Journal of
Clinical & Cellular Immunology

Paschou et al., J Clin Cell Immunol 2012, S2
DOI: 10.4172/2155-9899.S2-003

Review Article Open Access

The Role of T regulatory Cells (Tregs) in the Development and Prevention of Type 1 Diabetes

Stavroula A. Paschou[1], Stelios Tigas[1], Katerina Naka[2], George K. Papadopoulos[3]* and Agathocles Tsatsoulis[1]*

[1]*Department of Endocrinology, University of Ioannina, GR45110 Ioannina, Greece*
[2]*Department of Cardiology, University of Ioannina, GR45110 Ioannina, Greece*
[3]*Laboratory of Biochemistry and Biophysics, Faculty of Agricultural Technology, Epirus Institute of Technology, GR47100 Arta, Greece*

Abstract

In this article, we review the role of T regulatory cells (Tregs) in the development and prevention of type 1 diabetes. We first examine the definition of human Tregs, the generation of Tregs in the thymus and the periphery, their mode of action and their important role in the regulation of the immune response. We then examine the defects in Tregs observed thus far in type 1 diabetes and their role in the development of the disease. Finally, we point to possible clinical applications using Tregs as a therapeutic target for the prevention of type 1 diabetes.

Keywords: Tregs; Type 1 diabetes; Autoimmune regulator; Autoimmune diseases

Abbreviations: AIRE: Autoimmune Regulator; APECED: Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy; APS1: Autoimmune Polyendocrine Syndrome 1; IPEX Syndrome: Immune Deficiency-Polyendocrinopathy-Enteropathy-X-linked Syndrome; Teffs: $CD4^+$ T effector Cells; Tregs: $CD4^+$ T regulatory Cells

Introduction

Type 1 diabetes results from autoimmune self destruction of the pancreatic β cells leading to absolute insulin deficiency and requiring life-long insulin treatment. This autoimmune reaction is triggered by the environmental factors in genetically predisposed individuals. Although recent knowledge has contributed to our understanding of the autoimmune pathogenesis of type 1 diabetes, there remains no unifying theory of disease causation. However, it is accepted that autoimmune disease in general results from the dysregulation of the basic processes designed to maintain self tolerance [1,2]. In the few cases where it has been possible to examine the endocrine pancreas of newly-diagnosed type 1 diabetes patients, massive infiltration of mostly $CD8^+$ T lymphocytes was recorded in insulin-containing islets, but not in islets devoid of insulin. $CD4^+$ T cells, monocytes and B lymphocytes were also found in decreasing order [3-6].

Over the past few years, there has been a steadily increasing interest in regulatory T lymphocytes (Tregs) that exhibit several of the properties of the previously studied and so-called suppressor T cells [7,8]. In this review we will examine the generation of Tregs in the thymus and the periphery, their mode of action and importance in the regulation of the immune response. We will then examine the defects in Tregs observed thus far in type 1 diabetes and their role in the development of the disease. Finally, we will point to a few developments that may lead to possible therapeutic applications using Tregs as a therapeutic target for the prevention of type 1 diabetes.

The Definition of Tregs

Nearly 40 years ago immunologists postulated the concept of regulation of the immune response by T suppressor lymphocytes [9]. This was followed by a flurry of activity, identifying several phenotypic markers for the various cell types involved in the suppression of excessive immune responsiveness ($CD8^+$ suppressor effector cells, $CD4^+$ suppressor-inducer cells etc.), as well as several secreted suppressor factors [10,11]. The whole concept eventually fell into disrepute, mostly because of the lack of reproducible assays for these cells and lack of molecular identification of the factors involved [11,12]. The rebirth of the regulatory-suppressor cell originated from the seminal observation that thymectomy of neonatal mice on day 3 resulted in autoimmune gastritis, which could be corrected by transfusion of syngeneic $CD4^+CD25^+$ T cells, but not their $CD4^+CD25^-$ counterparts [7].

The phenotypic definition of human Tregs is still under discussion, and has been under continuous evolution. $CD4^+$ Treg cells have been most intensively studied. Nowadays, various phenotype markers are used not only to distinguish Tregs from other $CD4^+$ cells, but also to identify functional (sub) classes of Tregs. The high constitutive surface expression of the IL-2 receptor alpha chain (CD25) is generally considered as a characteristic feature of the vast majority of human Tregs and regulatory activity is enriched in $CD4^+CD25^{high}$ T cells [13-15]. Upon activation of T cells, independently of their regulatory capacity, CD25 can become up-regulated and highly expressed on Teffs as well. This puzzle of distinguishing between *bona-fide* Tregs and recently activated Teffs has been solved by a very effective utilization of the CD45RA marker (below). A considerable number of other surface markers have been reported to be expressed on human Tregs, such as CTLA-4 (CD152), L-selectin (CD62L), glucocorticoid-induced tumour necrosis factor receptor (GITR), TGF-β, CD95 and PD-L1 [16,17]. Recent studies have demonstrated that down-regulation of the IL-7 receptor α-chain (CD127) distinguishes Treg cells from activated T cells, facilitating the functional characterization of a more representative population [18].

