Interleukin-2 and type 1 diabetes: New therapeutic perspectives

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Abstract

A new sort of CD4+ T cells, so-called regulatory T cells (Tregs), has been described in 1996. Tregs are suggested to have an important function consisting in controlling autoimmune reactions. In humans, absence of Tregs induces the IPEX syndrome characterized by the presence of several autoimmune diseases. These cells depend on interleukin-2 (IL-2) for proliferating and controlling the T effector cells (Teff) reaction, but they do not have the capacity to produce IL-2. In type 1 diabetes (T1DM), a hypothesis is that a lack of IL-2 in pancreas could prevent Tregs action and lead to beta cells destruction. In NOD mice, low dose IL-2 treatment at the initial time of diabetes can rescue insulin secretion by restoring proteins expression that are necessary for Tregs regulatory function in the pancreas. Using low doses instead of high doses IL-2 prevents Teff activation which also depends on IL-2. These results led to conduct a dose-effect trial in human T1DM. This trial aimed at determining the therapeutic condition, which induces Tregs activation without major side effects, in a therapeutic perspective to recover insulin secretion at the apparition of diabetes.

Keywords: IL-2; Type 1 diabetes; Regulatory cells; Review

1. What are regulatory T cells (Tregs)?

Although multiple cells of the immune system have been identified as contributing to the pathogenesis of type 1 diabetes, a growing interest has recently been directed toward a population so-called regulatory T cells (Tregs). In 1996, Sakaguchi and al. could contribute to loss of self-tolerance in patients with type 1 diabetes.
demonstrated that transfer of cells depleted in CD4+ CD25+ T cells (which means cells co-expressing CD4 and α-chain of the interleukin (IL)-2 receptor complex called CD25), into nude mice, resulted in the onset of autoimmune diseases including diabetes [1]. Co-transfer of disease-inducing cells with these CD4+ CD25+ cells, prevented disease development. Tregs were then defined by this co-expression of CD4 and CD25 [2].

CD25 is a component of the IL-2 high affinity receptor that consists of three units, IL-2Rα (CD25), IL-2Rβ (CD122), and γc (CD132). The high affinity IL-2R expression is usually induced on T cells upon antigen activation, and very transiently observed. In contrast, Tregs are characterized by apparent constitutive expression of this receptor [3] and the CD25high phenotype marks Tregs. IL-2 dependent signal transduction is also quantitatively distinctive between different T cell subsets. CD4+ and CD8+ conventional T cells (Teffs) and CD4+ Tregs similarly activate Stat5 after IL-2R binding, but they distinctly activate the PI3K-Akt pathway [3]; PI3K-A pathway favours Teffs proliferation but opposes Tregs production.

As CD25 is also expressed on other lineages of T-cells, reagents have been developed that are capable of identifying a specific transcription factor more intimately associated with Tregs, transcription factor forkhead box P3 (FOXP3). The link between FOXP3 expression and Tregs homeostasis and function is well illustrated in humans by the rare polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, in which loss-of-function mutations in the FOXP3 gene lead to severe autoimmunity. In mice, FOXP3 deficiency induces lack of Treg cells, and overexpression of the FOXP3 protein leads to profound immune suppression [4]. Thus, Tregs are usually defined on the basis of their constitutive expression of high level of CD25 and FOXP3. But several groups suggest that another marker, the IL-7 receptor (CD127), may be used to identify Tregs, which are all FOXP3+ but could be either CD25high or CD25low or neg [5]: CD127 expression inversely correlates with FOXP3 and suppressive function. So, Tregs could be better defined as being CD25+ FOXP3+ CD127−.

At least two types of CD4+ Tregs have been described on the basis of their origin, function, and mechanisms of action. Natural Tregs (nTregs) are generated in the thymus and are specific for self-antigens. Induced Tregs (iTregs) stem from mature CD4+ CD25FOXP3− precursors at the periphery, following adequate antigenic stimulation in the presence of TGF-β and IL-2 [6,7]. iTregs can be distinguished from nTregs on the basis of FOXP3 DNA methylation in a defined region within the FOXP3 locus (the Treg specific demethylated region, TSDR) which is found to be constantly demethylated exclusively in nTregs [8].

