

# Central Role for Interleukin-2 in Type 1 Diabetes

Maigan A. Hulme, Clive H. Wasserfall, Mark A. Atkinson, and Todd M. Brusko

Type 1 diabetes presents clinically with overt hyperglycemia resulting from progressive immune-mediated destruction of pancreatic  $\beta$ -cells and associated metabolic dysfunction. Combined genetic and immunological studies now highlight deficiencies in both the interleukin-2 (IL-2) receptor and its downstream signaling pathway as a central defect in the pathogenesis of type 1 diabetes. Prior intervention studies in animal models indicate that augmenting IL-2 signaling can prevent and reverse disease, with protection conferred primarily by restoration of regulatory T-cell (Treg) function. In this article, we will focus on studies of type 1 diabetes noting deficient IL-2 signaling and build what we believe forms the molecular framework for their contribution to the disease. This activity results in the identification of a series of potentially novel therapeutic targets that could restore proper immune regulation in type 1 diabetes by augmenting the IL-2 pathway. *Diabetes* 61:14–22, 2012

## INTERLEUKIN-2 AND THE INTERLEUKIN-2 RECEPTOR

**Physiological role and function.** Understanding the role of interleukin (IL)-2 in the etiology of type 1 diabetes requires knowledge of the regulation of—as well as the structural and functional consequences of—IL-2 binding to its cognate receptor (IL-2R). IL-2 can be used by cells expressing either the intermediate-affinity ( $K_d = 10^{-9}$  M) receptor dimer of IL-2R $\beta$  (CD122) and the common  $\gamma$  chain ( $\gamma$ c; CD132), or the high-affinity ( $K_d = 10^{-11}$  M) trimeric IL-2R comprising IL-2R $\alpha$  (CD25), IL-2R $\beta$ , and  $\gamma$ c. The intermediate-affinity IL-2R is more broadly expressed on T cells, natural killer (NK) cells, and monocytes, whereas the high-affinity IL-2R is only constitutively expressed on regulatory T-cells (Tregs) (1). A simplified schematic of IL-2 signaling in both Tregs and conventional T cells (Tconv), or differentiated (i.e., Th1, Th17) effector T cells (Teffs) can be formed (Fig. 1). IL-2 binding initiates signal transduction following cross-phosphorylation of tyrosine residues in Janus-activated kinases (JAKs), leading to downstream phosphatidylinositol 3-kinase/Akt, mitogen-activated protein kinase/extracellular signal-related kinase, and signal transducer and activator of transcription (STAT5) activation.

**Functional impact of IL-2 signaling.** Downstream cellular response to IL-2 depends not only upon surface expression of the receptor but also upon local cytokine concentration, target cell population, and modification of the various response elements in this complex pleiotropic signaling pathway. For Tconv cells, high concentrations of IL-2 can cause activation-induced cell death (AICD), whereas moderate to low concentrations of IL-2 induce effector or memory phenotypes, respectively (2). IL-2 signaling is critical for the development, maintenance, and function of Tregs

(3). Despite this critical requirement, Tregs do not produce their own IL-2 and are dependent on Tconv or dendritic cells (DCs) for signals needed to maintain viability and function. Therefore, a reduction in IL-2 signaling in type 1 diabetes may contribute to Treg decline and the emergence of effector phenotypes.

**Receptor clustering and signal thresholds.** The IL-2 receptor is often modeled as a stand-alone structure consisting of the individual  $\alpha/\beta/\gamma$ c subunits complexed with IL-2 (Fig. 2A). This schematic conveys the notion that IL-2 receptors are diffusely distributed across the cell surface. In fact, high-resolution microscopy studies suggest clustering of receptors and signaling complexes adjacent to immunological synapses (4). Furthermore, a careful analysis of the X-ray crystallographic structure of the IL-2 tetrameric complex suggests that CD25/IL-2R $\beta$  may interact with the  $\gamma$ c chain on a neighboring receptor, allowing for assembly of a cell surface network of receptor complexes and increased responsiveness to IL-2 (5). This theory is supported by the observation of an extensive interaction between  $\gamma$ c and IL-2R $\alpha$  in the crystal structure, featuring high shape complementarity as well as hydrogen bonding (Fig. 2B and C) (5).