***Corresponding authors:** Agathocles Tsatsoulis, Professor of Medicine, Department of Endocrinology, University of Ioannina, GR45110 Ioannina, Greece, E-mail: atsatsou@uoi.gr

George K. Papadopoulos, Professor of Biochemistry and Biophysics, Laboratory of Biochemistry and Biophysics, Faculty of Agricultural Technology, Epirus Institute of Technology, GR47100 Arta, Greece, E-mail: gpapadop@teiep.gr, papadopg@gmail.com

Received May 30, 2012; **Accepted** July 31, 2012; **Published** August 06, 2012

Citation: Paschou SA, Tigas S, Naka K, Papadopoulos GK, satsoulis AT (2012) The Role of T regulatory Cells (Tregs) in the Development and Prevention of Type 1 Diabetes. J Clin Cell Immunol S2:003. doi:10.4172/2155-9899.S2-003

Intracellular expression of the FoxP3 transcription factor is the hallmark of Tregs, as the presence of this protein is necessary for their development. FoxP3 is a member of the forkhead or winged helix family of proteins and the respective gene is located on chromosome X [19]. FoxP3-mutant mice have the *scurfy* phenotype, characterized by massive lymphoproliferation, autoimmunity and death in the second to third month of life. In humans, mutations in the *FoxP3* gene lead to the IPEX (Immune deficiency-polyendocrinopathy-enteropathy-X-linked) syndrome. This is characterized by total absence of Tregs, food allergy, enteropathy, eczema and polyendocrinopathy, including neonatal type 1 diabetes and less often autoimmune thyroid disease [20,21]. The FoxP3 protein is also transiently expressed in human activated Teff cells (Teffs), albeit at a lower protein level than in Tregs. However, this transient expression does not confer any regulatory properties on such Teffs. Furthermore, in Tregs the region upstream of the *FoxP3* gene is completely demethylated, an indication of persistent and sustained expression of this master switch by Tregs. In contrast, this region is found to be methylated in Teffs. Therefore, DNA demethylation of the 5' upstream region and the STAT-5 responsive element in the human *FoxP3* locus can discriminate Tregs from conventional Teffs, even if the latter transiently express FoxP3 [22-24]. A recent detailed analysis showed that FoxP3^{+} CD4 T cells are composed of three phenotypically and functionally distinct subpopulations, depending on the expression of the CD45RA molecule: CD25^{+++}CD45RA^{-}FoxP3^{high}CD127-/^{low} activated Tregs (aTregs), CD25^{++}CD45RA^{+}FoxP3^{low}CD127-/^{low} resting Treg (rTregs) cells (with the former showing higher suppressive capacity *in vitro* compared to the latter) and a CD25^{++}CD45RA^{-}FoxP3lowCD127^{+} group of non-suppressive Teff cells [24]. It so happens that the CD25^{+++} population is over 90% CD45RA^{-} rendering this separation a very effective one [24]. Hence, the currently accepted way of recognizing CD4^{+} T cells with regulatory function is by the highest expression of CD25, the high intracellular expression of FoxP3, and the low or no expression of CD127 (CD4^{+}CD25^{high}FoxP3^{+}CD127^{low}/- cells).

Generation of Tregs

Tregs are generally classified into two categories, natural Tregs (nTregs) and adaptive or induced Tregs (iTregs). Natural Tregs primarily emerge from the thymus, whereas iTregs are generated in the periphery from naive T cells after antigen exposure [25]. Both T cell subsets share a similar phenotype, express intracellularly the transcription factor FoxP3 and possess suppressive capacity. Very recently, it has been shown that nTregs selectively express Helios, an Ikaros-family transcription factor [26].

The development of CD4^{++} T cells in the thymus rests upon the interaction of their antigen-specific T Cell Receptor (TCR) with self-antigen bearing MHC II proteins in antigen presenting cells (APCs), first in the thymic cortex and then in the medulla. Absence of such interaction leads to their death by neglect, low affinity interaction to positive selection, and high affinity interaction to negative selection [2]. By contrast, the intermediate affinity interaction induces the genetic program for Tregs. This includes up-regulation of the Treg-specific transcription factor FoxP3, the cell membrane molecules CD25, CTLA-4, down-regulation of IL-7Rα (CD127) and shutting off of the genes for IL-2 and the T_H1-, T_H2- and T_H17-specific cytokines (IFNγ, IL-4, and IL-6, respectively), as well as the respective unique transcription factors T-bet, GATA-3 and ROR-C (RORγt in the mouse) that determine the corresponding CD4^{++} T cell fates [27-32] (Figure 1). FoxP3 once induced, reinforces many of these processes ensuring thus the distinct phenotype and properties of CD4^{++} Tregs [32,33].

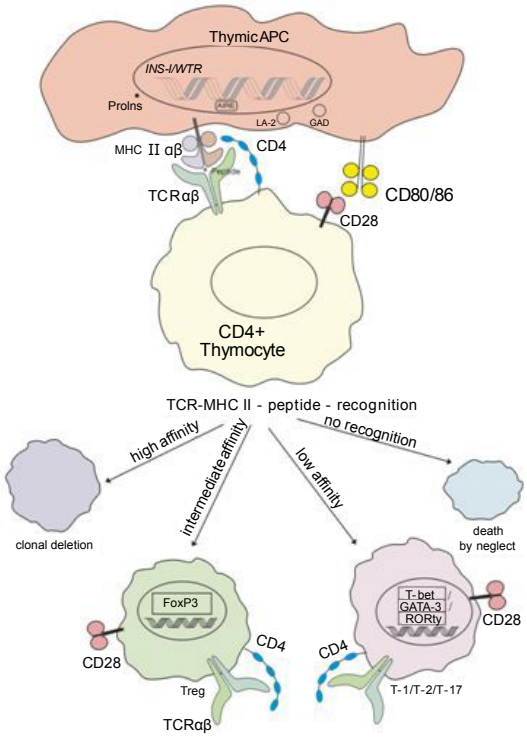

Figure 1: Thymic selection and generation of the various CD4^{++} T lymphocyte subsets: negative selection, nTreg selection and positive selection of thymocytes in the medulla as the outcome of high affinity, intermediate affinity, and low affinity interaction of TCR on immature thymocytes and MHC II plus peptide on medullary APCs. The differentiation into Th1/Th2/Th17 etc cells, takes place in the periphery. Tregs may also be selected in the periphery under the influence of factors such as TGFβ and IL-2, and are then called iTregs [40-43].

