Clinical application of regulatory T cells in type 1 diabetes


Regulatory T cells (Tregs) are responsible for the maintenance of peripheral tolerance. Animal studies have shown that administration of Tregs can prevent type 1 diabetes (DM1). Several clinical trials attempted to induce Tregs with various agents, and thus provide long-term tolerance of β cells in DM1. Nevertheless, most of these studies have focused on clinical parameters (e.g. C-peptide) and not Treg numbers nor their function after treatment. Therefore, it is not possible to conclude if the majority of these therapies failed because the drugs did not induce Tregs, or if they failed despite Treg expansion.

The current knowledge regarding Tregs, along with our experience in Treg therapy of patients with graft versus host disease, prompted us to use ex vivo expanded Tregs in 10 children with recent-onset DM1. No adverse effects in the treated individuals were observed. There was a significant increase in Treg number in peripheral blood immediately after the treatment administration, while the first clinical differences between treated and control patients were observed 4 months after Treg injection. Treated individuals had higher C-peptide levels and lower insulin requirements than non-treated children. Eleven months after diagnosis of DM1, there are still 2 individuals who are independent of exogenous insulin.

These results indicate that autologous Tregs are a safe and well-tolerated therapy in children with DM1, which can inhibit or delay the destruction of pancreatic β cells. Additionally, Tregs can be a useful tool for local protection of transplanted pancreatic islets. Isolation and expansion of antigen-specific Tregs is one of the directions for future studies on cellular therapy of DM1.

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Regulatory T cells

Regulatory T cells (Tregs) are a specific lymphocyte subset that, contrary to conventional T cells, do not actively fight infectious microorganisms, but suppress excessive responses of other immune cells (1–12) (Fig. 1).

The presence of such a cell population in the immune system was hypothesized in the 1970s by Gershon (13, 14). However, the evidence supporting the concept was provided 25 yr later in 1995, when Sakaguchi showed that a lack of cells with a CD4+CD25+ phenotype led to autoimmune-mediated multiple organ dysfunction (15). Currently, it is known that Tregs regulate cellular activity of the innate and adaptive immune system. Tregs can inhibit FcεRI-dependent degranulation of mast cells (Mcs) (Fig. 1A) in a cell contact-dependent manner, and thus can prevent/abrogate anaphylaxis.

It has been reported that the pathway is mediated via interactions between the Treg surface molecule, OX40, belonging to the tumor necrosis factor receptor family, and its ligand, OX40L, expressed on Mcs (3). Tregs are also effective inhibitors of antigen presenting cells (APC), thus, they not only suppress activated effector T cells (Teff), but also prevent their activation. It is well established that direct
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Fig. 1. Selected, basic mechanisms used by Tregs. Tregs act on both innate and adaptive immune response. (A) Tregs inhibit degranulation of mast cells (Mc) and thus dampen anaphylaxis in an OX40-OX40L-dependent manner. (B) Tregs inhibit maturation of dendritic cells (DC); immature DCs are weak stimulators of T cells; Tregs induce expression of indoleamine 2,3-dioxygenase (IDO) in DCs via the CTLA-4 (cytotoxic T lymphocyte antigen-4)-CD80/CD86 pathway; IDO inhibits T cell function. (C) Tregs inhibit activation of both helper (Th) and cytotoxic (Tc) effector T cells via cyclic adenosine monophosphate (cAMP) transferred through gap junctions. (D) Tregs induce apoptosis of monocytes/macrophages (Mono/Mac) and induce alternative activation of Mono/Mac; alternatively, activated Mono/Mac have a strong anti-inflammatory potential. (E) Tregs inhibit B cell proliferation and induce their apoptosis via a PD-1 (programmed death-1 receptor) PD-L1 (programmed death-1 ligand)-dependent manner; Tregs block plasma cell differentiation and affinity maturation of antibodies (Abs). (F) Tregs induce apoptosis of neutrophils (Neu) in a granzyme A- or B-dependent fashion; Tregs inhibit production of reactive oxygen intermediates (ROI) and cytokines by Neu. (G) Tregs inhibit proliferation and function (production of interferon gamma; IFN-γ and cytotoxicity) of natural killer (NK) cells via membrane-bound transforming growth factor beta (mTGF-β).