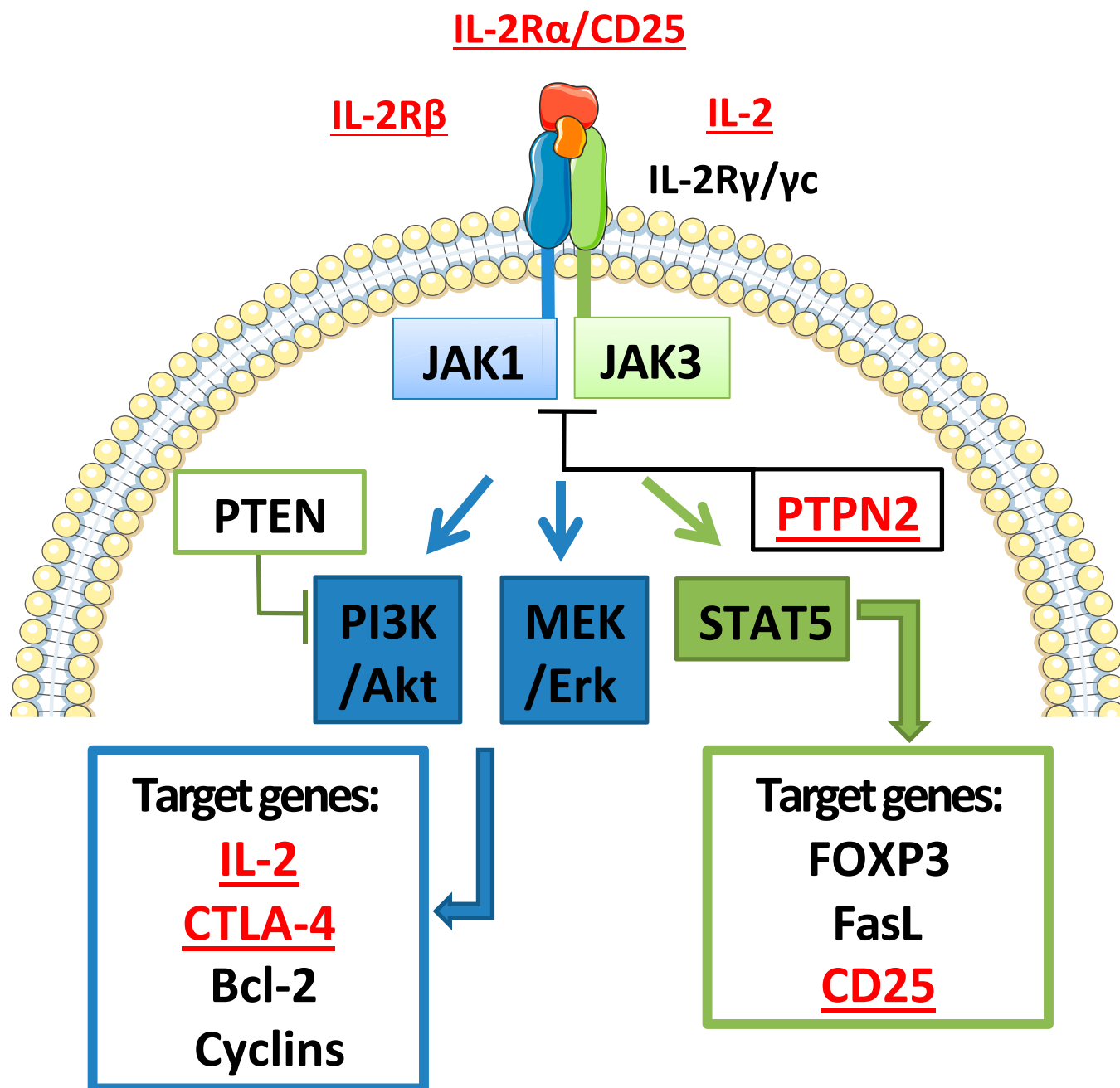
**Competition for  $\gamma$ c dictates cellular activity: a role for membrane CD25.**  $\gamma$ c serves as a signaling component in six distinct cytokine receptor assemblies, namely, the IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors (6). Additionally, IL-2 and IL-15 both use IL-2R $\beta$  to propagate downstream signaling. Cellular response to these cytokines is surprisingly variable despite this structural commonality. This is due, in part, to differential association of adaptor proteins and downstream STATs with the unique receptor subunits (Fig. 3) (6). There are, however, some functional redundancies (Fig. 3).

Competition among receptor subunits to use  $\gamma$ c depends on their expression level and the local cytokine environment. Experiments using fluorescence resonance energy transfer and confocal microscopy have shown that CD25 and IL-15R $\alpha$  are expressed within the same membrane microdomains (7). It has been theorized that multiple unique receptor subunits are expressed in close proximity to  $\gamma$ c, with rapid rearrangement to assemble the relevant receptor in response to cytokine presence (8). Extending these observations, the formation of a signaling zipper (Fig. 2C) could prevent the association of  $\gamma$ c with other cytokine receptor subunits, effectively sequestering it for CD25 and IL-2 signaling machinery. Conversely, proteolytic shedding of a soluble form of CD25 from the cell surface may prevent this signaling complex formation and alter downstream IL-2 response (9). The effect of CD25 loss likely differs by cell type; for example, Tregs may exhibit reduced function, whereas activated T cells might slow their progress toward AICD.

**IL-2 signaling in non-T cells.** Much of what is known about the IL-2R and its downstream activity has been determined in T cells, given its unique role in adaptive T cell responses. However, functional IL-2 receptors are expressed

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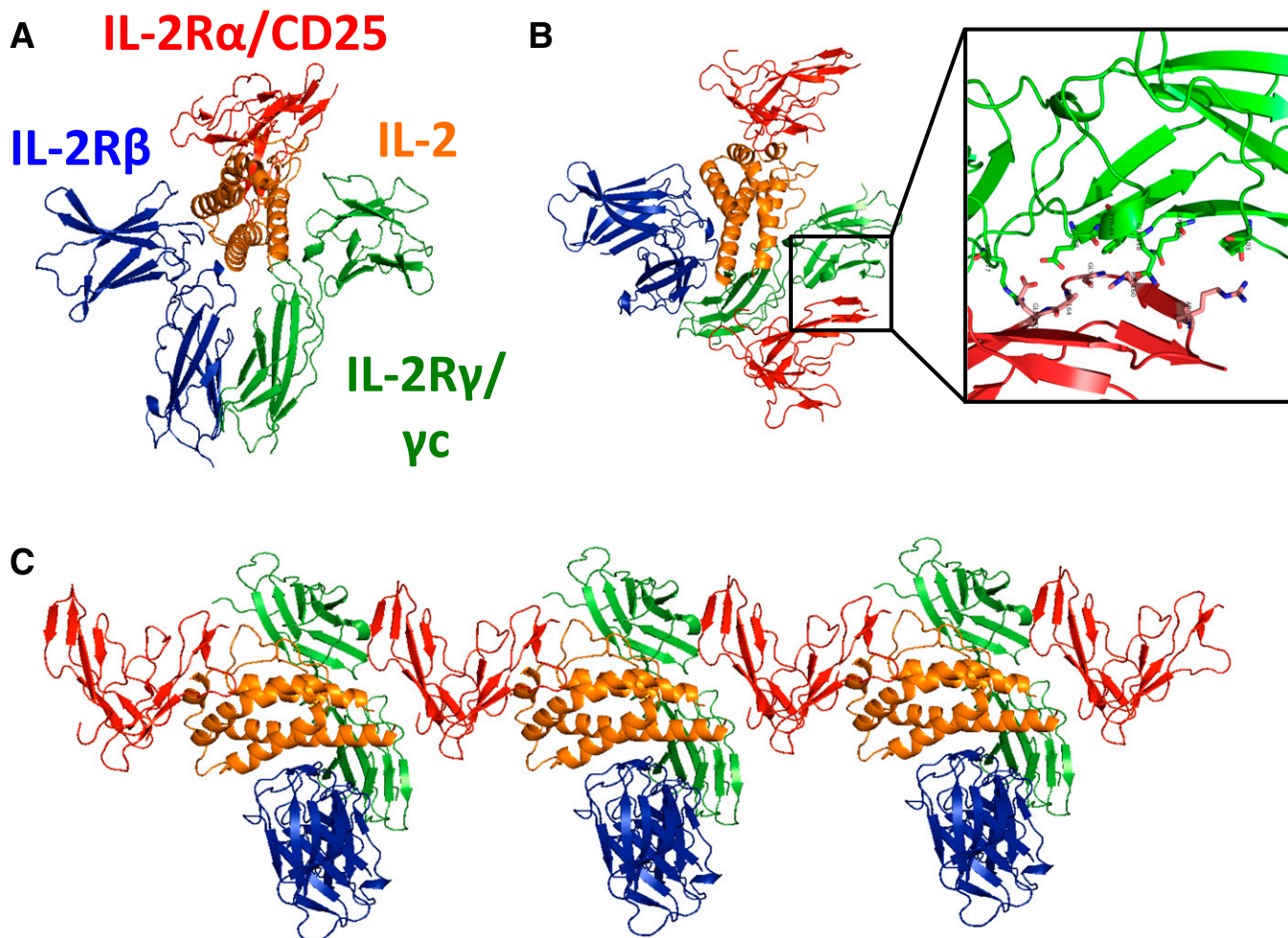
**FIG. 1.** A simplified schematic of IL-2 receptor signaling. In this diagram, and throughout this review, CD25 is shown in red, IL-2 in orange, IL-2R $\beta$  in blue, and  $\gamma$ c in green. Briefly, JAK1 and JAK3 are phosphorylated by IL-2R $\beta$  and  $\gamma$ c, and propagate downstream signaling through multiple pathways, which are uniquely regulated in Treg cells. Elements that are more active in Treg than in Teff or Tconv cells are shown in green (i.e., STAT5 and PTEN), while those that are more active in Teff or Tconv are shown in blue (i.e., phosphatidylinositol 3-kinase [PI3K]/Akt and mitogen-activated protein kinase [MEK]/extracellular signal-related kinase [Erk]). Proteins that are known to have genetic polymorphisms associated with type 1 diabetes risk are highlighted in red underlined text.