For several tissue-specific proteins their transcription in the thymus is under the control of the transcription factor Autoimmune Regulator (AIRE). Patients with mutations in this gene show defective expression of tissue-specific self-antigens in thymus, leading to autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), or autoimmune polyendocrine syndrome 1 (APS1) [34]. Such patients have defective suppressive function of their Tregs, most probably due to the significantly decreased expression of FoxP3 protein in these cells, as compared to controls [35].

Induced Tregs arise from CD4^{+}CD25^{-} precursor cells in peripheral lymphoid organs [32]. It is possible that in the periphery iTregs may develop probably from recent thymic CD4^{++} T cell emigrants that have high affinity for MHC II^{++} self antigen, yet have escaped selection [36,37]. Certainly, dendritic cells synthesize and present self-antigen (including all of the major auto-antigens for type 1 diabetes) to CD4^{++} T cells [38] and under certain circumstances can be tolerogenic [37], leading to the induction of Tregs, yet the specific mechanisms are under debate. There is now considerable evidence that IL-2 and transforming growth factor (TGF)-β are required for the preservation of iTregs, and that both factors are needed by nTregs and iTregs for the induction and continuous expression of FoxP3 [39-41].

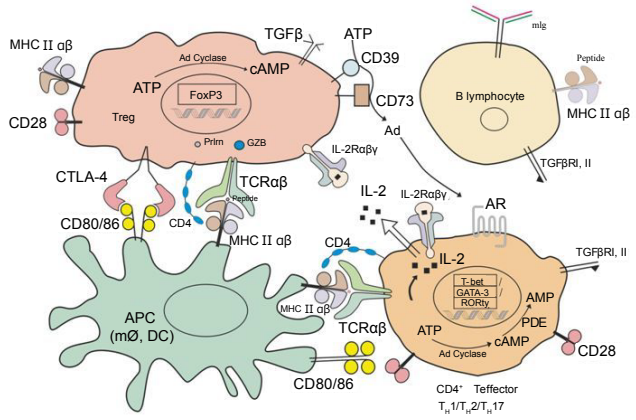

Figure 2: Simplified view of Tregs interacting with other immunocytes at a peripheral lymphoid organ or at a site of inflammation. Known immunocytes (other T cells, monocytes/macrophages, DCs, B lymphocytes) that may interact with Tregs are depicted as well as most candidate interaction molecules. It is now evident that DCs are very important for the maintenance of the regulatory function of Tregs [39,40], and there is evidence that CD8 T regulatory cells as well as B regulatory cells may play a role in the immune circuit [103,104]; neither population is shown here.

The Functional Role of Tregs

Tregs control the reactivity of self-reactive T effector cells that are not eliminated in the thymus and are thus responsible for maintaining peripheral self tolerance and immunological homeostasis. Initially, Tregs can control T cell activation, expansion and proliferation during lymph node priming. At this stage, Tregs colocalize with dendritic cells at the medullary-cortical junction at the T cell-B cell borders within the proximal lymph nodes [42]. In addition, Tregs can traffic to the site of inflammation and suppress the effector functions of immunocytes within the affected tissue [43].

The mechanisms used by Tregs to suppress immune responses are still unresolved, yet the prevailing view is that cell contact between Treg and Teff is obligatory [12-17,26-29]. This however, does not prevent subsequent bystander suppression as well, in the milieu generated by the thus activated Tregs [25,42]. In general, the activation of Tregs *in vivo* follows that of Teffs, and while capable of division, their functional programme consists of deactivation of the pathways found in Teffs, and up-regulation of pathways accumulating suppressive molecules in the cytoplasm, on their cell membrane and extracellularly (cAMP, CTLA-4, HLA-DR/DQ, TGFβ adenosine) [31,32,43,44] (Figure 2). *In vitro* activated human Tregs may directly kill activated $CD4^{++}$ and $CD8^{++}$ T cells in a perforin- or granzyme-dependent manner [45]. Although evidence for such cytotoxicity is lacking *in vivo,* patients with mutations in the perforin gene suffer from haemophagolytic lymphohistiocytosis (HLH), indicating a key involvement of perforin in immune regulation, perhaps via Tregs [46].

