The IL-2/CD25 Pathway Determines Susceptibility to T1D in Humans and NOD Mice

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Abstract Although the interleukin-2 (IL-2)/IL-2R signaling pathway has been the focus of numerous studies, certain aspects of its molecular regulation are not well characterized, especially in non-T cells, and a more complete understanding of the pathway is necessary to discern the functional basis of the genetic association between the IL-2-IL-21 and IL-2RA/CD25 gene regions and T1D in humans. Genetic variation in these regions may promote T1D susceptibility by influencing transcription and/or splicing and, hence, IL-2 and IL-2RA/CD25 expression at the protein level in different immune cell subsets; thus, there is a need to establish links between the genetic variation and immune cell phenotypes and functions in humans, which can be further investigated and validated in mouse models. The detection and characterization of genetically determined immunophenotypes should aid in elucidating disease mechanisms and may enable future monitoring of disease initiation and progression in prediabetic subjects and of responses to therapeutic intervention.

Keywords Type 1 diabetes · NOD mice · CD25 · IL-2RA · IL-2

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Genetic Control of Type 1 Diabetes

The discovery several decades ago of the association of type 1 diabetes (T1D) with the major histocompatibility complex (MHC) human leukocyte antigen (HLA) class II (chromosome 6p21) region convinced most investigators that T1D was an autoimmune disease [1–3]. This caused a fundamental shift in research focused on preventing T1D, and several trials using the immunosuppressive drug cyclosporine were conducted. Although cyclosporine could reverse T1D in new onset patients, an observation that reinforced the concept that immune cells mediate T1D, the disease emerged almost immediately after drug withdrawal, and moreover, long-term immunosuppression as a cure for T1D has more potentially life-threatening complications than T1D itself [4]. The search for more clues about the pathogenesis of T1D from cellular immunological studies and from the discovery of additional T1D genes characterized much of T1D research in the following decades. The search for other genes with effects on disease susceptibility as large as those of the HLA class II genes failed, and results from the nonobese diabetic (NOD) mouse model of T1D indicated that many genes outside of the MHC influence disease susceptibility [4]. The belief that a greater understanding of the genetics underlying T1D would illuminate disease etiology inspired several investigators to accumulate even larger numbers of DNA samples from T1D patients and controls as well as from T1D families to perform candidate gene association studies [5, 6].

Before recent advances from genome-wide association studies (GWAS) that have associated additional regions with T1D [7, 8], there were four non-MHC genes associated with human TID: the insulin gene (on chromosome 11p15) [9], the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene (on 2q33) [10], the PTPN22 gene

(on 1p13) [11–13], and the interleukin-2 receptor alpha chain (IL-2RA/CD25) gene region (on 10p15) [14, 15]. All four associations emerged from candidate gene studies, and studies on the CD25 gene, which encodes the inducible alpha subunit of the IL-2 receptor, were in part inspired by results from the NOD mouse model showing that a strong genetic effect for T1D mapped to the chromosome 3 region encompassing the IL-2 gene (*Idd3*) [16, 17]. Mice possessing T1D susceptibility alleles at *Idd3* have reduced IL-2 levels in comparison to mice with C57BL/6 (B6)-derived resistance alleles [18], with this allelic variation of the IL-2 gene having at least 46 associated SNPs near and in the gene, resulting in a twofold reduction in IL-2 expression [19]. Data supporting the hypothesis that particular SNPs upstream of the minimal promoter of the IL-2 gene can alter the transcriptional activity of the gene have also been presented [20].

In humans, the T1D-association in the gene region encompassing *IL2RA* was first discovered by Vella et al. [15], using a tag SNP approach [21, 22]: A strong statistical evidence for an association in the region was found ($P=6.5\times10^{-8}$), and this finding was later replicated by Qu et al. [23] in an independent family collection. The association of *IL2RA* with T1D has been further verified in a large-scale genetic fine-mapping study by Lowe et al. [14] that localized the T1D association in the region to two independent SNP groups (groups 1 and 2, Fig. 1). The groups span 14- and 40-kb overlapping regions that encompass *IL2RA* intron 1 and the 5′ regions of *IL2RA* and *RNA binding motif protein* (*RBM17*; OR=2.04, 95% CI=1.70–2.45, $P=1.92\times10^{-28}$). As the two SNP groups found by Lowe et al. [14] localized to a region centered on the 5′ region of both *IL2RA* and *RBM17*, one or both SNP groups could affect either of the genes; however, *IL2RA* is the favored candidate because of its known immunological relevance. Six positive regulatory regions and two negative regulatory elements have been found to be involved in the regulation of *IL2RA* expression [24]. Lowe et al. [14]