on activated B cells, DCs, NK cells, and eosinophils (10–12). Information about the role of IL-2 signaling in non-T cells is limited; yet existing data suggests a stimulatory role. Indeed, it has been shown that CD25<sup>+</sup> B cells have increased costimulatory and migratory capacity (10). NK cells display enhanced activity upon CD25 expression (11), and eosinophil degranulation appears to be triggered by IL-2 (12). The previous data provide clear evidence that high-affinity IL-2 signaling in non-T cells is rare, but may nonetheless impact a variety of immune cell subsets. What remains unclear is how these cells might be affected by therapeutic doses of IL-2 and what, if any, impact activation

of these cell subsets would have on the disease process in type 1 diabetes.

#### GENETIC DEFECTS IN THE IL-2 SIGNALING PATHWAY

**Linking genes with function in mice and humans.** Studies in the nonobese diabetic (NOD) mouse model and in humans with type 1 diabetes have identified multiple genes in the IL-2 signaling pathway that are associated with disease susceptibility (13) (Fig. 1). The clearest evidence delineating a role for IL-2 in maintaining immune tolerance derives from data generated in animal models.



**FIG. 2.** High-affinity IL-2 receptor structure and interreceptor association. **A:** Crystal structure of the quaternary IL-2 receptor structure, depicting CD25 (red), IL-2Rβ (blue) and γc (green) in interaction with IL-2 (orange). **B:** The enlarged panel highlights the area of interaction between γc and CD25 on a neighboring crystal unit. High complementarity and hydrogen bonding are features of this interaction. **C:** Depiction of the IL-2 signaling “zipper,” a repeating unit of high-affinity IL-2 receptors present in the solved crystal structure. The extensive complementarity of CD25 and γc interface in this structure suggests a physiologically relevant binding interaction.

Mice that lack IL-2, CD25, IL-2Rβ, or STAT5 all succumb to lymphoproliferative disease due to a marked Treg reduction. The administration of exogenous IL-2 or adoptive transfer of wild-type T cells to deficient animals results in the restoration of Tregs and peripheral immune regulation (1). The IL-2 locus in the NOD mouse (*Idd3*) and in humans (4q27) confer susceptibility to disease. In the NOD mouse, the resultant reduced IL-2 production is ameliorated in the presence of the protective (*Idd3.B6*) allele (14).

In humans, mutations in the Treg-defining transcription factor forkhead box P3 (*FOXP3*) result in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Notably, greater than 60% of these patients present with type 1 diabetes at or near birth (15). A syndrome similar to IPEX has also been described in a human subject with a mutation in *CD25* (16).

The previous examples highlight the importance of IL-2 signaling in immune regulation conferred by Tregs. However, the extremely high penetrance of autoimmunity observed in these examples is generally not found in polygenic autoimmune disorders such as type 1 diabetes, where the odds ratios of individual gene variants are relatively low (near 1.61 for *CD25* and 1.13 for *IL2*) (13). Despite the low

penetrance and relatively common occurrence of these variants in humans, the impact of having multiple gene defects that affect IL-2 signaling has not been determined. Intriguingly, the NOD mouse carries the majority of these IL-2 signaling deficiencies, while in humans it is unknown if multiple pathway defects synergize to cause type 1 diabetes.

#### DEFICIENT IL-2 SIGNALING PREDISPOSES TO AUTOIMMUNITY IN TYPE 1 DIABETES

**Escape from AICD.** AICD represents an IL-2-dependent mechanism through which peripheral tolerance is maintained within the T-cell compartment. NOD T cells are resistant to AICD when compared with T cells of both nonautoimmune C57BL/6 and diabetes-resistant NOR mice. The importance of IL-2 in AICD sensitization is demonstrated by restoration of AICD in Fas-deficient *lpr* mice through provision of IL-2 (17). It should be noted that other γc cytokines, namely IL-4 and IL-15, can inhibit the IL-2-dependent sensitization toward AICD (6). Therefore, genetic deficiencies in IL-2 signaling may skew the relative balance of cytokine signals derived from γc in Treg, Tcon, and Teff cells.