Cell-to-cell contact between Tregs and dendritic cells (DCs) suppresses DCs maturation. Consequently, DCs express low levels of costimulatory molecules (CD80 and CD86) required for the activation of naive T cells. Additionally, in a mechanism dependent on CTLA-4-CD80/CD86 interactions, Tregs induce the expression of indoleamine 2,3-dioxygenase (IDO) by DCs. IDO is a potent regulatory molecule that converts tryptophan into proapoptotic metabolites, resulting in the suppression of both helper (Th) and cytotoxic (Tc) T cells (Fig. 1C) (2). Another strategy used by Tregs to suppress T cells is the transfer of cyclic adenosine monophosphate (cAMP), a potent inhibitory second messenger, via membrane gap junctions (Fig. 1C).

Relatively little is known about the mechanisms which Tregs use to inhibit monocytes/macrophages. It was observed that, after coculture with Tregs, monocytes/macrophages differentiate towards alternatively activated macrophages (AAMs), and this process is partially dependent on interleukin 10 (IL)-10, IL-4, and IL-13. Nevertheless, the mechanism is not fully elucidated. AAMs are characterized by anti-inflammatory potential and immunoregulatory activity (Fig. 1D). Additionally, AAMs downregulate human leukocyte antigen (HLA)-DR, thus becoming weak antigen presenters (4). Tregs can also efficiently inhibit autoreactive B cells in an antigen-specific manner and are able to prevent production of autoantibodies. These mechanisms are primarily mediated through interactions between the surface molecules programmed death-1 (PD-1; expressed by B cells) and PD-1 ligand (PD-L1; on Tregs). In addition, Tregs can induce apoptosis of B cells and suppress their proliferation, as well as suppress plasma cell differentiation and affinity maturation of antibodies (Fig. 1E) (6, 10).

Tregs are also efficient suppressors of granulocytes. It was observed that Tregs effectively inhibit the production of reactive oxygen intermediates (ROI) and cytokines by neutrophils. These effects were partially dependent on IL-10, TGF-β, and direct contact. In addition, Tregs were found to induce neutrophil apoptosis in a granzyme-dependent manner (Fig. 1F) (8, 9). There are also multiple studies on Treg and natural killer (NK) cell interactions.
Currently, it is well established that Tregs are efficient inhibitors of interferon gamma (IFN-γ) production, proliferation, and cytotoxicity of NK cells. The main mechanism responsible for these effects seems to be signal transduction via membrane-bound TGF-β upon direct cell-to-cell contact (Fig. 1G) (11, 12).

The wide repertoire of mechanisms utilized by Tregs, and the variety of cell types that are their targets, serve the main goal of Tregs, which is maintenance of peripheral tolerance and prevention of autoimmune diseases. In addition, Tregs modulate immune responses induced by pathogens and environmental factors; thus, they do not become life-threatening reactions (2). This cell subset also plays a crucial role in the induction of tolerance to transplanted solid organs and controls allergic reactions (16–18). Numerous studies suggest that certain immunosuppressive drugs exert their therapeutic effects, at least partially, via stimulation of Treg activity and their generation/proliferation (19–22).

Tregs are generally divided into two groups: natural (nTregs) and inducible/adaptive (iTregs/aTregs). The first subset derives from the thymus, constitutively expresses high levels of CD25, is FoxP3+ (forkhead box P3 transcription factor), and mediates its suppressive function predominantly in a cell contact-dependent manner. The second group of Tregs is comprised of Tr1 and Th3 cells, which derive from conventional CD4+ T cells and act predominantly via IL-10 and transforming growth factor β (TGF-β) secretion, respectively (23, 24).

**Regulatory T cells in type 1 diabetes**

Studies of animals and humans lacking a certain immune cell subset are a valuable source of knowledge, as they show the real function and significance of the lacking cell population in the body. Owing to these types of observations, it is known that the lack of Tregs and/or impairment of their function lead to autoimmune diseases, including type 1 diabetes (DM1) (25–27).

One of the best-defined examples of this type of abnormality is IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), resulting from a mutation in the foxp3 gene, which encodes the transcription factor FoxP3, crucial for the activity of nTregs (25, 28). The characteristic symptoms of IPEX in neonatal age are atopic dermatitis, watery diarrhea, and DM1 (29).

In addition, it was observed that early intervention with ex vivo expanded Tregs can prevent the onset of DM1 in genetically predisposed animals, while Treg administration at the onset of DM1 induces regression of the pathological lesions in the pancreas (26, 30). Numerous studies on Treg function established the opinion that these cells could provide an opportunity for effective and targeted immunosuppression (31).