For another possible pathway, the transcription programme of Tregs includes diminution of TCR-induced downstream signaling and maintenance of a suppressive phenotype [32,33,47]. Specifically, the gene for the cAMP degrading enzyme phosphodiesterase 4 (*pde4*) is suppressed, while that of IL-7Rα is downregulated [31-33]. This leads to a considerable build-up of cAMP in Tregs. Upon proper Teff contact, Tregs establish communication with Teffs via gap junctions, transferring cAMP and rendering the latter cells inactive [48]. In addition, the CD39 and CD73 ectonucleases on the surface of Tregs use extracellular ATP to generate adenosine, which in turn activates the suppressive adenosine receptors on neighboring Teff cells [49]. CTLA-4, a membrane molecule whose gene locus is already linked to type 1 diabetes and autoimmunity (*IDDM12*) [50], binds with 20X higher affinity than CD28 to CD80/CD86 (B7 family) receptors, located on antigen-presenting cells (APCs) [3]. The CD28-CD80/86 interaction may function as the second signal to TCR—MHC II-peptide recognition. Thus, CTLA-4^{+} Tregs by tightly binding to CD80/86 receptors on APCs block the APC-Teff interaction necessary for activation and also send negative signals, preventing such activation. It has recently been shown that TGFβ, in its immature or mature form, is found on the surface of human Treg cells, bound to the membrane protein GARP (Glycoprotein A-repetitions predominant protein) [51]. There, immature TGFβ may be converted to its mature form by a variety of proteins, such as furin, thrombospondin and certain Arg-Gly-Asp—recognizing integrins [52,53]. The importance of TGFβ to the generation and maintenance of Tregs had long been demonstrated, and with this finding another potential mechanism of action of Tregs is revealed (Figure 2). The role of HLA-DR on the surface of $CD4^{+}CD25^{high}$ Tregs is worth mentioning, as it is the HLA-DR^{+} fraction of such cells that exhibits the most potent regulatory activity [54]. As in most autoimmune diseases, specific HLA-DR/DQ alleles are associated with susceptibility to type 1 diabetes [55].

Findings for Tregs in Type 1 Diabetes

Experimental animal studies

In the NOD mouse model of type 1 diabetes various defects have been noted in the Treg ($CD4^{++}CD25^{+}$) compartment. It appears that such cells are defective in suppressing the proliferation of Teff cells [56,57]. Remarkably, Tregs from 4 weeks old NOD mice are capable of suppressing T cell proliferation, yet Teffs from older NOD mice are refractory to such suppression [58]. Tregs can affect Teffs at several levels (proximal lymph nodes, sites of tissue inflammation), by controlling T cell trafficking to tissues as well as their reactivation whenever the first line of protection in the draining lymph nodes fails [42,43,58], and the islet micro-environment takes on characteristics of the lymphoid system [59]. Experimental studies in mice have shown that diabetes progression depends on a delicate balance between effector T_H cells and Tregs both in the pancreatic lymph nodes and within the inflamed pancreas [60,61]. After the onset of diabetes, autoimmunity progresses as the ratio between effector T_H cells and Tregs within the inflamed pancreas continuously increases [62]. On the other hand, TGF-β may also induce Tregs directly through the induction of FoxP3 and/or Treg proliferation, even at the site of tissue damage [41]. Interestingly, a transient pulse of TGF-β in islet cells of NOD mice during the priming phase of diabetes is sufficient to inhibit disease onset and stimulate expansion of intra-islet Tregs [63].

In many experimental models for type 1 diabetes immune tolerance was obtained following treatment with immunosuppressants, including old studies with polyclonal anti- T cell antibodies [64,65] or monoclonal antibodies targeting specific receptors or pathways (such as CD3, CD4, CD8, ICOS, CTLA-Ig etc) [66-69]. In most of these cases the immune tolerance relied on the expansion of Tregs rather than on deletion or anergy of effector T cells [69]. Also, in studies with NOD mice where possible β-cell autoantigens were administrated, it was pointed again the major role of Tregs in the induction of self tolerance, even the nature of Tregs was not very precise [70,71]. Long-term survival of pancreatic islet allografts induced by a soluble fusion protein composed of CTLA-4-Ig in mice depends on tryptophan catabolism

by dendritic cells, via the enzyme indolamine 2, 3–dioxygenase (IDO) [72,73], a known participant in the tolerogenic and Treg-inducing function of immature dendritic cells. Of great interest, $CD4^+CD25^+$ Tregs that adoptively transferred in NOD mice, effectively prevented or even reversed the disease [74].

Human studies

Unfortunately studies of Tregs in type 1 diabetes suffered from the lack of an acceptable criterion for the definition of Tregs. The first work to deal with Tregs in human type 1 diabetes showed a significantly decreased percentage of the $CD4^+CD25^+$ T cell fraction in young newly-diagnosed patients compared to older controls [75]. Subsequent works in newly-diagnosed patients as well as in patients with long-standing diabetes could not find such differences, even when distinguishing between $CD4^+CD25^+$ (activated Teffs) and $CD4^+CD25^{high}$ (Tregs); some of these works found a deceased regulatory function in type 1 diabetes patients, while others did not. As there is a continuum of CD25 intensities from $CD25^+$ to $CD25^{+++}$ ($CD25^{high}$), the distinction between two such populations was of necessity artificial. The Treg definition and separation outlined in [24], which is subsequent to all works estimating the percent of Tregs in type 1 diabetes patients, effectively solves this problem. Remarkably, the average values for these percentages differed considerably from one study to the other [76-78], with one study showing that there was an age-dependent increase in Tregs in controls [78]. Each of these works used essentially a different definition of Tregs. In a mini meta-analysis comparing these studies, the differences in testing for suppressor activity were pointed out and it was recommended that expression of FoxP3 should be used as a criterion, even though activated Teffs also express transiently lower levels of FoxP3 [79]. Yet, another study that grouped together all FoxP3-expressing cells (i.e. including those Teffs transiently expressing the protein), reported no differences in the frequency of such cells between type 1 diabetes patients and controls [80]. A different group showed that $CD4^+CD25^{high}$ Tregs from newly diagnosed type 1 diabetes patients and autoantibody-positive at risk subjects had a higher tendency for apoptosis, compared to such cells from age-matched controls or long-standing type 1 diabetes patients [81]. Two subsequent studies also pointed to refractoriness of Teff cells to the action of Tregs as one reason for the defective regulatory function observed in type 1 diabetes patients [82,83]. Interestingly, Tregs in one of these works [82], appear as $CD4^{dim}CD25^{high}$ cells, as also documented in a just published study by this same group and by us in a previous communication [84]. There has been no study enumerating Tregs after the seminal work by Miyara et al. in which issues regarding Foxp3 expression by Teffs and Tregs were settled, and active and resting Tregs were unequivocally defined [24]. Importantly, a histological study of pancreases from persons who died of type 1 diabetes as long as 6 months after clinical disease onset reported that $FoxP3^+$ cells were rarely found in $CD4^+$ T cell-infiltrated insulin-expressing islets, suggesting an inadequate presence of these cells at the site of inflammation and autoimmune attack [85].

