concentrations of IL-2. In addition to cell surface IL-2R α , IL-2R α can exist in a soluble form (sIL-2R α) that can be released from the cell surface (Figure 3), including in infectious disorders, transplantation rejection, and autoimmune inflammatory states, with an elevated amount of sIL-2R α being detected in certain hematologic malignancies. In some diseases, the concentration of

sIL-2R α correlates with disease activity and prognosis (Rubin and Nelson, 1990).

Interestingly, IL-2R α is expressed on a fraction of CD4⁺CD8⁺ thymocytes (Godfrey et al., 1993), but such IL-2R α expression may reflect the activation state or ability to trans-present IL-2 rather than responsiveness to IL-2. IL-2R α is also expressed by DC populations (Driesen et al., 2008), including after CpG or CD40 ligand stimulation of human plasmacytoid DCs (Naranjo-Gómez et al., 2007). Both cell surface and soluble IL-2R α are expressed by primary BDCA-1⁺ myeloid DCs stimulated with tumor necrosis factor α (TNF- α) and prostaglandin E2, as well as on tumor-associated DCs. These cells might sequester IL-2 and diminish T cell proliferation, thus contributing to immunosuppression by DCs (von Bergwelt-Baildon et al., 2006), but IL-2R α -expressing DCs can also trans-present IL-2, with agonistic effects, consistent with inhibition of T cell activation by antigen-specific DCs and diminished brain inflammation in multiple sclerosis patients treated with daclizumab, a humanized antibody to IL-2R α (Wuest et al., 2011).

Regulation of IL-2 Receptor Expression

The three IL-2R chains are independently regulated. IL-2R α is transcriptionally induced by stimulation via the T cell receptor (Leonard et al., 1985), by cytokines including IL-1, IL-2, IL-7, IL-12, IL-15, TNF- α , and transforming growth factor- β (TGF- β), by transactivator proteins Tax of human T cell leukemia virus-1 (HTLV-1) and TaxII of HTLV-2, and by activators of protein kinase C (Kim et al., 2006). Of six positive regulatory regions (PPR1, PPRII, PPRIII, PPRIV, PPRV, and PPRVI) in the IL-2R α gene (Figure 2B) (Kim et al., 2006), all but PPRIII contribute to antigen and mitogenic stimulation. PPRIII, PPRIV, and additional regions of the IL-2R α gene based on chromatin immunoprecipitation sequencing (ChIP-Seq) data (Lin et al., 2012) are IL-2-response elements that contain interferon- γ (IFN- γ) activation sequence (GAS) motifs and bind STAT5 proteins. Correspondingly, IL-2-induced IL-2R α expression is diminished in *Stat5a*^{-/-} and *Stat5b*^{-/-} mice (Imada et al., 1998; Nakajima

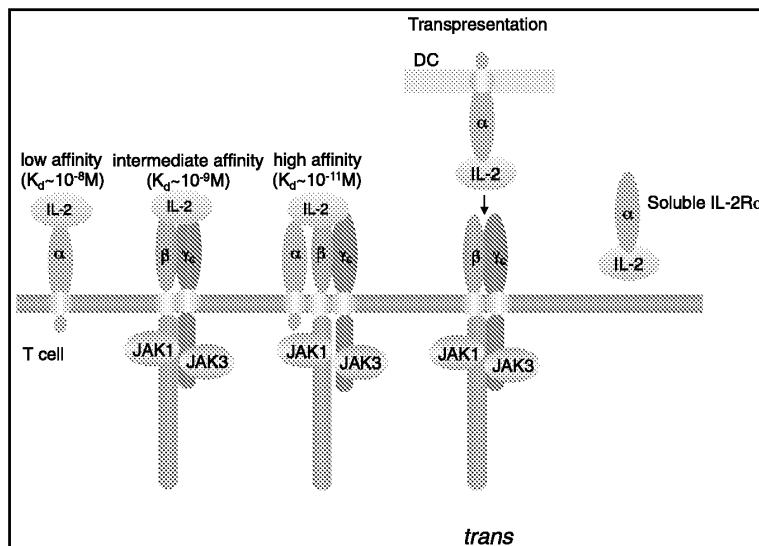


Figure 3. Classes of IL-2 Receptors
Shown are the three classes of IL-2 receptors (high-affinity, intermediate affinity, and low affinity), with receptor composition, K_d 's, receptor composition, and associated JAK kinases. Also shown is trans-presentation of IL-2 by a DC that expresses IL-2R α to a T cell that expresses IL-2R β and γ_c . On the right is the soluble IL-2 receptor (soluble IL-2R α) with bound IL-2. As discussed in the text, IL-2 is produced primarily by activated CD4 $^+$ T cells.