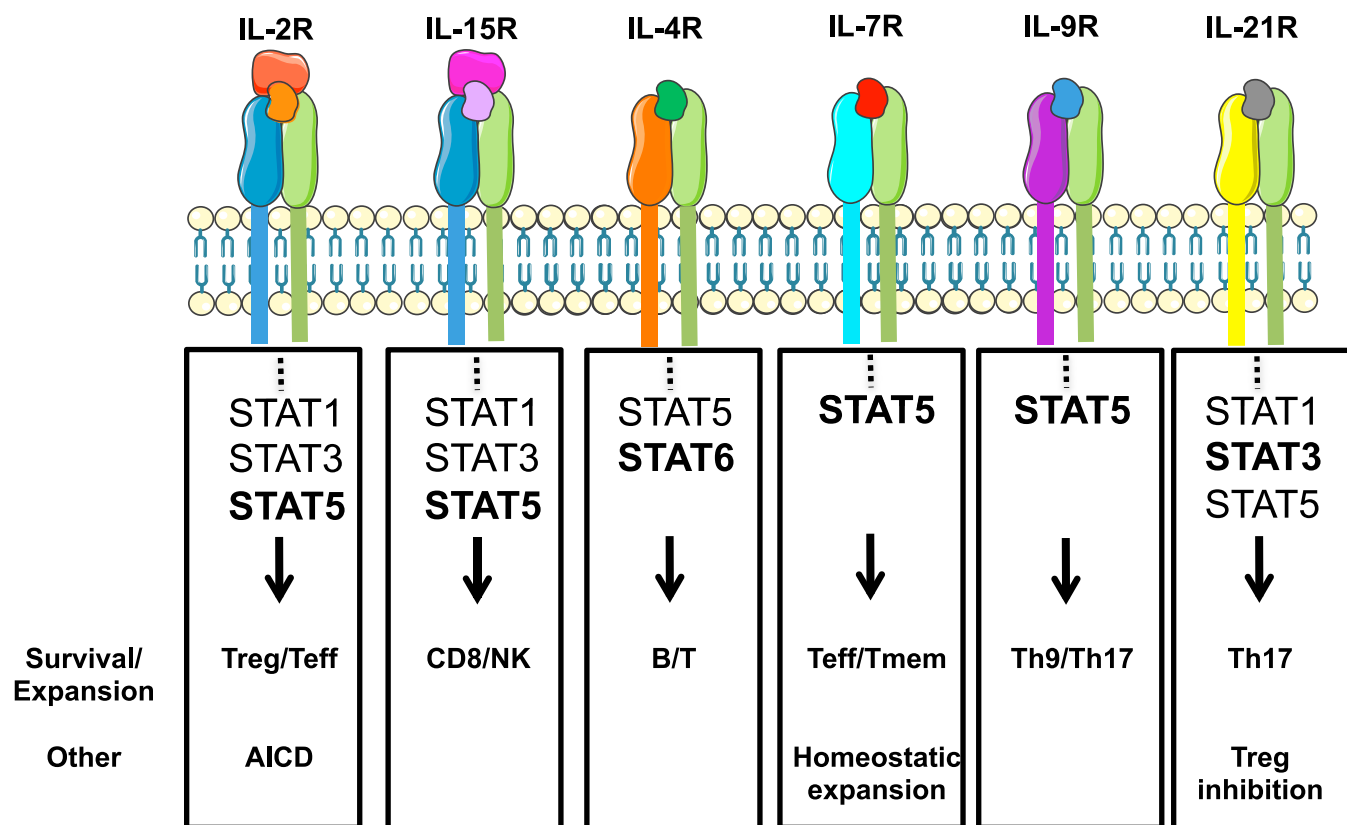


FIG. 3. Structure and function of the  $\gamma$ c cytokine receptor family.  $\gamma$ c promotes a variety of downstream effects across multiple cell types as a result of its diverse cytokine associations and differential STAT phosphorylation. The known main functions of each unique cytokine are listed with the most closely associated STAT protein in bold text. Tmem, memory T cells.

**Imbalance in Treg and effector T-cell activity.** T-cell subsets exist in a state of dynamic equilibrium. IL-2 produced by Tconv cells and DCs acts in a paracrine fashion to support the survival and function of Tregs. Type 1 diabetes subjects are reported to exhibit reduced IL-2 production and subsequent Treg dysfunction (18). Phenotypically, these defects include poor maintenance of FoxP3 protein expression, reduced in vitro suppression, and increased apoptosis (19). In mouse and human type 1 diabetes, apoptosis of Tregs caused by IL-2 deprivation is a hallmark of disease onset. NOD Tregs functionally decline during progression to disease. Moreover, aberrant Treg function seems to be exacerbated at the site of inflammation in the NOD. Islet infiltrates exhibit reduced frequency of Tregs, which are characterized by lower CD25 and Bcl-2 expression (19). Stable expression of CD25 in Treg is key in maintaining IL-2-mediated regulatory function (20). Additionally, cells from patients with type 1 diabetes have been shown to be resistant to Treg suppression (21). The detrimental effect of IL-2 insufficiency is thus twofold: the Treg repertoire suffers from neglect and activated autoreactive cells are able to persist unabated by AICD.

**Treg lineage plasticity.** Helper T-cell lineages are often classified into distinct subsets based upon expressed transcription factors, surface proteins, and cytokines. However, the sum of the environmental signals a T cell receives can induce a high degree of lineage overlap and cellular plasticity (22). IL-2 has recently been shown to have broad influence over T-helper subset generation through regulation of cytokine receptor and transcription factor expression (23). This influence includes constraint of Th17 development, as well as maintenance of stable FoxP3 expression in both

natural and adaptive Tregs. Emerging evidence now suggests the processes controlling T-cell fate are exquisitely dependent upon IL-2 (Fig. 4).

Multiple reports in the literature now suggest that a combination of intrinsic and extrinsic factors, including changes in the cytokine milieu may cause FoxP3 instability and subsequent Treg plasticity. These so-called ex-Tregs display reduced FoxP3 expression and acquire Teff functions (i.e., increased proliferation and inflammatory cytokine production) (24). Counterregulation of IL-2-dependent genes has been demonstrated in Th17 cells, in that IL-2 signaling through STAT5 directly competed with STAT3 binding to conserved target loci, prohibiting their activity (25). Significantly elevated IL-17 production has been seen in CD4<sup>+</sup>FoxP3<sup>+</sup> cells in new-onset type 1 diabetic subjects when compared with control subjects (26). Likewise, we recently reported that patients with type 1 diabetes exhibit increased frequencies of  $\gamma$  interferon<sup>+</sup>FoxP3<sup>+</sup> T cells in peripheral blood (27). This population exhibited reduced suppressive capacity and markers typical of an adaptive Treg (i.e., methylation of CpG residues in the Treg-specific demethylated region and lack of Helios expression). These data support the notion of altered adaptive Treg function in type 1 diabetes.