Currently, there are two main points of view on Tregs in DM1. On the one hand, there are reports that the number and function of Tregs in DM1 patients is decreased (32, 33). On the other hand, there is data which indicate that the main problem in DM1 is extensively activated autoreactive T cells that are resistant to physiologically acting Tregs (34–36). In our studies, we did not find differences in Treg function and number between healthy individuals and children with recent-onset DM1 (37). Nevertheless, we cannot rule out that some changes in Treg effectiveness appear as the disease progresses (38). This may result from long-term activation of Tregs upon the autoimmune reaction and the chronic inflammatory process which accompanies DM1 (39–41). Therefore, initiation of Treg therapy at the time of DM1 onset, when the function of these cells is still unchanged, is reasonable.

**Approaches to induce Tregs in diabetic patients**

Since the protective role of Tregs in DM1 has been suggested, several clinical studies attempted to potentiate their action and/or expand them in vivo with an aim to preserve β cells in recent-onset DM1 (42–53).

One of the very first approaches was based on oral insulin administration (42). It is well-known that immune response in the gut is precisely regulated; thus, pathogenic microorganisms are recognized and destroyed, while at the same time, physiological flora and dietary antigens are tolerated. About 20 yr ago, it was shown that oral antigen administration can suppress various experimental autoimmune diseases (54–57). In prediabetic mice, orally administered insulin was found to inhibit DM1 onset (42), and even reverse hyperglycemia (58). The effect resulted from induction of antigen-specific Tregs, which down-regulate autoimmunity via the inhibitory cytokines IL-10 and TGF-β (42, 55, 57, 58). As observations made in animal models suggested that oral insulin can attenuate anti-β cell response and thus delay onset of the disease, a study in humans was launched.

The trial recruited relatives of DM1 patients positive for anti-islet cell antibodies (ICA), with a low first-phase insulin response and a high 5-yr projection risk of DM1 onset (42). However, satisfactory results from animal models were not replicated in humans (42, 59). Further analysis revealed that individuals with high levels of anti-insulin autoantibodies (IAA; ≥80 nU/mL) at the time of study entry benefited from the treatment. Oral insulin administration delayed onset of DM1 in these patients for nearly 5 yr. At the same time, the therapy was found to accelerate development of DM1 in patients with lower baseline
IAA levels (<80 nU/mL). Nevertheless, after the discontinuation of therapy, the risk of DM1 onset in the treated patients and those receiving placebo equalized (42, 59). Thus, oral insulin administration did not induce the expected tolerance.

It is worth noting that, even though the aim of the study was to induce immune tolerance dependent on IL-10 and TGF-β, producing Tregs, neither the Treg number nor IL-10/TGF-β levels were measured in the patients. Therefore, the study did not reveal if the treatment was ineffective because oral insulin administration did not induce antigen-specific Tregs, as was expected, or if it was ineffective despite the generation of iTregs.

The next approach that aimed to inhibit the progression of DM1 and gain long-term tolerance via iTregs was a therapy with anti-CD3 antibody. There were two anti-CD3 antibodies used in the clinical trials: hOkt3y1 Ala-Ala (teplizumab) and ChAgyCD3 (otelixizumab). These humanized antibodies had mutated Fc fragments that prevented them from binding to Fc receptors (FcRs). This modification eliminated the risk of adverse effects resulting from cell activation via FcR stimulation. In addition, it has been suggested that these antibodies act in a biphasic mode. In phase 1, the antibody binds to the CD3 molecule and induces anergy/apoptosis of activated T cells. In phase 2, the drug increases the number of iTregs that produce anti-inflammatory IL-10 and TGF-β, inducing long-term tolerance (60, 61).

In the study of Herold et al., teplizumab was administered to 21 individuals with recent-onset DM1, while the control group comprised of 21 non-treated diabetic patients. After 6- and 12-month observation, the treated patients had higher levels of stimulated C-peptide than the control individuals. Moreover, the effect persisted 2 yr after the treatment, and the patients who received the drug had lower insulin requirements and lower levels of glycated hemoglobin (HbA1c). Depletion of T cells after teplizumab therapy was transient. The T cell number recovered around 1 month after the treatment. Clinical responders at months 1 and 3 after the therapy were characterized by increased numbers of CD8+ T cells as compared to the baseline values. No significant differences were found between the responders and non-responders in terms of CD4+ T cell numbers on day 90 as compared to the baseline values at the time of study entry. No results regarding Treg subsets were shown (43).