resequenced these regions and found no associated SNPs in any of the known regulatory elements, suggesting that disruption of one of these regions is not the cause of the disease association. However, the SNPs may potentially affect CpG dinucleotides [14], noting that CpG dinucleotide methylation is important in gene transcriptional regulation [25]. The genome-wide mapping technique used by Roh et al. [26] has also identified high acetylation levels in the *IL2RA* 5′ region in both resting and activated T cells, and one or more of the associated SNPs may be implicated in histone remodeling and, thus, in *IL2RA* transcription. It is also interesting to note that recently, the Wellcome Trust Case-Control Consortium completed a GWAS involving a scan of 500,000 SNPs across the genome, which showed a significant association between T1D and six previously unknown chromosome regions ($P<5\times10^{-7}$), including 4q27, which contains the IL-2 and IL-21 genes; further fine-mapping is required, however, to better localize the association within this region [8].

Influence of the IL-2/IL-2RA Pathway in Autoimmunity and Tolerance

The genetic evidence that the IL-2/IL-2R pathway is involved in the development of T1D in both humans and mice raises questions concerning how functional variation of this pathway can promote T1D susceptibility and whether the precise mechanisms involved are conserved between species. Moreover, might variation in these pathways alter susceptibility to autoimmune diseases in addition to T1D?

IL-2$^{-/-}$ knockout mice develop autoimmunity, including a type of inflammatory bowel disease [27–29], while mice deficient in CD25 show an initial normal lymphoid development, but subsequently develop peripheral lymphoid organ enlargement as polyclonal T and B cells undergo expansion and activation-induced cell death

Fig. 1 *IL2RA* locus and the T1D-associated SNP groups. The IL-2RA gene is found on chromosome region 10p15. Two groups of SNPs, found in the intron 1 and 5′ regions of *IL2RA* are independently associated with T1D (OR=2.04, 95% CI=1.70–2.45, $P=1.92\times10^{-28}$) [14]
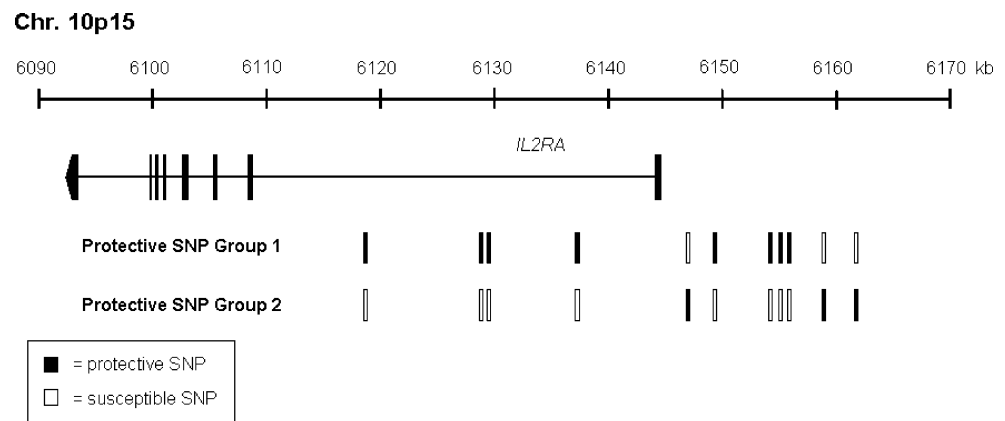
(AICD) is impaired, and with age autoimmunity develops [30]. These observations are consistent with the development of T1D in NOD mice associated with reduced IL-2 levels [18, 19], and furthermore, *Idd3* alleles are known to affect the development of a number of other autoimmune diseases, including autoimmune ovarian dysgenesis [31], experimental autoimmune encephalomyelitis [32], and Sjögren's syndrome-associated manifestations [33, 34]. In addition, experiments utilizing *Idd3* congenic mouse strains demonstrated that mice with *Idd3* susceptibility alleles have reduced sensitivity to tolerance induction to allogeneic [35] and xenogeneic [36] islet grafts. Similarly, protective alleles at *Idd3* and *Idd5* facilitated islet-antigen specific tolerance as compared with NOD mice; the proliferation of auto-reactive CD8⁺ T cells was greatly reduced in the pancreatic lymph nodes (PLNs) upon activation by cross-presented islet antigens and migration to the islets appeared to be eliminated [37].