Signaling Via the IL-2 Receptor

After IL-2 engagement, the IL-2 receptor complex is rapidly internalized, with IL-2R α located in early transferrin $^+$ endosomes that recycle to the plasma membrane, whereas IL-2R β and γ_c are targeted to Rab7 $^+$ vesicles that are sorted toward degradation (H  mar et al., 1995). Following receptor binding, IL-2 activates multiple signaling pathways (Figure 4). Heterodimerization of the IL-2R β and γ_c

et al., 1997). Whereas IL-2-mediated induction of the *IL2RA* gene is dependent on STAT proteins, its induction by TCR, TNF- α , and Tax proteins of HTLV-1 or HTLV-2, requires NF- κ B binding (Kim et al., 2006) (Figure 2B). NFAT sites are also present, consistent with diminished IL-2R α expression in *Nfatc2* $^{-/-}$ mice (Schuh et al., 1998). TGF- β cooperates with TCR signaling to act via PRRV, which has a SMAD ("small mothers against decapentaplegic") binding site (Kim et al., 2005). Like the *Il2* gene, SATB1 (special AT-rich sequencing-binding protein-1) binds to and suppresses the *Il2ra* gene, with elevated *Il2ra* expression in *Satb1* $^{-/-}$ double-positive (DP) thymocytes (Alvarez et al., 2000). Two negative regulatory elements, NRE-1 and NRE-2, are also present in the gene (Kim et al., 2006).

IL-2R β is expressed by multiple lymphohematopoietic populations of cells, including NK cells, resting T cells, monocytes, and neutrophils; on T cells, TCR stimulation, PMA, IL-2, and IL-4 each augments IL-2R β expression via both transcriptional and posttranscriptional regulation (Kim et al., 2006). The IL-2R β promoter binds the Ets family proteins, Ets1 and GABP, as well as Egr1 and Sp1 (Lin et al., 1993; Lin and Leonard, 1997) (Figure 2C), with decreased IL-2R β expression in *Ets1* $^{-/-}$ NK cells (Ramirez et al., 2012). The Ewing sarcoma gene-Wilms tumor suppressor (EWS-WT1) fusion transcription factor also binds to and augments IL-2R β expression (Wong et al., 2002). Interestingly, there is a run of Z-DNA within this gene, but its physiological significance is unknown (Kim et al., 2006). IL-2R β and IL-2R α expression was reported on fibroblasts (Gruss et al., 1996), but the significance of such expression also is unclear.

Like IL-2R β , IL-2R γ is constitutively expressed and mainly restricted to lymphohematopoietic cells (Cao et al., 1993). IL-2 and IFN- γ are each shown to enhance and TGF- β 1 to inhibit IL-2R γ expression in human monocytes (Bosco et al., 1994), but little is still known about *IL2RG* regulation (Figure 2D).

cytoplasmic domains leads to activation of Janus family tyrosine kinases, JAK1 and JAK3, with JAK1 associating with IL-2R β and JAK3 with γ_c (Boussiotis et al., 1994; Miyazaki et al., 1994; Russell et al., 1994). The JAK kinases activate each other and phosphorylate key residues in IL-2R β . Phosphorylation of Y338 in humans (Y341 in mice) allows association of the SHC adaptor protein (Friedmann et al., 1996), which provides a platform for Ras-MAP kinase activation and promotes cell growth. Phosphorylation of Y392 and Y510 in humans (Y395 and Y498 in mice) mediates recruitment of STAT1, STAT3, STAT5A, and STAT5B, with most potent and sustained activation of STAT5 proteins (Friedmann et al., 1996; Lin et al., 2012). IL-2 also activates the phosphoinositide 3-kinase (PI 3-kinase)-Akt-p70 S6 kinase signaling pathway (Lin and Leonard, 2000; Malek and Castro, 2010), which promotes cell growth and survival (Franke et al., 1997). This pathway is inhibited by rapamycin, which blocks the function of a serine and threonine kinase, mTOR (mechanistic target of rapamycin), which is activated by PI-3K-AKT pathway (Thomson et al., 2009). STAT5A and STAT5B dock on IL-2R β , are phosphorylated, dimerize, and translocate to the nucleus, where they bind to target genes essential for effector cell function and T cell growth (Friedmann et al., 1996; Lin et al., 2012), as well as differentiation (Liao et al., 2008, 2011). Generally, IL-2 is viewed as an activator, inducing more genes than it represses (Lin et al., 2012). Interestingly, a miRNA, miR-182, that is induced by IL-2 is an inhibitor of the transcription factor FOXO1, which blocks cell-cycle progression in resting T cells, thereby helping to mediate the clonal expansion of activated helper T cells (Stittrich et al., 2010).