As these initial results were very promising, phase III of the trial was launched, consisting of 516 individuals from 83 clinical centers from Europe, North America, Israel, and India. Patients were divided into four groups (groups 1–3 received different doses of the drug, group 4 was treated with placebo). However, after a 15-month observation, the study did not reach its primary and secondary end-points. There was no decrease in HbA1c levels from baseline and no improvement in C-peptide response after stimulation in the treated individuals. Nevertheless, the study suggested that teplizumab may be more effective in: (i) younger individuals, (ii) patients with high baseline C-peptide levels at the time of study entry, and (iii) those diagnosed with DM1 <6 wk before starting the therapy (44).

The studies with otelixizumab brought similar observations. Despite the initially promising results, higher C-peptide concentrations and lower insulin requirements 18 months after the treatment (45), the beneficial effect waned with time. Only the youngest patients benefited from the treatment up to 36 months after otelixizumab administration (46).

Adverse effects related to the treatment with anti-CD3 antibodies were usually mild and easily controlled with non-steroidal anti-inflammatory drugs and antihistamines. The most frequent side effects were headache, fever, rash, nausea, diarrhea, and vomiting. An increased frequency of infections was not observed in teplizumab studies. However, in otelixizumab-treated patients, transient reactivation of Epstein–Barr virus was reported. Neither of the antibodies caused sustained T cell depletion. The patients were fully immunocompetent as early as 1 month after the last injection of the drug. In approximately 40–50% of the patients, anti-idiotypic antibodies appeared that could potentially neutralize the effect of the therapy. Nevertheless, the phenomenon was observed after the last dose of the antibody and was not considered a problem because repeated treatments were not anticipated (43–46).

The next treatment that was suggested to induce Tregs in DM1 patients was glutamic acid decarboxylase (GAD)-alum (Diamyd; Diamyd Medical, Stockholm, Sweden), an autoantigen-based vaccine. The drug was composed of adjuvant (alum) and recombinant human glutamic acid decarboxylase 65 (GAD65), a specific isoform of GAD expressed in human pancreatic β cells. The aim of the therapy was to interfere with the process of GAD65 recognition by autoreactive T cells using the recombinant peptide and to induce long-term tolerance.

Results of the first studies with GAD-alum in patients with latent autoimmune diabetes of adults were very promising. The phase II dose-escalation clinical trial showed that a 20-µg dose of the vaccine was the most effective and preserved fasting and stimulated C-peptide levels for a 24-wk period. In addition, the patients who received 20-µg dose of GAD-alum had higher numbers of CD4+CD25+ T cells, which was interpreted as induction of Tregs (48). However, no additional markers of these T cells were analyzed. Therefore, it is not possible to determine if GAD-alum induced natural or adaptive
One of the most recent approaches to induce Tregs in vivo in DM1 patients was presented by Long et al. The trial recruited 10 individuals with DM1 4–48 months after diagnosis. The patients were treated with rapamycin and IL-2. Rapamycin is known to preferentially inhibit proliferation of Th1 and Th17 effector cells. Some studies also suggested that it can potentiate Treg function, while IL-2 is known to be a crucial cytokine for nTreg survival and proliferation (65). In addition, it has recently been reported that low-doses of IL-2 increase Treg numbers and exert beneficial effects on clinical outcome in some patients with autoimmune vasculitis (66) and graft vs. host disease (GVHD) (67). However, IL-2 and rapamycin co-administration did not induce clinical improvement in DM1 patients. Despite the increase in nTreg number, the therapy resulted in transient β cell dysfunction (65).

Isolation and ex vivo expansion of nTregs for clinical administration

As the knowledge regarding Tregs has been increasing rapidly, and multiple animal studies have confirmed the therapeutic potential of this cell subset, in 2007, nTregs was considered as a method for so-called ‘intelligent immunosuppression’. Autologous nTregs seemed to provide all the benefits of standard immunosuppressive drugs, without their adverse effects (31).

However, as nTregs are present in peripheral blood in very low numbers, there was a need for a clinical protocol for the expansion of human nTregs ex vivo. The main obstacles were weak proliferative potential of nTregs and decreasing immunosuppressive activity during culture in vitro.

In June 2008, after multiple in vitro and animal studies, we administered, for the first time in humans, ex vivo expanded nTregs to a patient with chronic GVHD, resistant to standard immunosuppression. nTreg infusion resulted in the regression of symptoms, which enabled the tapering of medication doses and improved the patient’s quality of life (68). Our team continues studies with nTreg therapy in GVHD.