#### IL-2 PATHWAY-TARGETED THERAPY FOR IMMUNOMODULATION

**Early efforts in IL-2-directed therapies.** The use of cyclosporine to suppress IL-2-mediated autoreactive T-cell activation in new-onset type 1 diabetes patients marked the advent of clinical immunotherapy in type 1 diabetes (28).

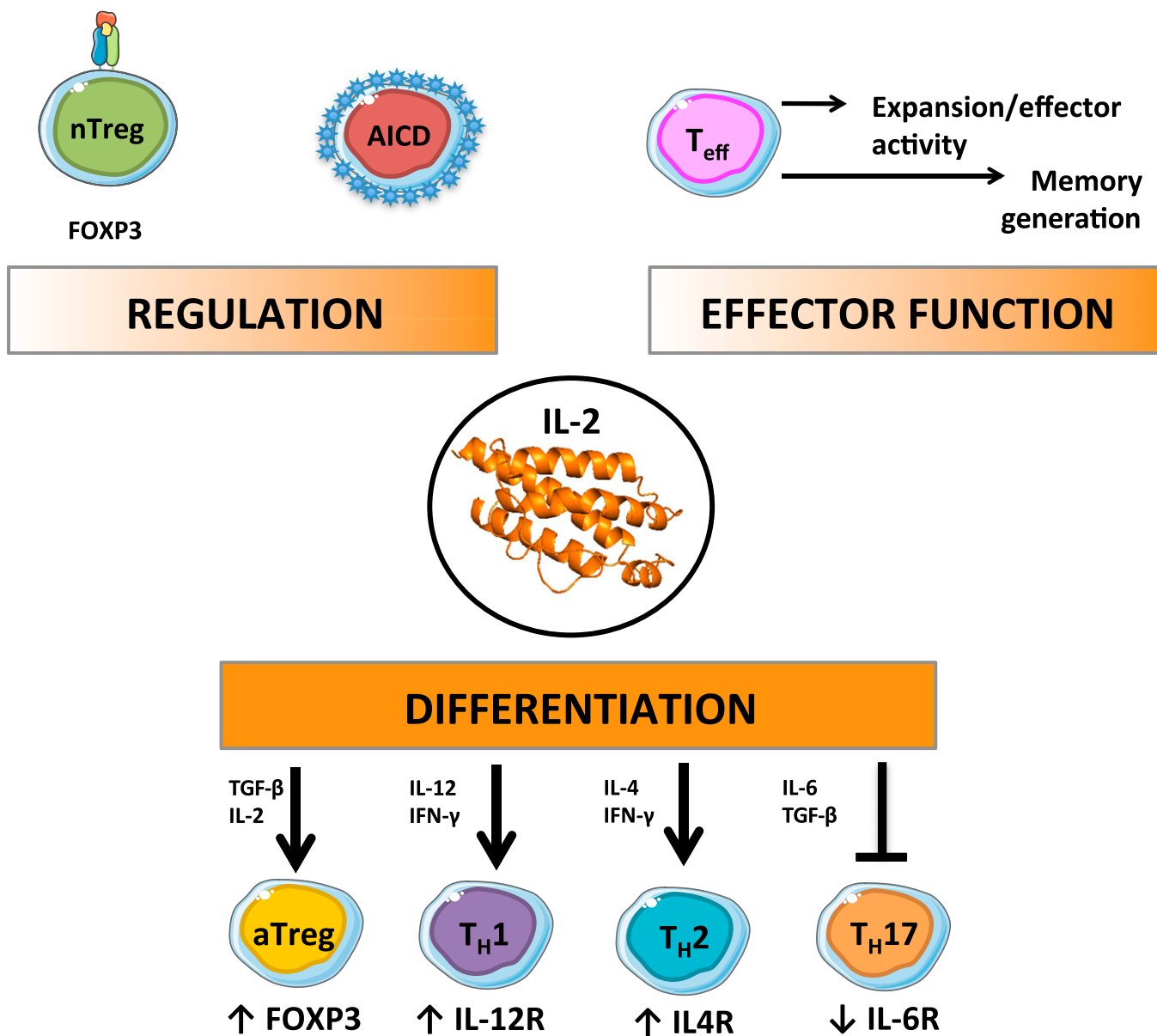


FIG. 4. IL-2 is central to immune regulation in the T-cell compartment. These functions can be dependent on concentration of IL-2, indicated where necessary by increased orange shading. IL-2 is known to be important to immune regulation through AICD sensitization and Treg maintenance (*upper left*), but more recent work highlights its importance in other areas. This cytokine is crucial in antigen-specific clonal expansion of T<sub>eff</sub>s and subsequent development of T-cell memory (*upper right*). Additionally, the presence of IL-2 is necessary for the development of Th1, Th2, and adaptive Treg subsets, but it arrests progression toward the proinflammatory Th17 phenotype (*lower section*).

This effort initially succeeded in halting T cell-mediated β-cell destruction, but the beneficial effect was only temporary because of concerns over drug toxicity and the effects of long-term immunosuppression. In 1995, the rediscovery of Tregs led to a paradigm shift in the field of autoimmunity research with the notion that immune tolerance could be generated by bolstering their activity (29). Importantly, the tolerance conferred by Tregs is dominant and durable. This implies that short-term treatments aimed at restoring or boosting Tregs could have long-term efficacy in maintaining tolerance, presenting a therapeutic opportunity for diseases such as type 1 diabetes where Treg dysfunction is implicated in disease pathogenesis.