In addition to their tyrosine phosphorylation-mediated dimerization, like STAT1 and STAT4 (Chen et al., 2003), STAT5 proteins can interact via their N-terminal region (N-domains), allowing tetramer or higher order oligomer formation and binding to tandem GAS motifs (Lin et al., 2012). The importance of STAT5 tetramers has been analyzed by generating mice in which *Il28* and *Phe81* in the STAT5 N-domain have been mutated to

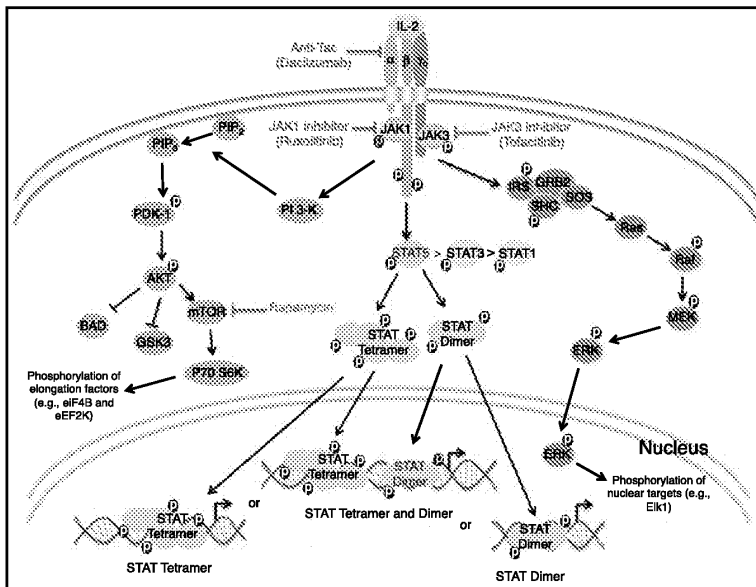


Figure 4. Schematic of Major IL-2 Signaling Pathways

Shown is the activation of PI3-K-AKT, JAK-STAT, and SHC-RAS-MAPK signaling pathways. Also shown are potential therapeutic points of control for IL-2 signaling, with anti-Tac (daclizumab), rapamycin, and JAK1 or JAK3 inhibitors being shown in red. The cartoon shows signaling by both STATs dimers and tetramers. The figure indicates that IL-2 activates more STAT5 than STAT3 and more STAT3 than STAT1. ERK refers to both ERK1 and ERK2. MEK refers to both MEK1 and MEK2.

marrow transplantation, affected individuals typically die in the first year of life (Cavazzana-Calvo et al., 2005; Leonard, 1996). Strikingly, *IL2RG* (encoding IL-2R γ) is mutated in humans with XSCID (Noguchi et al., 1993b). This finding was most unexpected, given normal T and NK cell development in humans with IL-2 deficiency or in *IL2^{-/-}* mice, leading to the hypothesis that IL-2R γ has roles beyond IL-2 (Noguchi et al., 1993b). Indeed, IL-2R γ was initially shown to be

alanines in the *Stat5a*, *Stat5b*, or both the *Stat5a* and *Stat5b* genes (Lin et al., 2012), allowing formation of STAT5 dimers but not tetramers. ChIP-Seq and gene expression analyses of cells from wild-type and mutant mice has allowed the identification of genes regulated by STAT5 dimers versus tetramers and the definition of dimer versus tetramer consensus motifs (Lin et al., 2012). Defective expression of certain genes, such as *Il2ra*, in the tetramerization-defective mice, can readily be attributed to known STAT5 tetramer binding sites, and these mice have decreased NK cell and CD8⁺ T cells and defective gene expression in response to IL-2 or IL-15. Moreover, these animals exhibit diminished virus-specific CD8⁺ T cell expansion, particularly early after infection with lymphocytic choriomeningitis virus (LCMV) or adenovirus 5, indicating that STAT5 tetramers are more important for early responses than for the later memory development and maintenance (Lin et al., 2012). An adoptive-transfer colitis model reveals that normal Treg cell-mediated suppression is also dependent on STAT5 tetramers (Lin et al., 2012), consistent with their requirement for IL-2 signaling (Malek and Castro, 2010; Malek et al., 2002). Thus, IL-2 function requires STAT5 tetramers as well as dimers.

IL2RG, X-Linked Severe Combined Immunodeficiency, and a Major Conundrum

Studies of the IL-2 receptor γ chain have revealed that the gene encoding this receptor protein, *IL2RG*, is located at the disease locus for X-linked severe combined immunodeficiency (XSCID) at Xq13 (Noguchi et al., 1993b). XSCID is a profound inherited immunodeficiency affecting approximately 1 in every 200,000 live births in the United States, representing the most common inherited immunodeficiency. XSCID is characterized by essentially absent T and NK cells; B cell numbers are relatively normal but are nonfunctional. Without successful bone

a critical component for the IL-4 and IL-7 receptors and subsequently for IL-9, IL-15, and IL-21 as well, hence its being renamed as the common cytokine receptor γ chain, γ_c (Leonard, 2001; Noguchi et al., 1993a) (Figure 1). The knowledge that JAK3 physically associates with γ_c led to speculation and confirmation that mutations in *JAK3* caused a similar clinical and immunological disease to XSCID (Macchi et al., 1995; Russell et al., 1995). The identification of *IL7R*-deficient SCID patients with a selective T cell defect (Puel et al., 1998) indicates that the T cell defect in XSCID results from defective IL-7 signaling; the ability of IL-15 to drive NK development (Waldmann, 2006) indicates that defective IL-15 signaling explains the lack of NK cells (Leonard, 1996), and analysis of mice in which both the *Il21r* and *Il4* genes were deleted revealed that defective signaling by the combination of IL-4 and IL-21 explained the defective B cell function in XSCID (Ozaki et al., 2002). Thus, although studies of IL-2 led to the discovery of the cause of XSCID, defective IL-2 signaling is not responsible for the major defects in that disease.