2. What is the role of IL-2 in Treg function?

Tregs potently suppress activation, proliferation and effector functions in both CD4+ and CD8+ T cells through cytokines and contact-dependent mechanisms. IL-2 plays a critical role on the peripheral survival and suppressive function of Tregs [9]. There is a functional link between IL-2R and control of FOXP3 expression: IL-2R-deficient mice contain immature Tregs that express a low amount of FOXP3, and forced expression of FOXP3 in IL-2Rβ-deficient mice restores Tregs production [3]. So IL-2 increases the expression of FOXP3 and like usual of its own receptor CD25 on Tregs.

But Tregs do not have the capacity to produce IL-2! This inability to produce IL-2 renders Tregs dependent on paracrine IL-2 for their development, homeostasis and function. The main IL-2 producing cells are activated conventional CD4+ and CD8+ T cells, but autoactive T cells themselves represent a possible source of IL-2. Physiologically, Tregs preferentially utilize IL-2 after antigenic challenge [10]. A model could be that autoreactive effectors T cells recognize autoantigens presented by dendritic cells, produce IL-2 when activated, thus activate specific Tregs which are already engaged by TCR, and Tregs in turn could suppress autoreactive T cells, thereby maintaining immune tolerance [3].

3. Implication of Tregs in animal models of autoimmune diabetes

The role of Tregs in the establishment and progression of T1DM has been intensely studied in Non Obese Diabetic (NOD) mice. Tregs constitute only 5% of the circulating CD4+ T cells in NOD mice, significantly lower than that observed in other strains [11]. NOD Tregs paradoxically increase in the peripheral lymph nodes at the time of diabetes onset, but consistently lower proportions of Tregs are found in the pancreas compared with lymphoid tissues [12]. Freshly isolated polyclonal Tregs delay diabetes when co-transferred with diabetogenic T cells into immune-compromised NOD mice [13]. Islet specific Tregs expanded in vitro and then injected to diabetic NOD mice, induce long-lasting reversal of hyperglycemia in 50% of mice [14]. Interestingly, in a modified transgenic model of autoimmune diabetes where mice are rendered completely devoid of Tregs, no differences in either the initial activation of conventional T effector cells in pancreatic draining lymph nodes, or the rate of islet T cells infiltration are observed, but this insulitis is immediately destructive, causing a dramatic progression to diabetes [15].

4. Does a defect in Tregs function or number in T1DM patients contribute to the disease pathogenesis?

It is now clear from several studies in humans that the number of peripheral CD4+ CD25+ T cells is normal in newly diagnosed [16] [17] or long lasting [18] T1DM patients. The mean frequency of circulating CD4+ CD25+ FOXP3+ is about 5% of CD4+ T cells. Others groups [5] [19] using FOXP3 expression as a marker for Tregs also found the same percentage of FOXP3+ T cells between control and T1DM individuals, and no decrease in the number of these cells in the first degree relatives of T1DM patients [20]. However, results concerning Tregs functions are disparate. Putnam et al. [18] (2004) suggested that the suppressor function of Tregs was normal in T1DM whereas others groups [16] [17] showed that, when co-cultured with CD4+ CD25− T cells, CD4+ CD25+ T cells from newly diagnosed T1DM patients displayed significantly impaired suppressor function when compared with a group of HLA-
age-matched control individuals. These results opened the question whether Tregs in T1DM patients were intrinsically less effective, or whether the responder Teffs cells were more resistant to suppression. In 2008 Schneider et al. [21] highlighted that Tregs coming from T1DM subjects function normally to suppress Teffs when the latter come from healthy controls, and that the T1D Teffs were resistant to suppression in the presence of Tregs coming from healthy subjects. These results suggested that the defects in suppressive function observed in vitro are not intrinsic to Tregs, but are predominantly the result of Teffs from T1DM patients that could be resistant to suppression.

However, above results all concern non-antigen specific Tregs, and very few groups have studied islet specific Tregs. Long et coll [22] have shown that Tregs specific for GAD can normally be induced from peripheral CD4 + CD25−FOX3+ T cells of T1DM patients, and these specific Tregs are capable of suppressing CD4 + T cells proliferation in vitro in an antigen specific manner. In conclusion, at least peripheral Tregs in T1DM patients are normal in number and function.