**IL-2 therapy in animal models of type 1 diabetes.** Given the abundance of evidence implicating defects in the IL-2 pathway as an etiological component of type 1 diabetes,

multiple studies have been conducted testing the ability of IL-2 supplementation to prevent and reverse type 1 diabetes in the NOD mouse (Table 1). Overall, these studies support the notion that exogenous IL-2 treatment can protect NOD mice from diabetes development. From a mechanistic standpoint, low-dose IL-2 treatment has been shown to increase CD25 and Bcl-2 expression by islet-resident Tregs, affording long-term protection from disease (19, Table 1). Conversely, high doses of IL-2 enhanced immune responses and exacerbated autoimmunity in the NOD mouse. The therapeutic efficacy of IL-2 can vary dramatically depending upon the dose, age at therapeutic intervention, and additional treatment agents (Table 1). These types of studies demonstrate the importance of proper dosing and temporal adjustment, as well as consideration of additional agents to act in concert with IL-2. These

TABLE 1

IL-2 as a therapeutic agent in the NOD mouse model. Outcome data are representative of the total study length, including follow-up, as reported in the original publications

Rx age	Formulation/ dose	Frequency	Rx duration	Concurrent Rx/dose	Rx group %euglycemic (success/total)	Control group %euglycemic (success/total)	Ref.
5 wk	RM/0.5 $\mu$ g	Daily	15 wk	N/A	80% (8/10)	56% (5/9)	19
10 wk	RM/0.5 $\mu$ g	Daily	10 wk	$\alpha$ -IL-2 mAb/5 $\mu$ g	95% (18/19)	39% (7/18)	19
10 wk	RH/5 $\mu$ g	Daily	5 d	$\alpha$ -IL-2 mAb/50 $\mu$ g	20% (2/10)‡	100% (10/10)	19
10 wk	RH/4 ng	Daily	15 wk	N/A	0% (0/9)	0% (0/9)	37
10 wk	RH/4 ng	Daily	15 wk	Rapa/0.1 mg/kg	67% (6/9)	0% (0/9)	37
10 wk	RH/4 ng	Daily	15 wk	Rapa/1 mg/kg	78% (7/9)	0% (0/9)	37
6 wk	RH/250 IU	2 $\times$ wkly	14 wk	N/A	50% (6/12)	0% (0/12)	49
6 wk	RH/250 IU	2 $\times$ wkly	14 wk	Poly I:C/50 $\mu$ g	80% (10/12)	0% (0/12)	49
10 wk	AAV,IL-2/inducible	Once§	3 wk	Dox/200 mg/kg	73% (11/15)	10% (1/10)	50
10 wk	AAV,IL-2/inducible	Once§	3 wk	AAV.TGF- $\beta$ /inducible Dox/200 mg/kg	60% (3/5)	10% (1/10)	50
Onset	RH/2.5 $\times$ 10 <sup>4</sup> IU	Daily	5 d	N/A	30% (7/24)	0% (0/9)	51
Onset	RH/2.5 $\times$ 10 <sup>4</sup> IU	Daily	10 d	N/A	37.5% (3/8)	0% (0/9)	51
Onset	RH/5 $\mu$ g	Daily	5 d	$\alpha$ -IL-2 mAb/50 $\mu$ g	25% (1/4)	0% (0/9)	51
Onset	RM IL-2 Fc/5 $\mu$ g	Daily	4 wk	Rapa/3 mg/kg	50% (10/20)	0% (0/150)	38
Onset	RM IL-2 Fc/5 $\mu$ g	Daily	2 wk	Rapa/3 mg/kg mutIL15.Fc/5 $\mu$ g	90% (18/20)	0% (0/150)	38
Onset	RM IL-2 Fc/5 $\mu$ g	Daily	4 wk	Rapa/3 mg/kg mutIL15.Fc/5 $\mu$ g	92% (37/40)	0% (0/150)	38

AAV, adeno-associated virus; d, days; Dox, Doxycycline; Fc, Fc fusion protein; mAb, monoclonal antibody; Rapa, rapamycin; RH, recombinant human; RM, recombinant murine; Rx, treatment; TGF- $\beta$ , transforming growth factor  $\beta$ ; wk, weeks; wkly, weekly. ‡Indicates accelerated incidence as compared with control group. §Indicates intramuscular route of delivery (others were intraperitoneal injection).

agents could be utilized to prolong IL-2 bioavailability, prevent its usage by off-target cell types, or provide additional immunosuppression.

**Clinical usage of IL-2 for the treatment of autoimmunity—a little goes a long way.** IL-2 therapy has a long clinical history in humans, which can provide invaluable insight to future therapeutic design. IL-2 treatment in humans has been used in the past for severe metastatic melanoma and renal carcinoma and has undergone several unsuccessful clinical trials for patients with HIV/AIDS (30). Treatment of cancer patients with high-dose IL-2 results in a robust response, but its use is limited by development of life-threatening toxicity and its short half-life in circulation. Unfortunately, lowering the dose to avoid undesirable side effects greatly reduces therapeutic efficacy, presumably as a result of increases in the Treg population that prevent antitumor immunity (31,32). This Treg increase, while detrimental to cancer immunology, provides a therapeutic opportunity for tolerance induction.

Low-dose IL-2 was recently used in a clinical trial for the treatment of graft versus host disease in patients after allogeneic hematopoietic stem cell transplantation with the notion of bolstering the Treg pool to prevent alloreactive T cell expansion (33). Patients treated with IL-2 exhibited increased Treg frequency, suggesting there may have been a preferential binding of IL-2 for the high-affinity IL-2R expressed on Tregs. Combination therapy using hematopoietic stem cell transplantation, low-dose IL-2, and donor CD4<sup>+</sup> lymphocyte infusion provided greater Treg increases than either IL-2 or lymphocyte infusion alone. Given these findings in graft versus host disease, IL-2-based therapeutic interventions in autoimmunity will require the identification of biomarkers of response in each cell subset, allowing for optimal and targeted trial design. A currently enrolling clinical trial of low-dose IL-2 should provide a wealth of information (34).