A most interesting development has been the remarkable result of about 50% lower insulin requirement and higher C-peptide, found in responding type 1 diabetes patients, 4 years after brief anti-CD3 (Otelixizumab®) treatment upon diagnosis [86]. These responders started with an initially higher C-peptide level. A thorough investigation of the effect of this treatment on T cells did not reveal any preferential sparing of any category of T cells, even though in an abstract it was claimed that the antibody had a sparing effect on Tregs [87,88]. As this is the most promising immune intervention in type 1 diabetes thus far, it deserves further attention, especially regarding the possible enhancement of Treg function and/or percent of cells.

While several studies have mapped all the genes associated with the pathogenesis of type 1 diabetes, it has been very difficult thus far to decipher a possible mechanism of action for any of them that would include their role in the generation and function of Tregs. Tregs from type 1 diabetes patients have been shown to be defective in their IL-2R signaling, compared to controls [89]. Just recently, a detailed study has shown that polymorphisms in the CD25 gene associated with susceptibility or with resistance to type 1 diabetes, could be linked to the level of expression of CD25 on Tregs and Teffs. Indeed using healthy controls it was shown that the disease-susceptible SNIPs were associated with significantly lower levels of expression of CD25 in aTregs, rTregs, and Teffs, and diminished IL-2 responsiveness in antigen-expressing CD4 T cells, and also associated with lower FoxP3 levels and lower levels of suppression of the proliferation of autologous Teffs [90]. These two studies make physiological sense, because Tregs cannot synthesize IL-2, rather they may obtain it from activated Teffs, after the latter have satisfied their own needs in the cytokine [43]. A lower level of CD25 in the membrane of Tregs would mean less efficient capture of IL-2 by the IL-2Rαβγc complex [91].

Also of interest for type 1 diabetes, is the observed emergence of host Tregs specific for the grafted tissue, and donor Tregs, specific for components of the host in transplantations [92]. There are no reports regarding Tregs after islet transplantation in humans, but the implications are obvious. Apparently, cyclosporine suppresses induced Treg generation from Teffs, while rapamycin supports it [93]. Interestingly, bone marrow transplant for correction of the IPEX syndrome in young males (whose symptoms included type 1 diabetes) resulted either in cure of type 1 diabetes via normal insulin secretion, and elimination of GAD antibody levels (4-month old child) or in a diminution of GADA levels and the daily insulin dose in another patient (1.5 years old); both patients evidenced appearance of sufficient Tregs after engraftment, that restored proper immune function [94,95].

Prospects in the Prevention of Type 1 Diabetes

Tregs are now well established as a new tool, not only for understanding type 1 diabetes pathogenesis, but also for giving new prospects in the prevention and treatment of the disease.

The defects of Tregs found in type 1 diabetes patients explain the loss of immune tolerance in these patients [75-83]. As the pathways for suppression by Tregs are elucidated and the roles of different molecules already found in Tregs become clearer, our understanding of their role in type 1 diabetes is expected to increase. The fact that bone marrow transplantation is accompanied by the appearance of functional Tregs at levels comparable to those in controls [94,95] offers hope of inducing tolerance via re-induction of a proper Treg repertoire. Methods for *ex-vivo* large scale production of antigen-non-specific or antigen-specific Tregs are already in place [96,97]; these could become a good starting place for the lasting blockade of β-cell destruction and/or successful islet transplantation, with optimized sorting strategies that could dramatically improve the isolation of highly potent Tregs [98]. The use of autologous Tregs cultured *ex-vivo* could of course lead to a re-appearance of disease after a temporary relapse, in a fashion that may be a re-enactment of what has been observed in a number of idiopathic juvenile rheumatoid arthritis patients, considering that type 1 diabetes has a very potent immune memory [99,100].

The journey towards prevention and cure of type 1 diabetes has been a long one and Tregs show promise of being part of the solution [101]. The recent discovery of a transposition genetic element that is found in marsupial mammals and plays a decisive role in the induction of Tregs in the periphery will bear watching for possible application in many autoimmune diseases [102]. Of course, the transition of such cell therapies from animal studies to human clinical trials is a real challenge and knowledge of the purity and stability of cell therapy products is essential prior to their introduction into patients. After all, we are still under the Hippocratic dictum of doing no harm.

Acknowledgements

The authors' research has been supported in part by a grant from the 3rd Community Support Framework (Archimedes I scheme, to GKP, 75% EU funds, 25% Hellenic State funds), and by a small grant of the Hellenic Endocrine Society (to AT and GKP).