Consistent with the conclusion that defective IL-15 signaling is responsible for the lack of NK cells in XSCID and JAK3 deficiency, defective expression of IL-2R β , which is shared by the receptors for IL-2 and IL-15, results in an NK-deficient form of SCID (Gilmour et al., 2001) (see Figure 1). Interestingly, mutation of *IL2RA* results not in SCID but an autoimmune syndrome (Sharfe et al., 1997) (see Figure 1), consistent with a selective defect in IL-2 signaling, which is required for normal Treg cell development (Malek and Castro, 2010). As expected, like humans with *IL2RB* deficiency, *Il2rb^{-/-}* mice also lack NK cells, and like humans with *IL2RA* deficiency, *Il2ra^{-/-}* mice exhibit an autoimmune syndrome. Interestingly, *Il2rg^{-/-}* mice have a major defect not only in T and NK cells but also in B cells, given the requirement for IL-7 for B cell development in mice but not humans (Leonard, 2001). These animals do not exhibit

autoimmunity given the profound developmental defects in T, B, and NK cells (Leonard, 2001).

IL-2 and IL-15 Are Two Closely Related but Biologically Distinct Cytokines

As discussed above, IL-2 and IL-15 are highly related to each other and both cytokines bind to receptors that contain IL-2R β and γ_c to transduce their signals, although they have distinct α chains (Waldmann, 2006). Their three-dimensional quaternary structures reveal that IL-15 binds to the IL-2R β and γ_c heterodimer in nearly identical fashion to that of IL-2, with similar gene induction profiles (Ring et al., 2012), but yet they are very distinctive biologically (Waldmann, 2006) (Figure 1), based in part on distinctive patterns of expression for IL-2 and IL-15, as well as IL-2R α versus IL-15R α . Despite the fact that both IL-2 and IL-15 share IL-2R β and γ_c as receptor components and activate common signaling pathways, they exhibit signaling differences (Castro et al., 2011; Cornish et al., 2006). For example, in freshly isolated CD8 $^+$ splenic T cells, IL-15 is more potent than IL-2 in driving cell-cycle progression and more effectively cooperates with IL-21 than does IL-2 (Zeng et al., 2005), whereas in antigen-activated CD8 $^+$ T cells, IL-2 more potently increases protein synthesis and cell proliferation than does IL-15 (Cornish et al., 2006). This effect of IL-2 correlates with rapid expansion of short-lived effector cells, whereas IL-15 favors the maintenance of long-lived, memory phenotype cells (Waldmann, 2006). As noted above, both cytokines can use trans-presentation although this mechanism is more utilized by IL-15.

Because of the sharing of IL-2R β , a monoclonal antibody (Mik β 1) to this protein can inhibit not only IL-2 signaling but also IL-15 signaling. Indeed, it was reported that abnormal IL-15 responses might contribute to the survival and expansion of certain leukemias and/or lymphomas, and moreover, Mik β 1 blocked trans-presentation of IL-15 and now is being used in a phase I clinical trial for T cell large granular lymphocytic leukemia (T-LGL) (Waldmann et al., 2012).

IL-2 and Treg Cells

Treg cells play vital roles in preventing autoimmunity, limiting inflammatory responses, and maintaining immune homeostasis (Littman and Rudensky, 2010; Sakaguchi et al., 2008; Shevach, 2009). A hallmark of Treg cells is expression of the forkhead transcription factor FOXP3, which is necessary but not sufficient for Treg development (Figure 5). TCR-induced CpG hypomethylation of conserved noncoding DNA sequences (CNSs) critically regulates FOXP3 expression and is important for establishing Treg cell identity (Kim and Leonard, 2007; Ohkura et al., 2012; Zheng et al., 2010). Most FOXP3 $^+$ Treg cells are CD4 $^+$ CD25 $^+$ cells (Sakaguchi et al., 2008; Shevach, 2009). In addition to thymic-derived natural Treg cells, naive peripheral CD4 $^+$ T cells can become CD4 $^+$ FOXP3 $^+$ -inducible Treg cells when stimulated with anti-CD3, TGF- β , and IL-2, with a critical role for IL-2 in their generation and expansion (Davidson et al., 2007; Zheng et al., 2007). The suppressive actions of Treg cells in part can be attributed to their ability to efficiently compete with effector CD4 $^+$ T cells for the available IL-2 (Busse et al., 2010; Pandiyan et al., 2007). Interestingly, this can result in apoptosis of the effector cells, in part through an increased expression of proapoptotic protein BIM and a decreased phosphorylation of the