However, several challenges arise when translating IL-2 into clinical use for the treatment of type 1 diabetes. An optimal IL-2 dose may be difficult to predict for a heterogeneous patient population, and it is likely that an adjunct

therapy will be needed to improve outcome, as suggested by animal studies (Table 1). In this regard, rapamycin (Sirolimus) presents an attractive option because it blocks cell-cycle progression and cytokine signal transduction through inhibition of the mammalian target of rapamycin (mTOR), another downstream IL-2 signaling component (Fig. 5). mTOR activation is required for generation of effector T cells from naïve cells, and its absence or inhibition causes activated naïve T cells to default to a regulatory lineage (35). Rapamycin monotherapy in type 1 diabetes subjects resulted in increased Treg suppression, and exhibited beneficial effects on long-term type 1 diabetes patients, including an increase in c-peptide and reduction in insulin autoantibodies and exogenous insulin requirements in responders (36). Thus, the concept was that combining IL-2 with rapamycin would provide a Treg growth factor while blocking Teff activity. This combination is indeed capable of preventing diabetes in NOD mice, as well as conferring lasting protection to islet grafts (37). Similarly, a combination of a mutant IL-15Fc plus IL-2Fc and rapamycin has been shown to induce long-term islet allograft acceptance in the stringent NOD islet allograft model, by eliminating Teff and promoting Treg development (38). A recent phase I clinical trial using IL-2 and rapamycin in patients with type 1 diabetes reported an accelerated yet transient loss of c-peptide, despite observable responses in terms of cell number and pSTAT5 in follow-up studies monitoring both Tregs and NK cells (39). These preliminary findings once again highlight the critical need to optimize dose and timing to limit off-target effects of IL-2.

**Modified versions of IL-2 allow for lower dose and optimal delivery.** Modified mutant versions of IL-2 have been developed to preferentially bind the trimeric IL-2R, allowing avoidance of off-target cell types bearing intermediate-affinity receptors. One such effort demonstrated a mutant IL-2 form with  $\sim$ 3,000-fold greater selectivity (relative to wild-type IL-2) for T cells bearing the trimeric IL-2R complex (40). While originally proposed for cancer immunotherapy,

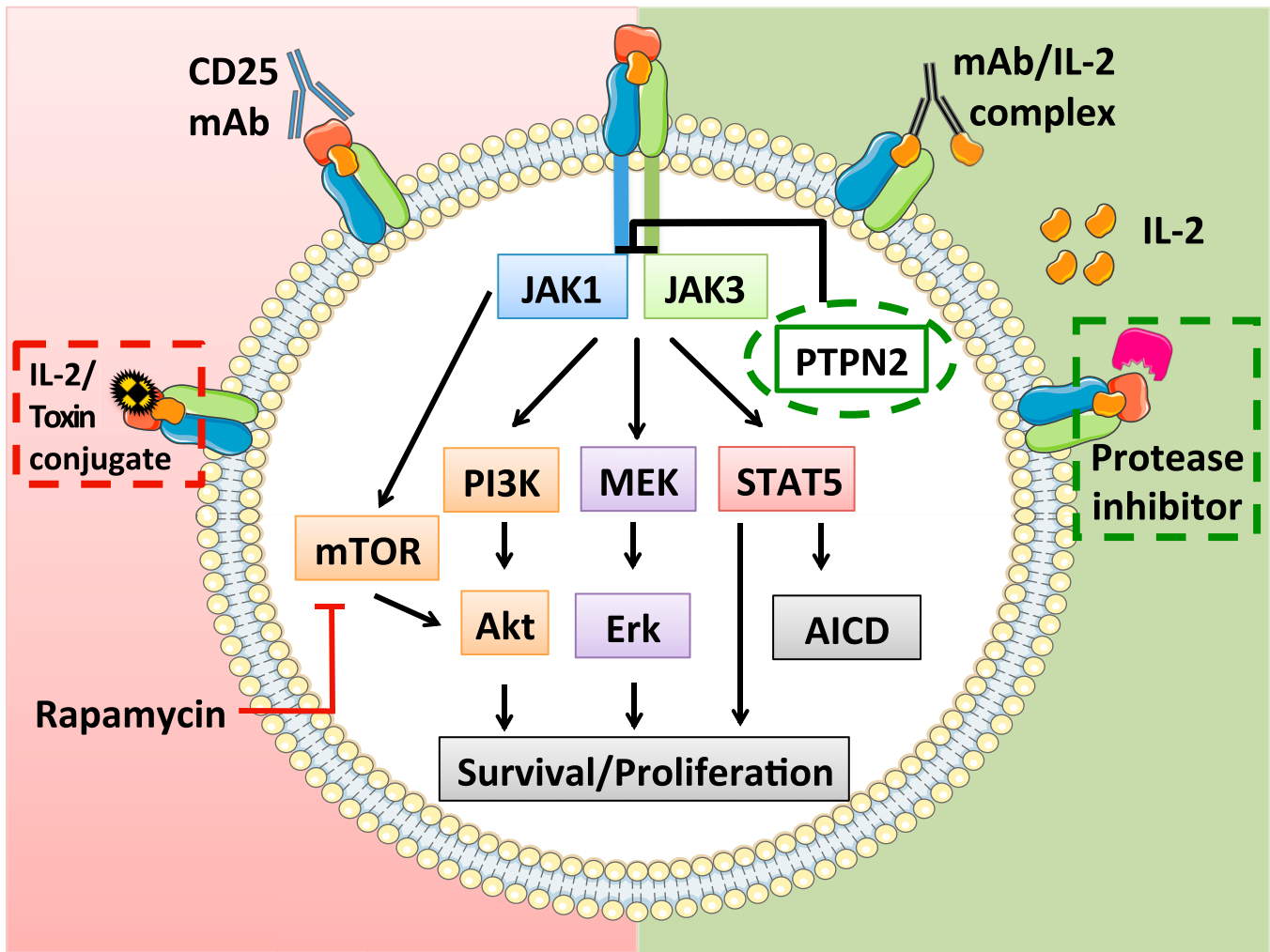


FIG. 5. IL-2 pathway–targeted interventions in immunomodulatory therapy. Therapeutic agents on the right (shaded green) enhance IL-2 signaling, whereas those on the left (shaded red) are designed to inhibit IL-2 signaling downstream of its receptor. Treatments suggested in this review that have no clinical history in type 1 diabetes therapy are outlined in dashed boxes. mAb, monoclonal antibody.

such a modified version of IL-2 could substantially increase the ability to selectively target Tregs in vivo.