Owing to the positive results of this trial, lack of adverse effects directly after nTreg administration and at each time point after the treatment (no increased frequency of infections, no primary disease reactivation, etc.), we decided to use nTregs to preserve β cell function in children with recent-onset DM1 (clinical trial reg. no. NKEBN/8/2010; phase I) (Fig. 2).

As nTregs are a rare cell population, according to our protocol, we needed approximately 250 mL of blood to separate and expand them to the therapeutically relevant numbers. Therefore, appropriate venous access and the required blood volume are the first limiting factors for the therapy, and only children above 5 yr can be recruited to the study. nTreg yield
Fig. 2. Therapy with natural regulatory T cells (nTregs): outline of the procedure. (A) Patient is admitted to the pediatric ward for drawing of blood. (B) Blood is processed in blood bank into Buffy coat and plasma. (C) Buffy coat and plasma are sent to the good manufacturing practice (GMP) facility for nTreg isolation, expansion, and certification (GMP facility of Medical University of Gdansk in Poland is shown). (D) nTregs are expanded under GMP conditions in presence of artificial antigen presenting cells (APC), which are beads coated with anti-CD3 and anti-CD28 antibodies, autologous serum, and interleukin-2 (IL-2) (schematic view). Under the described conditions, nTregs proliferate vigorously. Just after the nTreg isolation, their number is very low and cells occupy just few wells on 96-well plate (day ‘0’). Cells are cultured up to 14 d. Within this period, they proliferate to the clinically relevant numbers (day ‘10’ - 10-d culture is shown). (E) When the required nTreg number is reached, cells are collected, counted, and washed to remove the whole culture medium. (F) At this time, the patient is admitted to the hospital again and receives nTregs in a slow intravenous infusion (nTregs are suspended in 250 mL of 0.9% NaCl).

immediately after the sorting is very low and, before administration to the patient, their number must be multiplied. For example, if the total postsort number of nTregs is $1 \times 10^6$, they must be multiplied by a 1000 to few 1000-fold to obtain the dose of $20 \times 10^6$ nTregs/kg of body weight (kg b.w.). Multiplication of nTregs can be performed in a so-called expansion culture, which lasts for up to 2 wk. Within this period, nTregs are incubated with IL-2 and beads coated with anti-CD3 and anti-CD28 antibodies, which are extremely strong activators of nTreg proliferation (37) (Fig. 2). Nevertheless, the expansion is challenging, as only a very pure population of sorted nTregs can be successfully multiplied. Thus, purity is also one of the most crucial factors affecting the clinical results of the therapy. We confirmed in our preclinical in vitro studies that Teff proliferate more vigorously than nTregs (69). Therefore, even small initial impurities (low percentage of Teff within the isolated nTregs), may become a serious problem, leading to the expansion of potentially harmful Teff, but not therapeutic nTregs. For this reason, we chose fluorescence-activated cell sorting (FACS) for the final step of nTreg isolation. This method guarantees the highest purity of cell isolation (approximately 100% of nTregs after sorting), which can be sustained through the entire expansion, and thus, the final cell product still keeps the characteristics of nTregs (37).

There are several other safety issues that must be addressed during the production of nTregs for clinical applications. Isolation and expansion of nTregs for therapy must be performed at a facility complying with the requirements of good manufacturing practice (GMP) (Fig. 2). The reagents used for the cell culture should also be kept to these standards. In our laboratory, all reagents used for expansion, polystyrene beads coated with anti-CD3 and anti-CD28 antibodies (Invitrogen, Carlsbad, CA, USA),
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Therapy of type 1 diabetic children with nTregs

The main inclusion criterion that we used to recruit children with DM1 for nTreg therapy was a fasting C-peptide level of $>0.4 \text{ ng/mL}$ in two consecutive measurements. C-peptide levels reflect the production of endogenous insulin and, indirectly, $\beta$ cell mass. Therefore, relatively high C-peptide levels indicate that there are still some active $\beta$ cells that can be spared from the autoimmune attack, which is the main idea of the therapy with nTregs. Other inclusion criteria were age 5–18 yr, up to 2 months since DM1 diagnosis, presence of at least one type of anti-islet antibody in high titer (GAD65, ICA, IAA, and IA2), body mass index range of 25th–75th percentiles for a particular age, and adequate venous access. The exclusion criteria were any cytopenia or low hemoglobin levels, presence of the HLA-DQB1*0602 allele, positive test for HBV, HCV, HIV, Treponema pallidum, or other active infections, history of neoplasm, excessive anxiety related to the procedure, and other chronic diseases requiring pharmacological treatment. The control group comprised of 10 children with DM1 fulfilling the same inclusion criteria as the research group, but not treated with nTregs.