In humans, van Heel et al. [38] have found evidence of an association in the *IL2-IL21* region with celiac disease ($P=1.3\times10^{-14}$), and this group also found an association in this region with rheumatoid arthritis and confirmed the initial T1D association found by Todd et al. [8], in an independent case-control cohort [39]. Todd et al. [8] also found evidence of an association in this region with autoimmune thyroid disease (Graves' disease). Similarly, apart from its association with T1D, the *IL2RA* region has been found to be associated with Graves' disease [40], rheumatoid arthritis [7], and multiple sclerosis [41] through GWAS, and rare *IL2RA* mutations cause severe autoimmune disease [42, 43].

Thus, there is increasingly strong evidence supporting the hypothesis that genetic variants in the IL-2/IL-2R pathway play a critical role in the balance between tolerance and autoimmunity. Although for many of the above autoimmune diseases fine-mapping is required to find the most associated SNP or group of SNPs, once each independent allele is identified, the process of understanding the mechanism by which the likelihood of breaking self-tolerance is altered by genetic variation at the *IL2-IL21* and/or *IL2RA* regions will require identification of genotype–phenotype correlations for each allele. It is even possible that an allele that is protective for one autoimmune disease would be neutral or confer susceptibility to another depending on aspects of the two disease processes such as disease location, the effector cells and mechanisms used to destroy the targeted self-tissues, and the subsets of regulatory cells engaged to counter each disease. To begin to understand how genetic variation may alter function and lead to disease susceptibility or resistance, we need to consider what is already known about the molecules in question, and about the cellular consequences of their interaction.

IL-2/IL-2R Transcriptional Regulation: Splicing

As the two groups of T1D-associated SNPs in the *IL2RA* region are found in noncoding regions, they could influence overall CD25 transcript levels or splicing; indeed, levels of alternatively spliced CTLA-4 forms vary by genotype and are associated with T1D susceptibility in both humans and mice [10]. A genotype-associated alteration in the mRNA splice form levels of *BANK1*, a gene associated with systemic lupus erythematosus, has also been reported [44].

The full-length, 55-kDa CD25 glycoprotein is encoded by eight exons. The first studies indicating that CD25 has alternative splice forms identified a splice form lacking exon 4 (Δ4) [45, 46]. The Δ4 protein isoform was further characterized by Neeper et al. [47]: Unlike full-length CD25, the Δ4 isoform was incapable of binding IL-2. Horiuchi et al. [48] also provided evidence for another alternatively spliced mRNA from *IL2RA* encoding an isoform lacking the amino acids encoded by exons 5 to 7, which includes the transmembrane region, and is presumably secreted.

A study by Eicher et al. [49] used different IL-2RA/CD25 constructs lacking specific exons to investigate some of the characteristics of the regions encoded by the various exons in IL-2R subunit oligomerization. This work is of particular interest in light of the potential existence of several CD25 isoforms. The trimeric IL-2R comprises CD25 (Fig. 2a), the IL-2Rβ chain (IL-2RB/CD122) (which is shared with IL-15R), and the common γ chain (IL-2RG/CD132) (which is also shared by IL-4, IL-7, IL-9, IL-15, and IL-21 receptors). By using CD25 constructs, it was determined that a deletion of exon 4, but not exons 5 or 6, resulted in an α chain that showed an absence of the IL-2-induced oligomerization response typically observed when full-length CD25 was coexpressed with IL-2RB or with IL-2RB and IL-2RG. Interestingly, it was also observed that, when full-length CD25 was expressed alone, a high baseline reporter signal for oligomerization was detected, indicating that CD25 can undergo a spontaneous, cytokine-independent oligomerization, and this phenomenon was confirmed by fluorescence resonance energy transfer (FRET) analysis. The spontaneous CD25 homo-association was attenuated upon deletion of exon 6, but not exons 4 or 5 [49]. Hence, it seems likely that CD25 splice isoforms may differ in certain functional aspects compared to the full-length form, and it remains to be determined whether any splice isoform variation is correlated with T1D susceptibility.

Although alternatively spliced IL-2 mRNAs have not been studied in the context of autoimmunity, two alternatively spliced IL-2 mRNAs have been identified in humans. These two produce proteins lacking the amino acids encoded by exons 2 and 3, respectively, and act as