References

1. Cahill GF Jr, McDevitt HO (1981) Insulin-dependent diabetes mellitus: the initial lesion. N Engl J Med 304: 1454-1465.
2. Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464: 1293-1300.
3. Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PG, et al. (1985) In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 313: 353-360.
4. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS (1986) The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia 29: 267-274.
5. Hänninen A, Jalkanen S, Salmi M, Toikkanen S, Nikolakaros G, et al. (1992) Macrophages, T cell receptor usage, and endothelial cell activation in the pancreas at the onset of insulin-dependent diabetes mellitus. J Clin Invest 90: 1901-1910.
6. Uno S, Imagawa A, Okita K, Sayama K, Moriwaki M, et al. (2007) Macrophages and dendritic cells infiltrating islets with or without beta cells produce tumour necrosis factor-alpha in patients with recent-onset type 1 diabetes. Diabetologia 50: 596-601.
7. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 155: 1151-1164.
8. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133: 775-787.
9. Gershon RK, Kondo K (1970) Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 18: 723-737.
10. Dorf ME, Benacerraf B (1984) Suppressor cells and immunoregulation. Annu Rev Immunol 2: 127-157.
11. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2005) Immunobiology. (6th edn) Garland Science, London, New York.
12. Steinman L (2007) A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 13: 139-145.
13. Cao D, Malmström V, Baecher-Allan C, Hafler D, Klareskog L, et al. (2003) Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. Eur J Immunol 33: 215-223.
14. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA (2001) CD4+CD25high regulatory cells in human peripheral blood. J Immunol 167: 1245-1253.
15. de Kleer IM, Wedderburn LR, Taams LS, Patel A, Varsani H, et al. (2004) CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. J Immunol 172: 6435-6443.
16. Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G (2001) Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. J Exp Med 193: 1303-1310.
17. Nakamura K, Kitani A, Strober W (2001) Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 194: 629-644.
18. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, et al. (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 203: 1701-1711.
19. Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299: 1057-1061.
20. Ziegler SF (2006) FOXP3: of mice and men. Annu Rev Immunol 24: 209-226.
21. Wan YY, Flavell RA (2007) Regulatory T-cell functions are subverted and converted owing to attenuated Foxpf3 expression. Nature 445: 766-770.
22. Baron U, Floess S, Wieczorek G, Baumann K, Grützkau A, et al. (2007) DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. Eur J Immunol 37: 2378-2389.
23. Floess S, Freyer J, Siewert C, Baron U, Olek S, et al. (2007) Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol 5: e38.
24. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, et al. (2009) Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity 30: 899-911.
25. Bluestone JA, Abbas AK (2003) Natural versus adaptive regulatory T cells. Nat Rev Immunol 3: 253-257.
26. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, et al. (2010) Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol 184: 3433-3441.
27. Saoudi A, Seddon B, Fowell D, Mason D (1996) The thymus contains a high frequency of cells that prevent autoimmune diabetes on transfer into prediabetic recipients. J Exp Med 184: 2393-2398.
28. Stephens LA, Mottet C, Mason D, Powrie F (2001) Human CD4(+)CD25(+) thymocytes and peripheral T cells have immune suppressive activity in vitro. Eur J Immunol 31: 1247-1254.
29. Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, et al. (2001) Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. J Exp Med 193: 1285-1294.
30. Fontenot JD, Rudensky AY (2005) A well adapted regulatory contrivance: regulatory T cell development and the forkhead transcription factor FoxP3. Nature Immunol 6: 331-337.
31. Zheng Y, Rudensky AY (2007) Foxp3 in control of the regulatory T cell lineage. Nat Immunol 8: 457-462.
32. Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, et al. (2007) Foxp3-dependent programme of regulatory T-cell differentiation. Nature 445: 771-775.
33. Zheng Y, Josefowicz SZ, Kas A, Chu TT, Gavin MA, et al. (2007) Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. Nature 445: 936-940.
34. Peterson P, Nagamine K, Scott H, Heino M, Kudoh J, et al. (1998) APECED: a monogenic autoimmune disease providing new clues to self-tolerance. Immunol Today 19: 384-386.
35. Kekäläinen E, Tuovinen H, Joensuu J, Gylling M, Franssila R, et al. (2007) A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Immunol 178: 1208-1215.
36. Danke NA, Koelle DM, Yee C, Beheray S, Kwok WW (2004) Autoreactive T cells in healthy individuals. J Immunol 172: 5967-5972.
37. Li MO, Wan YY, Flavell RA (2007) T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity 26: 579-591.
38. Pugliese A, Brown D, Garza D, Murchison D, Zeller M, et al. (2001) Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. J Clin Invest 107: 555-564.
39. Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, et al. (2008) Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity 28: 687-697.

40. Nakamura K, Kitani A, Strober W (2001) Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 194: 629-644.

41. Pyzik M, Piccirillo CA (2007) TGF-beta1 modulates Foxp3 expression and regulatory activity in distinct CD4+ T cell subsets. J Leukoc Biol 82: 335-346.

42. Bluestone JA, Tang Q (2005) How do CD4+CD25+ regulatory T cells control autoimmunity? Curr Opin Immunol 17: 638-642.

43. Haribhai D, Lin W, Relland LM, Truong N, Williams CB, et al. (2007) Regulatory T cells dynamically control the primary immune response to foreign antigen. J Immunol 178: 2961-2972.

44. Apostolou I, von Boehmer H (2004) In vivo instruction of suppressor commitment in naive T cells. J Exp Med 199: 1401-1408.

45. Grossman WJ, Verbsky JW, Barchet W, Colonna M, Atkinson JP, et al. (2004) Human T regulatory cells can use the perforin pathway to cause autologous target cell death. Immunity 21: 589-601.

46. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, et al. (1999) Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 286: 1957-1959.

47. Carson BD, Ziegler SF (2007) Impaired T cell receptor signaling in Foxp3+ CD4 T cells. Ann N Y Acad Sci 1103: 167-178.