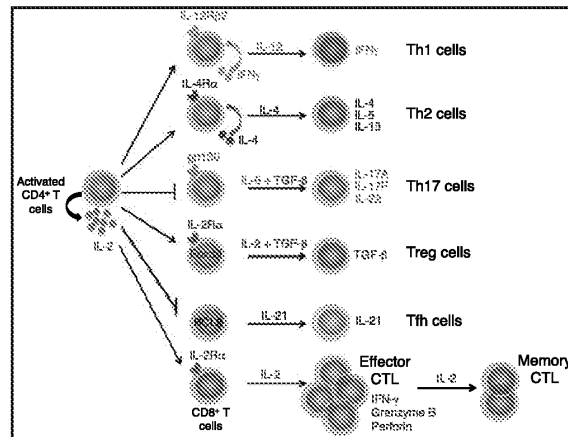


Figure 5. IL-2 is important in many types of T effector cell differentiation

Shown is the induction by IL-2 of IL-12R β 2 to promote Th1 cell differentiation, of IL-4R α to promote Th2 cell differentiation, and of IL-2R α to promote Treg cell differentiation. Conversely, IL-2 represses expression of gp130 (and IL-6R α) while inducing T-bet (data not shown) to repress Th17 cell differentiation. IL-2 is also a repressor of Tfh differentiation based on its repression of BCL6 expression. Finally, IL-2 promotes the differentiation, expansion, and the cytolytic activity of cytotoxic T cells.

proapoptotic protein BAD due to lower AKT activity (Pandiyan et al., 2007).

An essential role of IL-2 in controlling immune homeostasis was first suggested by the spontaneous lethal autoimmunity found in mice lacking expression of IL-2, IL-2R α , or IL-2R β (Leonard, 2001). Adoptive transfer of wild-type CD4 $^+$ CD25 $^+$ T cells into *Il2rb* $^{-/-}$ mice prevents autoimmunity, demonstrating a critical role for IL-2 for Treg cell development and function in vivo (Antony et al., 2006; Malek and Castro, 2010; Malek et al., 2002). Consistent with this, in the nonobese diabetic (NOD) mouse model of type 1 diabetes, intraislet Treg cell dysfunction due to defective IL-2 production results in the breakdown of self-tolerance, but administering low doses of IL-2 and IL-2 mAbs (Boyman et al., 2006), which form stable immune complexes, increased Treg cell survival and decreased the incidence of diabetes (Tang et al., 2008), without activating effector CD8 $^+$ T cells. Similarly, pretreatment of mice with IL-2-anti-IL-2 complexes boosts Treg cell numbers and ameliorates experimental allergic encephalitis, myasthenia gravis, and graft rejection (Boyman and Sprent, 2012). Beneficial effects of IL-2 have also been observed in approximately half of patients with glucocorticoid refractory chronic graft-versus-host disease, with expansion of Treg but not conventional T cells (Koreth et al., 2011). Similarly, low-dose IL-2 increased Treg cells in patients with autoimmune vasculitis secondary to hepatitis C virus infection (Saadoun et al., 2011).

IL-2 and T Helper Cell Differentiation

CD4 $^+$ T cells can differentiate into multiple helper T cell populations upon antigen exposure, including Th1, Th2, Th9, Th17, and Tfh cells (Crotty, 2011; Jabeen and Kaplan, 2012; Littman and Rudensky, 2010; Paul and Zhu, 2010; Szabo et al., 2003) (Figure 5). IL-12 drives the differentiation of Th1 cells, which

produce IFN- γ ; IL-4 drives differentiation of Th2 cells, which produce IL-4, IL-5, and IL-13; the combination of IL-4 and TGF- β drives differentiation of Th9 cells which produce IL-9; IL-6 + TGF- β drives differentiation of Th17 cells, which produce IL-17A, IL-17F, and IL-22; and IL-6 + IL-21 promotes the differentiation of Tfh cells. These differentiation pathways are not necessarily terminal, however, and subsets of Th cells can acquire the ability to produce other Th cell-specific cytokine(s) (O'Shea and Paul, 2010).

Th1 Cell Differentiation

Th1 cells mediate host defense to viruses and intracellular pathogens and contribute to the development of pathogenic inflammatory diseases based on their production of IFN- γ . IL-12 via STAT4 is essential for driving Th1 cell differentiation and inducing T-bet, promoting the survival and proliferation of Th1 cells, a transcriptionally permissive chromatin structure at the *Ifng* locus, and enhanced IL-12R β 2 expression (Afkarian et al., 2002; Szabo et al., 2003; Zhang and Boothby, 2006). IL-2 induces IFN- γ , both by inducing T cell proliferation (Bird et al., 1998) and via a STAT5-dependent mechanism (Shi et al., 2008). Moreover, IL-2 via STAT5 also rapidly induces IL-12R β 2 (Liao et al., 2011), augmenting responsiveness to IL-12. Retroviral expression of IL-12R β 2 could restore diminished Th1 cell differentiation in *Il2*^{-/-} T cells; thus, IL-2-induced IL-12R β 2 is critical for Th1 cell differentiation. IL-2 also induces *Tbx21*, which encodes T-bet, but T-bet alone could not restore Th1 cell differentiation (Liao et al., 2011). An essential role for IL-2 in Th1 cell differentiation in vivo has also been reported using *Il27*^{-/-} mice. These mice have elevated production of IL-2, and when infected with *T. gondii*, they developed a lethal inflammatory disease associated with increased IFN- γ that is diminished by IL-2 blockade (Villarin et al., 2006).