Fig. 2 The IL-2R subunits, their affinities for IL-2, and the expression of the cytokine and its receptor by multiple immune cell types. **a** CD25 (IL-2Rα) alone has a low affinity for IL-2, it is incapable of intracellular signaling and may be found at the cell surface as a monomer or homodimer. Upon immune-cell activation sCD25 is generated by proteolytic cleavage. The high-affinity trimeric IL-2R comprises CD25, IL-2Rβ, and the common γ chain (IL-2Rγ). The heterodimer of IL-2Rβ and IL-2Rγ is also capable of binding IL-2, but with an intermediate affinity. **b** Although the IL-2/IL-2R interaction has been most studied in T cells, IL-2 and its high or intermediate affinity receptors are expressed by various immune cell types
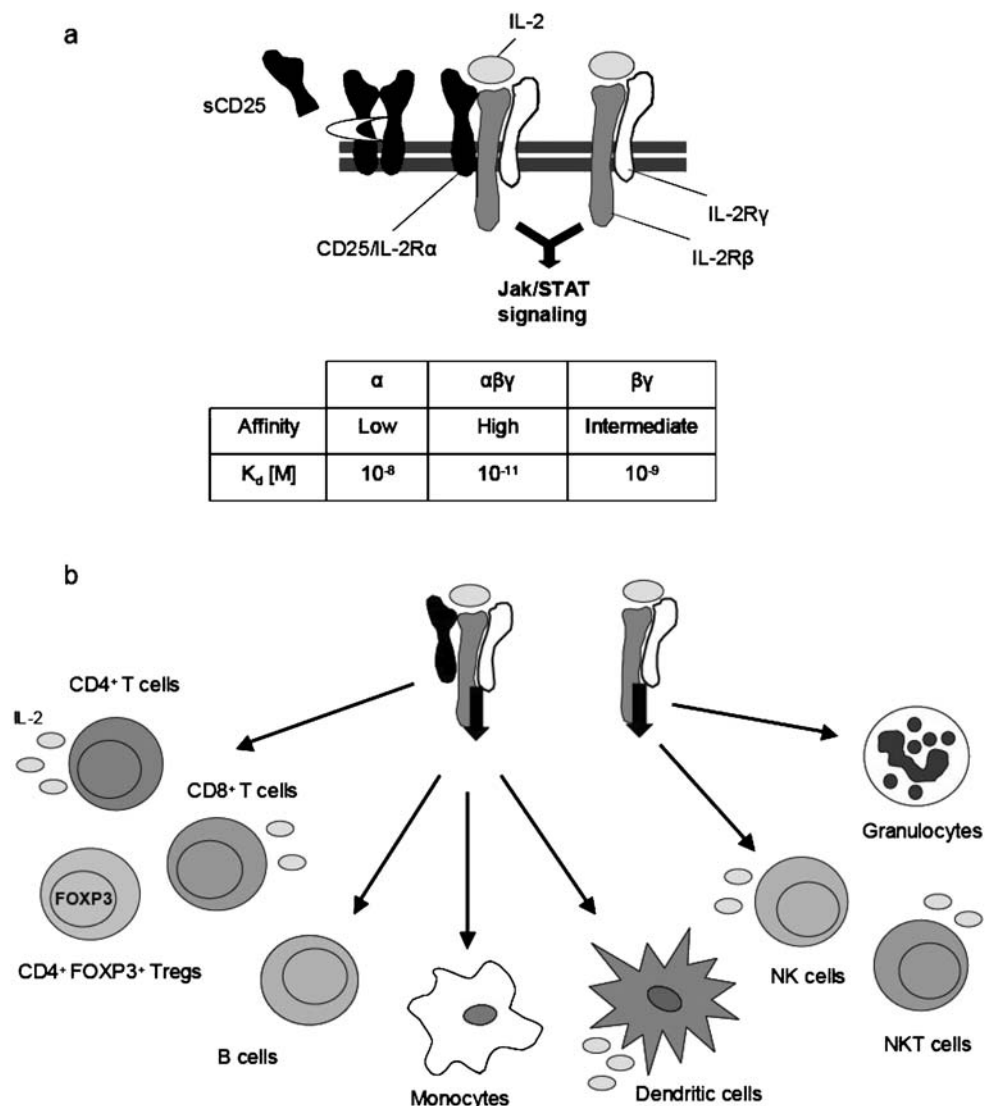
competitive inhibitors of full-length IL-2, blocking its ability to costimulate T cell proliferation and bind to the trimeric IL-2R, in a dose-dependent fashion [50].

The IL-2/IL-2R Complex and Downstream Signaling

CD25 alone has a low affinity ($K_d \sim 10$ nM) for IL-2 [51]. The dimer of IL-2RB and IL-2RG is also capable of binding IL-2 with an intermediate affinity ($K_d \sim 1$ nM) [46]. The high-affinity trimeric IL-2R complex has a K_d of ~ 10 pM [52] (Fig. 2a). Rickert et al. [53] solved the crystal structure of IL-2 bound to CD25. CD25 has two extracellular domains (D1 and D2) that are homologous to a set of β-sandwich protein domains known as sushi domains, complement control protein repeats or short consensus repeats [54, 55]. The structure of the trimeric IL-2R ectodomains bound to IL-2 has been solved by Wang et al. [70] and, subsequently, by Stauber et al. [56]. Interest-

ingly, CD25 does not contact either IL-2RB or IL-2RG (noting that only the ectodomains of the receptor chains were present); Wang et al. [57] proposed that the basis of the cooperativity of the trimeric receptor is a decrease in entropy whereby CD25 binds and concentrates IL-2 at the cell surface for presentation to IL-2RB and IL-2RG, or alternatively, a CD25-induced alteration in IL-2 conformation may occur, hence stabilizing the complex [57].