48. Bopp T, Becker C, Klein M, Klein-Hessling S, Palmetshofer A, et al. (2007) Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med 204: 1303-1310.

49. Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, et al. (2007) Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood 110: 1225-1232.

50. Anjos S, Polychronakos C (2004) Mechanisms of genetic susceptibility to type I diabetes: beyond HLA. Mol Genet Metab 81: 187-195.

51. Stockis J, Colau D, Coulie PG, Lucas S (2009) Membrane protein GARP is a receptor for latent TGF-beta on the surface of activated human Treg. Eur J Immunol 39: 3315-3322.

52. Wang R, Zhu J, Dong X, Shi M, Lu C, et al. (2012) GARP regulates the bioavailability and activation of TGFβ. Mol Biol Cell. 23: 1129-1139.

53. Tran DQ, Andersson J, Wang R, Ramsey H, Unutmaz D, et al. (2009) GARP (LRRC32) is essential for the surface expression of latent TGF-beta on platelets and activated FOXP3+ regulatory T cells. Proc Natl Acad Sci U S A 106: 13445-13450.

54. Baecher-Allan C, Wolf E, Hafler DA (2006) MHC class II expression identifies functionally distinct human regulatory T cells. J Immunol 176: 4622-4631.

55. Moustakas AK, Papadopoulos GK (2002) Molecular properties of HLA-DQ alleles conferring susceptibility to or protection from insulin-dependent diabetes mellitus: keys to the fate of islet beta-cells. Am J Med Genet 115: 37-47.

56. You S, Belghith M, Cobbold S, Alyanakian MA, Gouarin C, et al. (2005) Autoimmune diabetes onset results from qualitative rather than quantitative age-dependent changes in pathogenic T-cells. Diabetes 54: 1415-1422.

57. Ochando JC, Yopp AC, Yang Y, Garin A, Li Y, et al. (2005) Lymph node occupancy is required for the peripheral development of alloantigen-specific Foxp3+ regulatory T cells. J Immunol 174: 6993-7005.

58. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL (2002) CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 420: 502-507.

59. Ludewig B, Odermatt B, Landmann S, Hengartner H, Zinkernagel RM (1998) Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. J Exp Med 188: 1493-1501.

60. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, et al. (2000) B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity 12: 431-440.

61. Green EA, Choi Y, Flavell RA (2002) Pancreatic lymph node-derived CD4(+) CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. Immunity 16: 183-191.

62. Bour-Jordan H, Salomon BL, Thompson HL, Szot GL, Bernhard MR, et al. (2004) Costimulation controls diabetes by altering the balance of pathogenic and regulatory T cells. J Clin Invest 114: 979-987.

63. Peng Y, Laouar Y, Li MO, Green EA, Flavell RA (2004) TGF-beta regulates in vivo expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. Proc Natl Acad Sci U S A 101: 4572-4577.

64. Monaco AP, Wood ML, Russell PS (1966) Studies on heterologous antilymphocyte serum in mice. III. Immunological tolerance and chimerism produced across the H2-locus with adult thymectomy and antilymphocyte serum. Ann NY Acad Sci 129: 190-209.

65. Wood ML, Monaco AP, Gozzo JJ, Liegeois A (1971) Use of homozygous allogeneic bone marrow for induction of tolerance with antilymphocyte serum: dose and timing. Transplant Proc 3: 676-679.

66. Penaranda C, Tang Q, Bluestone JA (2011) Anti-CD3 therapy promotes tolerance by selectively depleting pathogenic cells while preserving regulatory T cells. J Immunol 187: 2015-2022.

67. Nanji SA, Hancock WW, Luo B, Schur CD, Pawlick RL, et al. (2006) Costimulation blockade of both inducible costimulator and CD40 ligand induces dominant tolerance to islet allografts and prevents spontaneous autoimmune diabetes in the NOD mouse. Diabetes 55: 27-33.

68. Zhang QW, Rabant M, Schenk A, Valujskikh A (2008) ICOS-Dependent and -independent functions of memory CD4 T cells in allograft rejection. Am J Transplant 8: 497-506.

69. Cobbold SP, Adams E, Graca L, Daley S, Yates S, et al. (2006) Immune privilege induced by regulatory T cells in transplantation tolerance. Immunol Rev 213: 239-255.

70. Tian J, Atkinson MA, Clare-Salzler M, Herschenfeld A, Forsthuber T, et al. (1996) Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. J Exp Med 183: 1561-1567.

71. Elias D, Meilin A, Ablamunits V, Birk OS, Carmi P, et al. (1997) Hsp60 peptide therapy of NOD mouse diabetes induces a Th2 cytokine burst and downregulates autoimmunity to various beta-cell antigens. Diabetes 46: 758-764.

72. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, et al. (2002) CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol 3: 1097-1101.

73. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, et al. (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 297: 1867-1870.

74. Bluestone JA, Tang Q (2004) Therapeutic vaccination using CD4+CD25+ antigen-specific regulatory T cells. Proc Natl Acad Sci U S A 101 Suppl 2: 14622-14626.

75. Kukreja A, Cost G, Marker J, Zhang C, Sun Z, et al. (2002) Multiple immunoregulatory defects in type 1 diabetes. J Clin Invest 109: 131-140.

76. Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, et al. (2005) Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. Diabetes 54: 92-99.

77. Putnam AL, Vendrame F, Dotta F, Gottlieb PA (2005) CD4+CD25high regulatory T cells in human autoimmune diabetes. J Autoimmun 24: 55-62.