Th2 Cell Differentiation

Th2 cells control humoral immunity to extracellular parasites and mediate allergic inflammatory responses (Paul and Zhu, 2010). In vitro Th2 differentiation requires the TCR-mediated activation of naive CD4⁺ T cells in the presence of anti-IFN- γ , IL-2, and IL-4, with IL-2 promoting chromatin accessibility at the *Il4* locus (Cote-Sierra et al., 2004). TCR stimulation induces IL-4R α expression by an IL-2 and STAT5-dependent mechanism, promoting cellular responsiveness to IL-4, and transduction of *Il4ra* into *Il2*-deficient T cells can rescue Th2 cell differentiation in the absence of IL-2 (Liao et al., 2008). IL-2-induced STAT5 binds throughout the *Il4-Il13-Il5* Th2 cell-associated cytokine locus cluster, at hypersensitive site 2 (HS2), HS3, and HS5, as well as within the locus control region B and C elements in the *Rad50* gene (Liao et al., 2008). Thus, IL-2 via STAT5A and STAT5B directly regulates IL-4R α and IL-4 expression to promote Th2 cell differentiation. Interestingly, other cytokines that activate STAT5, including IL-7 and IL-15, also induce IL-4R α on T cells, suggesting that depending on the cellular context, other cytokines can also prime CD4⁺ T cells for Th2 cell differentiation (Liao et al., 2008).

Th17 Cell Differentiation

Th17 cells produce IL-17A, IL-17F, and IL-22, which are important for anti-bacterial and anti-fungal immunity, as well as in

autoimmune diseases, including multiple sclerosis, psoriasis, diabetes, rheumatoid arthritis, and Crohn's disease (Littman and Rudensky, 2010). Differentiation of Th17 cells can be induced by IL-6 + TGF- β via an IL-6 to IL-23 to IL-21 cytokine cascade (Littman and Rudensky, 2010). IL-2 signaling decreases Th17 cell generation, and when immunized, host mice receiving adoptively transferred *Il2*^{-/-} CD4⁺ T cells generate more Th17 cells than did mice receiving wild-type cells (Laurence et al., 2007). It has been proposed that IL-2-activated STAT5 competes with STAT3 for binding to sites in the *Il17a* locus, thus inhibiting *Il17a* transcription (Yang et al., 2011), but direct STAT5 inhibition of *Il17a* transcription has not been shown. IL-2 also inhibits expression of both IL-6R α and gp130, with increased expression of these receptors in *Il2*^{-/-} T cells (Liao et al., 2011), and retroviral transduction of *Il6st*, which encodes gp130, partially overcomes IL-2-induced inhibition of IL-17A production (Liao et al., 2011). Nevertheless, IL-2 partially inhibits IL-17A even when gp130 is constitutively expressed, indicating that IL-2 also inhibits Th17 cell differentiation by a receptor-independent mechanism (Liao et al., 2011). In this regard, IL-2 also induces expression of *Tbx21*, which inhibits Th17 cell differentiation (Liao et al., 2011), consistent with the ability of T-bet to interact with Runx1 and to prevent association of Runx1 with ROR γ t, which is essential for Th17 differentiation (Lazarevic et al., 2011). Interestingly, in Th17 cells, *Tbx21* retroviral transduction increases IFN- γ production, suggesting that IL-2 stimulation can promote a Th17-to-Th1 cell shift in these cells (Liao et al., 2011). Although IL-2 can inhibit Th17 cell differentiation, adults recently infected with HIV-1 maintain peripheral Th17 cell numbers when treated with IL-2 (Ndhlovu et al., 2010), and IL-2 can expand Th17 cells once generated, which helps to explain benefits of anti-IL-2 receptor-based immunotherapy in uveitis and scleritis, where Th17 cells are pathogenic (Amadi-Obi et al., 2007). Thus, IL-2 has complex actions on Th17 cells, inhibiting their differentiation but promoting their expansion.

Tfh Cell Differentiation

Tfh cells reside in germinal centers and promote immunoglobulin class switch and antibody affinity maturation by B cells (Crotty, 2011). Tfh cells are CD4⁺ T cells and both secrete and respond to IL-21, augmenting their expression of ICOS, CXCR5, and BCL6. IL-2 via STAT5 induces BLIMP1, which suppresses Tfh differentiation and germinal center formation (Ballesteros-Tato et al., 2012; Johnston et al., 2012); IL-2 also inhibits BCL6 in Th1 cells, which suppresses a Tfh-like gene profile (Oestreich et al., 2012).