When IL-2-induced oligomerization of the trimeric IL-2R occurs, this results in the activation of the JAK1 and JAK3 cytoplasmic protein tyrosine kinases that are associated with the intracellular domains of IL-2RB and IL-2RG, respectively. These kinases then phosphorylate certain IL-2RB tyrosine residues that act as docking sites for signal transducer and activator of transcription (STAT)5a and STAT5b, for example [58]. The IL-2-induced activation of numerous signaling pathways eventually results in the transcription of target genes that contribute to the many functions associated with the IL-2/IL-2R pathway [59–61].

There is also some evidence that CD25 on one cell can present IL-2 to IL-2RB/IL-2RG dimers on another cell, in a high-affinity, intercellular interaction [62]. Even though the signal transduction cascades downstream of the IL-2/IL-2R binding event have been quite extensively studied in T cells, much remains to be elucidated with respect to the status of these downstream pathways in non-T cells.

The IL-2/IL-2R Pathway and T cell Function

The IL-2/IL-2R signaling pathway is known to have a pleiotropic role in T cell biology, and the in vivo necessity for this signaling pathway according to T cell type and function has been recently reviewed [61]. In 1980, Mier and Gallo [63] identified IL-2 as an autocrine factor necessary for the long-term culture of CD4$^+$ T cells, and the involvement of IL-2 in T cell responses is widely accepted. The activation of T cells through their T cell receptor (TCR) along with costimulatory signaling results in the production of IL-2 and the expression of the IL-2R at the cell surface. The interaction of the cytokine with its receptor results in clonal expansion and the differentiation of effector cells [61, 64], as this interaction drives a STAT5-dependent positive feedback loop that induces CD25 upregulation and, hence, increased signaling through augmented IL-2 capture by its receptor on the activated T cells [65]. IL-2R-induced signaling also leads to repression of IL-2 transcription, whereby IL-2 negatively regulates its own production [66, 67]. In addition to downregulating its own generation, IL-2 alters the immune response by causing T cells to become susceptible to apoptosis following a further strong TCR cross-linking that increases the expression of death-mediating molecules in the Fas and tumor necrosis factor (TNF) pathways, in a process termed AICD [68].

Although these findings support the involvement of the IL-2/IL-2R pathway in the development of immune responses, IL-2- or CD25-deficient mice are still capable of developing effective immune responses in vivo upon antigenic challenge [61, 69], suggesting that this pathway may actually be dispensable. One difficulty in interpreting studies using such mice is that the autoimmunity associated with the lack of IL-2 or CD25 may provide a confounding setting in which a requirement for IL-2 may be circumvented. Yet this does not seem to be the case: For example, TCR-transgenic IL-2- or CD25-deficient mice (that in some cases have been further crossed with Rag knockout mice) [70, 71] have little or no autoimmune disease, but they are still capable of developing effective immunity despite the lack of IL-2/IL-2R signaling, suggesting that this signaling pathway may indeed have a redundant role in the elicitation of an immune response [61, 69]. However, there is some evidence that IL-2/IL-2R signaling may be mandatory in the CD8$^+$ memory T cell responses associated with long-lasting T cell immunity [72, 73], and in light of the possibility of compensatory mechanisms to become enhanced when IL-2 or CD25 are deficient, the true contribution of IL-2/IL-2R signaling in T cell responses in vivo remains to be determined.

Apart from its function in stimulating effector T cells, the IL-2/IL-2R pathway also has a role in the maintenance of self-tolerance. CD25 is constitutively expressed at high levels on CD4$^+$ Foxp3$^+$ regulatory T cells (Tregs), which suppress putatively autoreactive peripheral T cells that escape thymic deletion during the establishment of central tolerance [74–77], and Treg depletion causes autoimmunity in mouse models [78]. Foxp3 is a transcription factor that is fundamental for Treg production and function [79–81]: Targeted ablation of the Foxp3 gene results in severe autoimmune manifestations in mice, and in humans, natural mutations of the orthologous gene lead to immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX) syndrome [82–84] and X-linked autoimmunity-allergic dysregulation syndrome [85]. A notable feature of Tregs is their inability to produce IL-2, even upon TCR stimulation [86], and this transcriptional repression is mediated by Foxp3 and its interaction with NFAT and AML1/Runx1 [87, 88]. The interaction of Foxp3 with these transcription factors is generally responsible for the establishment of the Treg signature, which includes the high expression of CD25 [87, 88]. The incapacity to produce IL-2 themselves along with their high CD25 levels renders Tregs uniquely sensitive to paracrine IL-2, and this raises questions regarding the detailed mechanisms by which the IL-2/IL-2R pathway contributes to Treg development and function.