78. Brusko TM, Wasserfall CH, Clare-Salzler MJ, Schatz DA, Atkinson MA (2005) Functional defects and the influence of age on the frequency of CD4+ CD25+ T-cells in type 1 diabetes. Diabetes 54: 1407-1414.

79. Tree TI, Roep BO, Peakman M (2006) A mini meta-analysis of studies on CD4+CD25+ T cells in human type 1 diabetes: report of the Immunology of Diabetes Society T Cell Workshop. Ann N Y Acad Sci 1079: 9-18.

80. Brusko T, Wasserfall C, McGrail K, Schatz R, Viener HL, et al. (2007) No alterations in the frequency of FOXP3+ regulatory T-cells in type 1 diabetes. Diabetes 56: 604-612.

81. Glisic-Milosavljevic S, Waukau J, Jailwala P, Jana S, Khoo HJ, et al. (2007) At-risk and recent-onset type 1 diabetic subjects have increased apoptosis in the CD4+CD25+ T-cell fraction. PLoS One 2: e146.

82. Lawson JM, Tremble J, Dayan C, Beyan H, Leslie RD, et al. (2008) Increased resistance to CD4+CD25hi regulatory T cell-mediated suppression in patients with type 1 diabetes. Clin Exp Immunol 154: 353-359.

83. Schneider A, Rieck M, Sanda S, Pihoker C, Greenbaum C, et al. (2008)

The effector T cells of diabetic subjects are resistant to regulation via CD4+ FOXP3+ regulatory T cells. J Immunol 181: 7350-7355.

84. Paschou SA, Vartholomatos G, Petsiou A, Kolaitis N, Giotaki E, et al. (2010) The deficiencies of T regulatory lymphocytes (Tregs) in cell amount, expression and coordination of suppresion-related proteins at type 1 diabetes onset are only partially remedied in long term patients. Diabetologia 53: S182.

85. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG (2009) Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol 155: 173-181.

86. Keymeulen B, Walter M, Mathieu C, Kaufman L, Gorus F, et al. (2010) Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. Diabetologia 53: 614-623.

87. Keymeulen B, Candon S, Fafi-Kremer S, Ziegler A, Leruez-Ville M, et al. (2010) Transient Epstein-Barr virus reactivation in CD3 monoclonal antibody-treated patients. Blood 115: 1145-1155.

88. Apostolou I, Guild J, Vaickus L, Rosenzweig M (2010) Otelixizumab differentially modulates human regulatory and non-regulatory T cells. Diabetologia 53: S183.

89. Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, et al. (2010) Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes 59: 407-415.

90. Garg G, Tyler JR, Yang JH, Cutler AJ, Downes K, et al. (2012) Type 1 diabetes-associated IL2RA variation lowers IL-2 signaling and contributes to diminished CD4+CD25+ regulatory T cell function. J Immunol 188: 4644-4653.

91. Stauber DJ, Debler EW, Horton PA, Smith KA, Wilson IA (2006) Crystal structure of the IL-2 signaling complex: paradigm for a heterotrimeric cytokine receptor. Proc Natl Acad Sci U S A 103: 2788-2793.

92. Graca L, Cobbold SP, Waldmann H (2002) Identification of regulatory T cells in tolerated allografts. J Exp Med 195: 1641-1646.

93. Gao W, Lu Y, El Essawy B, Oukka M, Kuchroo VK, et al. (2007) Contrasting effects of cyclosporine and rapamycin in de novo generation of alloantigen-specific regulatory T cells. Am J Transplant 7: 1722-1732.

94. Baud O, Goulet O, Canioni D, Le Deist F, Radford I, et al. (2001) Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. N Engl J Med 344: 1758-1762.

95. Rao A, Kamani N, Filipovich A, Lee SM, Davies SM, et al. (2007) Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. Blood 109: 383-385.

96. Long SA, Walker MR, Rieck M, James E, Kwok WW, et al. (2009) Functional islet-specific Treg can be generated from CD4+CD25- T cells of healthy and type 1 diabetic subjects. Eur J Immunol 39: 612-620.

97. Putnam AL, Brusko TM, Lee MR, Liu W, Szot GL, et al. (2009) Expansion of human regulatory T-cells from patients with type 1 diabetes. Diabetes 58: 652-662.

98. Brusko TM, Putnam AL, Bluestone JA (2008) Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. Immunol Rev 223: 371-390.

99. Delemarre E, Roord S, Wulffraat N, van Wijk F, Prakken B (2011) Restoration of the immune balance by autologous bone marrow transplantation in juvenile idiopathic arthritis. Curr Stem Cell Res Ther 6: 3-9.

100. Sibley RK, Sutherland DE, Goetz F, Michael AF (1985) Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest 53: 132-144.

101. Bach JF, Chatenoud L (2011) A historical view from thirty eventful years of immunotherapy in autoimmune diabetes. Semin Immunol 23: 174-181.

102. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY (2012) Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell 150: 29-38.

103. Ablamunits V, Herold KC (2008) Generation and function of human regulatory CD8+ T cells induced by a humanized OKT3 monoclonal antibody hOKT3gamma1 (Ala-Ala). Hum Immunol 69: 732-736.

104. Giannoukakis N, Trucco M (2012) A role for tolerogenic dendritic cell-induced B-regulatory cells in type 1 diabetes mellitus. Curr Opin Endocrinol Diabetes Obes 19: 279-287.

This article was originally published in a special issue, **Prevention of Type 1 diabetes by regulation of the immune system** handled by Editor(s). Dr. M. Lucrecia Alvarez, Translational Genomics Research Institute, USA