IL-2 and Effector-Memory Cytolytic CD8⁺ T Cell Differentiation

Besides its actions on Th cell populations, IL-2 drives development of naive CD8⁺ T cells into effector and memory cytolytic T-lymphocytes (CTL) upon antigen stimulation in the context of infection or inflammation, with induction of IFN- γ , perforin, and granzymes (Pipkin et al., 2010). IL-2 increases expression of the transcription factor Eomes, which together with STAT5 upregulates *Prf1* expression (Pipkin et al., 2010). IL-2 also potentially suppresses BCL6 (Pipkin et al., 2010) and IL-7R α (Xue et al., 2002). Resting CD8⁺ T cells express IL-2R β and γ_c , so concentrations of IL-2 sufficient to titrate intermediate affinity receptors

can induce proliferation of these cells, which interestingly requires formation of STAT5 tetramers (Lin et al., 2012), as well as the proliferation of activated CD8⁺ T cells that express high-affinity IL-2 receptors.

In LCMV infection, in contrast to WT cells, *Il2ra*^{-/-} CD8⁺ T cells do not expand to secondary antigen exposure even though they robustly respond to primary viral infection, indicating a critical role for IL-2 in development of the memory response (Williams et al., 2006). During infection, CD25 expression is initially high but then becomes heterogeneous; cells that downregulate CD25 early in infection receive a less prolonged or “intense” IL-2 signal, upregulate IL-7R α and CD62L, and eventually become long-lived memory cells, whereas cells expressing higher numbers of CD25 molecules undergo more rapid proliferation, exhibit a terminally differentiated effector phenotype, and are eliminated by apoptosis (Kalia et al., 2010; Williams et al., 2006). A critical role for endogenous IL-2 production in the antiviral response has also been demonstrated using *Il2*^{-/-} mice (Cousens et al., 1995). Moreover, autocrine production of IL-2 by CD8⁺ T cells is important for the development of memory CD8⁺ T cells in vivo (Feau et al., 2011). Overall, these studies support the model that both the duration and strength of the IL-2 signal is important, with contributions of IL-2 to the generation of effector cells as well as memory cell differentiation.

IL-2 and Adult T Cell Leukemia-Lymphoma (ATL)

Human T cell lymphotropic virus, type 1 (HTLV-1) is a retrovirus that causes adult T cell leukemia-lymphoma (ATL) and tropical spastic paraparesis-HTLV-1 associated myelopathy (TSP-HAM). In the early phase of ATL, there is a period of autocrine growth of leukemic CD4⁺ T cells, with expression of IL-2 and functional IL-2 receptors, and anti-Tac mAb to human IL-2R α can decrease proliferation of these cells during this phase of ATL (Waldmann et al., 2001). Over time, however, IL-2 production is lost, although cell surface IL-2R α expression persists, and this later phase is associated with IL-2-independent growth. Interestingly, ATL cells exhibit Treg cell-like suppressor activity (Waldmann et al., 1984). In many patients, this later phase is associated with a constitutively-activated JAK-STAT pathway (Migone et al., 1995). Humanized anti-Tac (daclizumab or Zenopax) (Figure 4) was approved by the FDA to prevent renal allograft rejection but has also been used to treat patients with ATL (Waldmann, 2007) and relapsing-remitting multiple sclerosis (Martin, 2012); whereas the antibody alone was effective in a very limited number of individuals, coupling the antibody to immunotoxin-linked mAbs or arming it with ⁹⁰Y are approaches that have been used to enhance effectiveness (Waldmann, 2007).

IL-2 as a Therapeutic Modality

IL-2 immunotherapy has been used for many years, with FDA approval in 1992 for metastatic renal cell carcinoma and in 1998 for metastatic melanoma. IL-2 treatment can result in complete remission of 5%–10% of patients with these diseases, with lack of recurrence for as long as 25 years and potential cures of 70% of these individuals, with complete tumor regression (Rosenberg, 2012). However, this represents only a small fraction of the patients. Vaccination with a melanoma peptide vaccine can significantly improve effectiveness (Schwartzentruber et al., 2011), and the addition of adoptive cell transfer

approaches have improved cure rates for metastatic melanoma to between 20% and 40% (Rosenberg, 2012). Such treatment involves the isolation of tumor infiltrating lymphocytes (TILs) grown from a patient's tumor and expansion of these autologous TILs prior to their reintroduction (Rosenberg, 2012). A limitation of IL-2 is its toxicity, including severe capillary leak syndrome that can accompany such treatment. In mice, endothelial nitric oxide synthase appears to play a critical role in the development of IL-2-induced capillary leak syndrome (Sarniowski et al., 2011).

Interestingly, although IL-2 is a potent T cell growth factor, in animal models in which adoptive immunotherapy has been performed, IL-2 is less effective as an anti-tumor agent than IL-21, which inhibits expression of IL-2R α on CD8⁺ T cells, potentially decreasing the responsiveness of cells to IL-2 (Hinrichs et al., 2008). The decreased antitumor effect has been potentially attributed to the more potent induction of terminal effector CTL differentiation by IL-2, whereas IL-21-treated CD8⁺ T cells persist for longer periods of time in vivo.