Natural Tregs develop in the thymus, and this development requires binding of self-antigen to the TCR and costimulation through CD28 [89, 90], the latter contributing to Foxp3 induction and to the production of IL-2 by other T cells that may act as a paracrine supply of the cytokine for the developing Tregs [91]. IL-2 has been shown to increase Foxp3 levels [92], and as the development and maintenance of Tregs rely on the sustained, high expression of Foxp3, the IL-2/IL-2R pathway may indeed be involved in Treg generation and lineage commitment. Interestingly, utilizing an ins-HEL TCR transgenic mouse model, it has been demonstrated that IL-2 plays a critical role in the level of autoantigen-specific Tregs that are selected in the thymus and thus found in the periphery, and it has been suggested that IL-2 promotes the development of Tregs with a high avidity for self, which may be more efficient in suppressing autoreactive T cells [93]. Apart from the thymic-derived Tregs, conventional T cells can become Foxp3$^+$-induced Tregs in the periphery [94], and considering the potential

role of IL-2/IL-2R signaling in natural Treg development, it seems likely that this pathway may be involved in the generation of these induced Tregs. In addition, the IL-2/IL-2R interaction is required by Tregs in the periphery for the maintenance of their homeostasis and competitive fitness in vivo [95].

CD4$^+$ CD25^{hi} Foxp3$^+$ Tregs and T1D

Given the importance of Tregs in peripheral tolerance, the involvement of the IL-2/IL-2R pathway in their development and function, and the genetic association of components of this pathway with T1D in both humans and mice, the Treg population has been much studied in the context of this autoimmune disease.

Treg depletion or blocking of the B7/CD28 pathway (which decreases Treg frequency) in NOD mice leads to accelerated disease onset [89, 90], as does IL-2 neutralization [96]. Furthermore, T1D resistance in aged NOD mice has been correlated with Treg expansion in PLNs [97, 98], and Foxp3-deficient NOD mice show earlier onset and a higher incidence of T1D compared to normal NOD mice [99]. More recently, Tritt et al. [100] have investigated the frequency and function of Tregs in the primary and secondary lymphoid tissues in the NOD mouse model; the researchers found no differences in Treg numbers between T1D-susceptible and T1D-resistant mice, and although the Tregs did not affect the priming or initial expansion of diabetogenic T cells, they did influence the differentiation of these cells in the PLNs.

Yamanouchi et al. [19] provided direct evidence that IL-2 affects Tregs in the context of T1D by showing that diabetes susceptibility associated with a NOD allele at *Idd3* is indeed because of allelic variation of the IL-2 gene, with an approximately twofold reduction in expression correlating with disease. Engineered haplodeficiency of IL-2 gene expression also reduced IL-2 production by T cells and resulted in dysregulatory effects similar to those of the naturally occurring *Il2* diabetes susceptibility alleles. Moreover, either the natural or engineered deficiency of IL-2 production correlated with reduced Treg function. In accordance with this, Tang et al. [101] found a progressive decline in the number of Tregs relative to effector T cells in inflamed NOD mouse islets (but not in the PLNs), with the intra-islet Tregs expressing decreased amounts of CD25 and Bcl-2, indicating a Treg decrease in numbers by apoptosis, potentially because of an IL-2 deficiency, as IL-2 can induce both CD25 and Bcl-2 in T cells [102]. The administration of low-dose IL-2 was capable of promoting Treg survival (noting that these Tregs had high levels of CD25) and preventing diabetes.