5. Hence finally, is there any problem with Tregs in T1DM?

It is important to note that circulating Tregs frequency and function may not be representative of cell populations at the site of inflammation. Recently a group had the opportunity to study human pancreatic lymph nodes (PLNs) coming from long standing T1DM patients who were candidates for a pancreas or kidney/pancreas transplantation [23]. These results showed that:

• in PLNs of T1D patients thymic-derived nTregs frequency is normal, but surprisingly some of these cells do not express FOXP3 protein;
• Tregs (CD25bright, fax sorted) isolated from the PLNs of diabetic patients have an impaired suppressive capability whereas Teffs from PLNs are not refractory to suppression;
• surprisingly in vitro depletion of Tregs from the PLNs of diabetic patients leads to a reduced proinsulin specific cell response rather than an increased one;
• and PLNs of diabetic patients are enriched in Th17 proinflammatory T cells.

Altogether these results suggest that in the pancreatic draining lymph nodes of T1DM patients, T cells that are epigenetically imprinted to be regulatory do not function as such, and turn off FOXP3 expression, leading to defective control of Th17 cells which have an established role in several autoimmune diseases. These data are in favor of a dysfunction of Tregs at the site of inflammation, which could be secondary to an insufficient capacity, in unknown conditions, to maintain expression of FOXP3.

The validity of this hypothesis is reinforced by Marwaha et al. results who described in peripheral blood of children with new-onset T1DM [24] an increased proportion of a surprising subset of T cells that express a low amount of FOXP3 protein, an intermediate amount of CD25, and secrete the proinflammatory protein IL-17. These cells have no suppressive capability in vitro. This kind of cells was already described in systemic lupus subjects. Are these cells primary activated effector T cells that transiently express FOXP3, or rather could they derive from Tregs that failed to maintain sufficient level of FOXP3 expression, and subsequently have converted to a proinflammatory IL-17 secreting phenotype? [25]. A hypothesis is that a local deprivation in IL-2 could have favoured this conversion.

6. Is IL-2 linked to Tregs dysfunction in autoimmune diabetes?

Several arguments suggest that IL-2 deprivation could be partly responsible for Tregs dysfunction in T1DM. NOD mice present a qualitative diminution of IL-2 production [26] and a genetic predisposing factor to T1DM development is linked to IL-2/IL-2R gene polymorphism in NOD and humans [27] [28]. Furthermore Tregs in the NOD inflamed islets have a concomitant reduction of CD25 expression [29] and CD25 expression is under the control of IL-2.

In humans, peripheral CD4+CD25high cells coming from recent T1DM patients or subjects at high risk for T1DM (first degree relatives with 2 or 3 autoantibodies) show significantly increased apoptosis markers [30] and modulation of several apoptosis genes [31] compared with long standing T1DM and T2D subjects. These changes in gene expression mimic that observed in conditions of in vitro IL-2 deprivation in healthy humans, which induces a strong apoptosis of Tregs cells but not of Teff cells [31]. This suggests that the expression signature in Tregs from T1DM patients may be partially induced by a cytokine deficient milieu. Furthermore, maintenance of FOXP3 expression in CD4 + CD25+ in adult T1DM subjects is diminished in vitro compared with age matched controls. Persistence of FOXP3 expression is maintained in the presence of IL-2 in controls, but significantly decreased in T1DM. This could be possibly explained by a defect in IL-2R signaling [32]. Altogether these data suggest that lack of IL-2 or dysfunction in IL-2 action could be, at least in part, responsible for Tregs dysfunction in T1DM.

7. Could IL-2 be a therapeutic option in T1DM?

As the binding of IL-2 to CD25 and subsequent STAT5 signalling are essential for the survival of Tregs in the periphery, it was suggested that the selective loss of the Treg cells/Teff cells balance in the inflamed islets in NOD mice could be due to apoptosis as a consequence of local IL-2 deficiency. Studies were initiated to determine whether a correction of this IL-2 deficiency would improve Tregs survival and confer disease protection. Unfortunately a high-dose IL-2 treatment enhanced functions of pathogenic Teff cells and, despite a higher number of Tregs, resulted in accelerated autoimmune tissue destruction [29]. However because Tregs constitutively express the high affinity IL-2R CD25, authors hypothesized that a lower dose of IL-2 might selectively act on Tregs with minimal effect on CD4+ and CD8+ Teff cells. Indeed they found that a low
IL-2 dose led to a moderate increase in Tregs percentages with a mild increase in Teff cells, and that a treatment with this low dose prevented diabetes development in the majority of female NOD mice [29]. These results confirmed the hypothesis that the problem was not an intrinsic defect of NOD Tregs, but rather a reduced availability of IL-2 in the pancreas. Then the question was if an increase in the number and function of pancreatic Tregs could cure already established diabetes. The answer was positive when it was shown that only five days of low-dose IL-2 administration at diabetes onset could induce long-lasting remission in the treated animals [12]. At this low dose, IL-2 treatment did not modify the peripheral homeostasis of haematopoietic cells, did not increase the number of pancreatic Tregs, but induced an increase in the expression of CD25 and FOXP3 of these cells. The proportion of Teff was unaltered in the pancreatic inflammation, but IL-2 treatment induced an important reduction of IFN-γ production specifically in pancreatic CD8+ T cells.