Recently, “super-IL-2” variants of IL-2 have been developed that exhibit enhanced binding to IL-2R β and do not require IL-2R α (Levin et al., 2012). As compared to wild-type IL-2, they induce greater expansion of cytotoxic T cells but less expansion of Treg cells, with reduced pulmonary edema in mice when administered in vivo (Levin et al., 2012). Based on the crystal structure, the mutations that result in the augmented function are mainly in the core structure and stabilize IL-2, reducing the flexibility of a helix in the IL-2R β binding site and resulting in a conformation that is normally achieved only in the presence of IL-2R α (Levin et al., 2012). Presumably for this reason, the IL-2 superkine induces vigorous signaling and phosphorylation in the absence IL-2R α (Levin et al., 2012). More studies are needed to determine whether this or another engineered IL-2 has therapeutic advantages over wild-type IL-2.

Inhibition of IL-2 Signaling as a Therapeutic Modality

In addition to daclizumab, other modes of IL-2 inhibition are possible. As noted above, IL-2 activates JAK1 and JAK3, with JAK1 associating with IL-2R β and JAK3 with γ_c (Russell et al., 1994), and it was proposed that inhibitors of JAK3 would be immunosuppressive (Russell et al., 1995). Two JAK inhibitors are now available for clinical use. Ruxolitinib (Jakafi) inhibits JAK1 > JAK2 (Harrison et al., 2012) and has been approved by the FDA for treating myelofibrosis, with ongoing clinical trials for primary polycythemia and primary thrombocythemia. Tofacitinib (Changellian et al., 2003; Kremer et al., 2009), which inhibits JAK3 > JAK1 and JAK2 and has also been approved for the treatment of rheumatoid arthritis, based on successful clinical trials (Kontzias et al., 2012), with ongoing Phase III clinical trials for the treatment of psoriasis (Figure 4). Other JAK inhibitors are being evaluated for use in rheumatoid arthritis and other diseases as well. It is important to note that these agents inhibit not only IL-2, but also other cytokines that utilized the inhibited JAK kinases. Finally, IL-2 mutants with inhibitory activity as well as structural-based medicinal chemistry approaches (Wilson and Arkin, 2011) may also potentially yield useful inhibitors of IL-2.

Concluding Remarks and Perspective

As discussed herein, IL-2 mediates diverse pleiotropic actions, promoting T cell proliferation, survival, cytolytic activity, NK cell

activity, development of Treg cells, and AICD. Moreover, by inducing IL-12R β 2 and IL-4R α but suppressing gp130 expression, IL-2 increases responsiveness to IL-12 and IL-4 to promote Th1 and Th2 cell differentiation while inhibiting the IL-6 to IL-23 to IL-21 cytokine cascade that drives Th17 cell differentiation, and furthermore, its induction of IL-2R β not only increases responsiveness to IL-2 but also to IL-15, with potential impact on CTL and NK activity. Thus, IL-2 does not by itself determine the eventual differentiation but rather facilitates or inhibits responsiveness to other cytokines, in part by promoting or inhibiting different cytokine cascades. Consistent with its potent proliferative activity for T cells, IL-2 promotes the expansion of multiple populations, including even Th17 cells whose differentiation it inhibits. The amount of IL-2 produced, when and where IL-2 receptors are expressed, the affinities (high- or intermediate-affinity) of these receptors, and the other cytokines present in the cellular milieu, are critical in determining the biological outcome orchestrated by IL-2 during an immune response. Moreover, different populations of responding cells can potentially compete for IL-2, and Treg cells expressing high affinity IL-2Rs can efficiently compete for limited amount IL-2 with effector cells that express intermediate affinity IL-2Rs (Busse et al., 2010; Pandiyan et al., 2007). Furthermore, when IL-2 production by effector cells is abrogated during acute massive inflammation in lethal *T. gondii* infection, there is a marked decrease in Treg cell numbers and function that was reversed by exogenous IL-2 (Oldenhove et al., 2009). Another complexity is the possible competition among γ_c family cytokines. For example, if one γ_c family cytokine is more abundant than others, it might quantitatively recruit γ_c to its receptor, thereby effectively sequestering γ_c and diminishing cellular responsiveness to the other cytokines. Overall, we have tremendous knowledge regarding how IL-2 and its receptor are regulated as well as related to its structure, its signaling pathways, and its relationship to the five other cytokines that share γ_c . These basic research findings have led to the development of new therapeutic approaches potentially applicable to a range of diseases, either based on the administration of IL-2 or by using antagonists of IL-2 production or signaling. How and when it is desirable to modulate the IL-2 response is context-dependent. For example, promoting effector activity may be a desired response for treatment of cancer, whereas blocking effector responses or augmenting Treg cell activity may be beneficial in preventing autoimmunity or massive inflammatory responses, as noted above related to *T. gondii*. Thus, directed manipulation of the activities of this cytokine, which has major actions both related to effector T cells and in tolerance, could have implications for controlling complex immune responses and for immunotherapy of a range of diseases. Moreover, future studies directed toward comprehensively mapping the IL-2 signaling network and further elucidating the molecular mechanisms of IL-2 action could facilitate the development of better agonists and more specific antagonists to efficiently modulate immune responses.

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