In humans, several studies have reported either numerical [103] or functional [104, 105] defects in the Treg population in T1D patients, while one study suggests no Treg-related defects at all [106]. Tree et al. [107] have collectively considered these conflicting results and suggest that the numerical deficiency in Tregs observed by Kukreja et al. [103] may be because of the methodology by which this cell population was defined and the fact that the ages of their T1D patients and controls were significantly different. Recently, Brusko et al. [108] have more extensively investigated Treg frequency and absolute numbers in long-standing and new-onset T1D patients, their relatives and healthy controls with no significant differences being found. Notably, Treg frequency was determined to be age independent [108], and there is some evidence that Tregs are maintained by the rapid turnover of memory populations in vivo [109]. Although, in mice, Foxp3 is only expressed in Tregs [95], in humans, transiently activated effector T cells can also express the transcription factor [110], and thus, definitive Treg identification requires functional testing for suppressive capability. In relation to Treg functional defects in T1D patients, Tree et al. [107] indicate that the discordance between studies may be because of the different stimulatory conditions utilized in the assays measuring suppressive capacity. It has been shown that the strength of stimulation used to activate effector T cells can greatly affect the suppressive capacity of Tregs [111], with increasing T cell signal strength reducing the Treg suppressive capability [112]. Hence, Brusko et al. [104] and Lindley et al. [105] may have been able to observe a defect in Tregs from T1D patients because of the precise stimulatory conditions used. To date there has been no investigation of Treg number or function in relation to genetic variation at the *IL2RA* or *IL2-IL21* regions in humans.

Non-regulatory T cells and T1D

Apart from a potential IL-2/IL-2R-associated defect in Treg numbers and/or function, an alternative, although not mutually exclusive, possibility is that an IL-2/IL-2R-associated defect in effector T cells renders them resistant to Treg suppression, therefore, resulting in a breakdown of peripheral tolerance that promotes susceptibility to T1D. Indeed, an acquisition of resistance to suppression by effector T cells with time as diabetes progresses in NOD mice has been observed [113–115]. A study by Waithman et al. [116] indicates that the provision of IL-2 signaling through the use of an activatory anti-IL-2 antibody-IL-2 complex can drive tolerant, naïve T cells toward autoimmunity, and memory T cells were also found to upregulate CD25 subsequent to self-antigen recognition. These results

suggest that enhanced IL-2 signaling can induce autoimmunity, and when considered along with the IL-2 deficiency associated with T1D susceptibility at *Idd3*, these otherwise paradoxical findings imply that there is a fine balance between tolerance and autoimmunity and that there is a necessity for the maintenance of an optimal level of IL-2/IL-2R signaling, given the multiple functions of this pathway.

The role of nonregulatory T cells in human T1D has focused primarily on the antigen-specificity of the effector T cells, although a correlation has been identified between genotype at the *IL2RA* region and soluble CD25 (sCD25) [14]. sCD25 is considered to be a marker of immune-cell activation as increased amounts, above the basal circulating level, are observed during acute graft rejection or infections [117]. Although alternatively spliced mRNA species can encode non-membrane bound CD25 as discussed above, it has been postulated that most of the sCD25 found in the blood is generated by proteolytic cleavage as T cells become activated and proliferate [118], and there is conflicting evidence regarding its monomeric [119] or dimeric [120] nature under physiological conditions. Tregs proliferate in vivo more quickly than do non-Treg CD4$^+$ T cells [109, 121–123] suggesting the possibility that Tregs contribute to blood levels of sCD25. Competitive binding studies indicate that sCD25 binds IL-2 with low affinity [124], although in vivo functional properties remain to be elucidated. The half-life of sCD25 is about 40 min in the circulation [125], and it has been suggested that sCD25 inhibits bioactive IL-2 [126–128]. Kobayashi et al. [125] have evaluated the extent of the protection from degradation conferred by sCD25 on IL-2. sCD25 was found to protect IL-2 from in vitro inactivation.

Lowe et al. [14] measured the total sCD25 concentration in plasma samples from 1,357 T1D cases and found that susceptibility at the *IL2RA* region was significantly associated with lower sCD25 levels ($P=6.28 \times 10^{-28}$). The functional significance of this lower level of sCD25 in T1D patients with susceptibility alleles at *IL2RA* is unknown, but it could suggest a reduced T cell activity and/or proliferation, which is potentially indicative of a lowered state of T cell responsiveness in vivo, and which might, very speculatively, be consistent with the *Idd3*-associated IL-2 deficiency observed in NOD mice. Such hyporesponsive T cells have been reported in T1D patients in vitro [129] and in rodent autoimmune-prone models ex vivo [130]. However, much remains to be determined regarding the significance of the *IL2RA* genotype-sCD25 correlation; any functionality of sCD25 is unknown, as is the actual cellular origin of it, as there is evidence that non-T cells can also produce it (see the next section).

The IL-2/IL-2R Pathway in Other Immune Cell Types

Even though the function of the IL-2/IL-2R pathway has been most studied in T cells historically, there is now increasing evidence from work in both humans and mice that this pathway has a much broader immunological function (Fig. 2b) that needs to be investigated with respect to the function of the genetic variants in the IL-2/IL-2R pathway that influence T1D and other autoimmune diseases.