Patients from the treated group received either $10 \times 10^6$/kg b.w. or $20 \times 10^6$/kg b.w. of expanded nTregs. Technically, the transfer was performed after complete washout of the culture media and reagents. Subsequently, nTregs were suspended in 250 mL of 0.9%NaCl (Polfa, Starogard Gdanski, Poland), and administered in a slow infusion to the patient (Fig. 2).

No adverse reactions related to the treatment were observed. In all the patients, a significant increase in nTreg frequency in peripheral blood was observed immediately after the injection. The first clinical differences between the treated and control patients were observed 6 months after DM1 diagnosis (4 months since administration of nTregs). After this period, children who received nTregs had nearly twofold higher levels of fasting C-peptide than nontreated individuals. The results were clinically relevant, as treated patients required twofold lower doses of exogenous insulin than the control individuals, while HbA1c and glucose levels were comparable in both groups (37). Among patients treated with nTregs, there are still two individuals who do not require exogenous insulin 11 months after DM1 diagnosis.

There is one similar trial recruiting adults with DM1, currently underway in USA. However, the results are not yet available. We are looking forward to the completion of this trial, which hopefully will further encourage nTreg-based therapies in DM1.

Perspectives for the future clinical use of nTregs

Most of the experimental therapies of DM1 focused on patients with preserved C-peptide. While for the patients with long-lasting DM1 and undetectable C-peptide, the only chance for insulin independence is pancreas or pancreatic islet transplantation. However, pancreas transplantation is associated with a high risk of severe complications (70, 71), while islet survival after transplantation is still a big challenge (72–78). Main limitations of a positive outcome in islet recipients include not only immune rejection, but also reactivation of the autoreactive response toward transplanted islets (79–81). Furthermore, even immunosuppression used for islet protection is toxic for the graft (80, 82). Currently, no treatment exists that could provide permanent $\beta$ cell tolerance and islet protection in DM1 recipients (83–85).

As nTregs are able to inhibit both auto- and alloreactivity (2, 86–88), we found these cells a perfect tool to provide protection for transplanted islets from rejection and anti-$\beta$ cell response that reactivates in DM1 recipients. Therefore, we coated human pancreatic islets with live nTregs using a polymer of ethylene glycol (poly(ethylene glycol))-N-hydroxysuccinimide ester; biotin-PEG-NHS) and streptavidin as a binding bridge. Fluorescence and confocal microscopy confirmed the feasibility of the approach (nTregs covered the islets with a uniform layer). In addition, functional tests in vitro showed that the coating procedure does not...
and persistent tolerance in the treated patients. Further experiments showed that surface-bound nTregs were able to protect the islets against allogeneic effector cells, and one of the mechanisms was associated with the inhibition of IFN-γ production. These results show that Tregs may be used successfully as a fully biocompatible protection for pancreatic islets. However, there is a need for animal studies to confirm safety and efficacy of this technique in vivo before any clinical trial in humans would be launched.

Summary

The results of available studies strongly indicate that success of the treatment in DM1 correlates with the level of nTregs. The agents used to increase the number of these cells, as well as the studies on adoptive transfer of expanded nTregs, are currently a main interest of the research groups working on therapies for DM1. Our results indicate that autologous nTregs are a safe and well-tolerated therapy of DM1 in children. Higher C-peptide concentrations in the treated individuals as compared to the control group indicate that nTregs inhibit the autoimmune process of pancreatic β cell destruction. This is the first study of this type in diabetic patients; therefore, it is hard to predict how long the beneficial effect of nTreg administration will last. For the same reason, it is difficult to define the factors necessary for the treatment success. According to our experience, it seems that one of the most important criteria for future recruitment is short disease duration/prediabetes and high C-peptide level. The number of nTregs administered and infusion of the additional doses of nTregs are other important issues that need to be addressed in future studies. Finally, one of the directions for future therapies with nTregs is isolation and expansion of antigen-specific cells. Elaboration of the technique that would enable this approach could result in the higher efficacy of cellular therapy of DM1 and persistent tolerance in the treated patients.

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References

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