Additional modifications to improve half-life and decrease required dose include the formation of antibody:cytokine (anti-IL-2:IL-2) complexes, which are purported to extend the half-life of IL-2 in circulation. Interestingly, utilization of different monoclonal antibodies in complex formation allows for targeting of specific cell subsets based on IL-2R affinity. Certain complexes, for example, caused selective Treg expansion and suppression of allergic airway inflammation in a mouse model (41). Site-specific cytokine delivery would also allow for reduced systemic toxicity. Cancer therapy involving antibody-cytokine fusion proteins, called immunocytokines, show promise in allowing targeted cytokine delivery to tumor tissue. The notion of targeting is particularly desirable for immunosuppressive therapy.

**IL-2 signaling blockade differentially affects Treg and Teff populations.** Agents designed to ablate total T cells have shown some success in models of autoimmune disease, but more selective T-cell targeting may avoid some of the risks of global immunosuppression. In this vein, anti-CD25 monoclonal antibodies (Daclizumab and Basiliximab), have been used to selectively deplete cells expressing high

affinity IL-2R, namely, Tregs and activated Teffs. Their use allowed lower dosage of immunosuppressive drugs such as cyclosporine in addition to showing success in transplant, leukemia, and autoimmune diseases. Multiple studies show no alteration in frequency or function of Tregs following anti-CD25 antibody treatment, particularly over a short therapeutic course (32). However, others report reduced Treg frequency and function (42,43). These differences likely result from variability of phenotypic markers used to define Tregs. A study in subjects with multiple sclerosis showed therapeutic efficacy of Daclizumab despite a reported Treg decrease; however, a subset of patients developed secondary inflammation (43). Such a functional decline in remaining Tregs could provide complications for administration in type 1 diabetes. However, Daclizumab treatment did not alter clinical outcomes or result in increased inflammation either in combination with mycophenolate mofetil in new-onset type 1 diabetes patients (44) or with Exenatide in subjects with longstanding disease (45).

Recent work emphasizes the importance of DCs in the action of Daclizumab in tolerance induction (46). Pretreatment of DCs with Daclizumab prior to coculture with T cells prevented antigen-specific proliferation. T-cell

pretreatment did not have a significant effect, suggesting that DCs use membrane CD25 to present IL-2 to T cells in *trans* and promote their early expansion (46). The effects of CD25 blockade on the DC-Treg interaction, and the importance of *trans*-presentation in vivo, are as yet undetermined.

Denileukin (Diftitox), a fusion protein consisting of IL-2 fused to diphtheria toxin, was designed to eliminate CD25-expressing leukemia and lymphoma cells, and has been used in an attempt to selectively deplete Tregs in other cancer therapies. However, clinical administration has varying efficacy in Treg depletion, similar to reported data for Daclizumab (47). Prevention of IL-2 signaling, therefore, presents a definite opportunity to ablate activated T cells, but the effect on Tregs remains an important consideration for therapeutic design.

#### ALTERNATIVE APPROACHES TO THERAPEUTICALLY TARGET THE IL-2 SIGNALING PATHWAY

##### **Finding new ways of intervening in type 1 diabetes.**

We earlier postulated that reduced CD25 expression on Tregs weakens IL-2 signaling; additionally, lowered IL-2 responsiveness in activated T cells allows for AICD avoidance. Therefore, another therapeutic opportunity may lie in enhancing surface CD25. Protease inhibitors to prevent receptor cleavage provide an avenue to maintain existing CD25 expression. Alternatively, cytokines such as IL-10 and transforming growth factor- $\beta$  induce expression of CD25 and may serve as effective additional therapeutic agents.

**Personalized medicine and pathway-targeted interventions.** The multicenter genome-wide association studies have led to the discovery and validation of over 50 non-HLA regions that significantly affect the risk for type 1 diabetes. Although the individual contributions of each of these variants may be low, the disease significance of pathway defects may be profound, particularly if several variants impact on a single pathway. It is imperative to study gene-gene interactions and their phenotypic effects along the entire IL-2 pathway to determine if the presence of multiple susceptible alleles would have a synergistic effect on risk for autoimmunity.

PTPN2 is a purported negative regulator of IL-2 signaling through its inhibition of JAK1 and JAK3 (Fig. 5). Decreased protein was observed in T cells of subjects carrying the type 1 diabetes-associated risk allele, a finding that, somewhat counter intuitively, correlated with a reduction in IL-2 response (48). This finding highlights the need for further investigation into the mechanism by which PTPN2 influences IL-2 signaling and may reveal this protein as a therapeutic target for immunomodulation.

It may be feasible in the future to stratify individuals at high risk of developing type 1 diabetes based on their individual pathway defects into clinical intervention trials that would correct a specific immunological deficiency (e.g., IL-2 signaling). Evidence in support of such a tailored approach in type 1 diabetes was provided by the early Diabetes Prevention Trial-Type 1, wherein individuals who exhibited higher titer autoantibodies to insulin responded optimally to oral insulin therapy.

#### CONCLUSIONS

IL-2 is unique in its ability to fine-tune the immune system through diverse mechanisms, due in part to complex regulation of its receptor system as well as downstream

signaling. Components of the IL-2 signaling pathway are found to be deficient in both humans and the NOD model of disease. Although no single genetic deficiency in the pathway is independently sufficient to cause disease, this cluster of disease-associated alleles strongly implicates the IL-2 pathway as a whole in the development of autoimmunity. Indeed, therapeutic interventions directed at augmenting this pathway have been beneficial in treating the NOD mouse, primarily through functional enhancement of Tregs.

The therapeutic history of IL-2 in humans emphasizes the importance of proper dosing to avoid systemic toxicity and achieve desired outcomes, particularly in the context of autoimmune inflammation mediated by T cells. The delicate balance that this cytokine maintains through contraction of activated cells by AICD and Treg maintenance presents a challenge for its administration in the clinical setting. An excess of IL-2 tips the balance in favor of pathogenic autoreactive T cells, allowing them to proliferate beyond the reaches of the Treg repertoire. Redundancies in the  $\gamma$ c cytokine family present an additional hurdle to targeting the IL-2 pathway. It is then likely that successful pathway-targeted therapy in type 1 diabetes must involve a detailed understanding of IL-2R signaling on multiple immune cell types in order to direct pathway-specific agents to fine-tune IL-2 response—providing Treg support while putting a damper on activated autoreactive cells.

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