Aside from T cell-related IL-2 production, dendritic cells (DCs) also produce IL-2 upon activation with microbial stimuli [131], and natural killer (NK) and NKT cells are capable of generating the cytokine as well [96, 132, 133]. Although the functional consequences of NK- and NKT-cell IL-2 production are not clear, DC-associated IL-2 secretion plays an important role in T cell priming: Granucci et al. [131] found that mouse IL-2$^{-/-}$ DCs were impaired in their ability to induce CD4$^+$ and CD8$^+$ T cell proliferation. Feau et al. [134] subsequently showed that human myeloid and plasmacytoid DCs could also produce IL-2, and they showed that this production was regulated by IL-15 in both humans and mice. The ability of DCs to produce IL-2 raises the question of whether the cytokine may affect these cells in an autocrine manner, rather than acting only as a paracrine factor. Both human and mouse DCs have been found to express CD25 at their plasma membrane [135, 136]. Work by Naranjo-Gomez et al. [137] indicates that CD25 can be quickly upregulated on CpG- and CD40L-activated human plasmacytoid DCs within 6 h of activation, and the administration of exogenous IL-2 increases the secretion of pro-inflammatory cytokines such as TNF-α, as well as plasmacytoid DC survival. Mnasria et al. [138] used lipopolysaccharide (LPS) to upregulate CD25 at the surface of myeloid DCs, and the addition of antagonistic anti-CD25 antibodies to the LPS stimulation resulted in decreased production of pro-inflammatory cytokines and impaired ability of the DCs to prime T$_H$1 cells. Hence, IL-2/IL-2 signaling is important in DCs themselves, for their survival and for the generation of appropriate inflammatory and adaptive immune responses.

Physiologically, the intercellular presentation of IL-2 may play a role in disseminating IL-2-mediated T cell/DC activation and in activating bystander IL-2RB/IL-2RG-expressing cells. Such cells include NKT cells [139], and NK cells, whose activity is augmented upon IL-2 administration [140–142], and it has more recently been shown in mice that myeloid DC-derived IL-2 directly acts on NK cells, stimulating these cells to produce interferon-γ (IFNγ), that in turn promotes NK-cell cytotoxicity [143]. Resting neutrophils also possess the intermediate-affinity IL-2R, and the IL-2-mediated effects on these cells include resistance to apoptosis [144], enhanced anti-fungal activity [145], and increased protein synthesis and cytokine pro-

duction [146–148]. Jung et al. [149] reported that CD25 is expressed at the surface of activated B cells and that recombinant IL-2 stimulates their proliferation, thus confirming the existence of a direct effect of IL-2 on B cells, as had been previously proposed by Swain et al. [150] and Parker [151]. Monocytes also constitutively express the intermediate affinity IL-2R [152], and CD25 expression can be induced at the cell surface upon activation with various stimuli, including LPS [153, 154]; moreover, after activation, the supernatants of activated monocytes have high levels of sCD25 [153]. Consistent with these findings are the gene expression profiling results of Martinez et al. [155] regarding monocyte-to-macrophage differentiation and polarization. These results show that classically polarized M1 macrophages, which are induced by IFNγ with or without microbial stimuli such as LPS or cytokines such as granulocyte-macrophage colony-stimulating factor, have higher CD25 transcript levels than nonclassically activated M2 macrophages.

The IL-2/IL-2R interaction may also have functions outside of the immune system. IL-2 is able to augment endothelial cell proliferation and hence vascularization [156]. Other studies have shown that IL-2 affects enterocyte ion secretion and proliferation [157], and it has also been demonstrated to be an important neuroregulatory molecule implicated in neurite survival and extension, oligodendrocyte proliferation, hypothalamic-pituitary function and analgesia [158].

Conclusion

Although the IL-2/IL-2R signaling pathway has been the focus of numerous studies, certain aspects of its molecular regulation are not well characterized, especially in non-T cells, and a more complete understanding of the pathway is necessary to discern the functional basis of the genetic association between the IL-2-IL-21 and IL-2RA/CD25 gene regions and T1D in humans. Genetic variation in these regions may promote T1D susceptibility by influencing transcription and/or splicing and hence IL-2 and CD25 expression at the protein level in different immune cell subsets; thus, there is a need to establish links between the genetic variation and immune cell phenotypes and functions in humans, which can be further investigated and validated in mouse models. The detection and characterization of genetically determined immunophenotypes should aid in elucidating disease mechanisms, and may enable future monitoring of disease initiation and progression in prediabetic subjects and of responses to therapeutic intervention.

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