In conclusion low-dose IL-2 in NOD mice cures already established diabetes, at least partly by increasing regulatory function of Tregs at the site of inflammation. What can be done in humans? Several options are possible. Long et al. [22] showed that islet-specific Tregs can be generated in vitro from T1D patients, and injecting islet specific Tregs can reverse established diabetes in NOD mice. However at present the lack of good manufacture practice procedures to obtain antigen-specific Tregs in humans precludes the translation of such approach to the clinic. Stimulating the patient’s own Tregs compartment to down-regulate the autoimmune process represents a more accessible alternative. The association of IL-2 with rapamycin (sirolimus) was recently tested in recent-onset T1DM because it had been previously shown that rapamycin in vitro could selectively expand Tregs and deplete Teff cells [19], and in vivo rapamycin treatment could increase suppressive capability of Tregs [33]. Unfortunately in a recent trial, when rapamycin was associated with IL-2 in recent-onset T1D, it led to an accelerated loss of C-peptide (Long S and al, abstract presented at ADA 2011), probably because of an intrinsic toxic effect of rapamycin on beta-cell, and/or on insulin sensitivity.

Another option is to give IL-2 alone. In human it was shown that high doses of IL-2 (15 doses of 700 000 IU/kg) induce a significant increase of CD4+ CD25high T cells expressing FOXP3, and these cells exhibited suppressive function in vitro [34]. But these high doses also activate Teff cells what is not favourable in autoimmune diseases. They are only used in severe metastatic cancers to stimulate anti-cancer immunity, because they also have important side effects like pulmonary oedema. Conversely low doses of IL-2 are supposed to selectively enhance Tregs number and function. They were recently used in the treatment of graft versus host disease, and in this context patients exhibited increase Tregs frequency without serious side effects. They were also used in vasculitis induced by the hepatitis C virus, where low dose IL-2 treatment showed no T effectors activation nor HCV viremia increase, but was followed by an increase in the percentage of Tregs, with reduction in cryoglobulinemia in 9 of 10 patients [35]. In adult T1D, a preliminary trial with various low doses of IL-2 during 5 days is conducted to find the lowest dose capable of inducing a significant increase in Tregs number (clinicaltrials.govNCT01353833), and a therapeutic trial aiming at preserving C-peptide in recent-onset T1D is coming.

8. Are low-doses IL-2 safe?

No serious events were observed in several short duration studies but, like for all new drugs, only long duration trials will give us full answer on benefit/risk ratio. An important issue is about anti-cancer immunity. Tregs have been shown to accumulate in tumors, and their number increases with tumour progression. What that means (is this a benefit or deleterious reaction to tumor induced inflammation?) is still being elucidated because in vivo, increased Tregs number correlates either with reduced or with better survival in humans, depending on cancers [36]. Some groups showed that depleting Tregs helps to increase tumour-specific effector T cells responses [37] [38] but the beneficial impact of this strategy on survival has not already been clearly proved in humans. In mice [39,40], therapy with low-dose IL-2/anti-IL-2 complexes or using an engineered IL-2, greatly improves tumour immunotherapy and reduce tumour volume despite an increase number of Tregs. These results suggest that control of tumour volume is overall dependent on the absolute number of antitumour immunity cells. Lastly in a model of forced expression of IL-2 in mice, growth of carcinogen induced tumours is not accelerated despite an increased number of Tregs (Klatzman and al, manuscript in preparation).

In conclusion, a new hypothesis in T1DM autoimmune mechanisms is that Tregs, at the site of insulitis, could fail to maintain expression of proteins crucial for their suppressive function, possibly because of a lack of sufficient IL-2 amount. The recent discovery of the specific capacity of Tregs to be sensitive to low dose IL-2, opens a new area in T1DM immunotherapy.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

Grinberg-Bleyer


