

# IL-10/TGF- $\beta$ -Treated Dendritic Cells, Pulsed with Insulin, Specifically Reduce the Response to Insulin of CD4+ Effector/Memory T Cells from Type 1 Diabetic Individuals

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## Abstract

**Introduction** Diabetogenic autoreactive T cells with effector/memory characteristics are described in type 1 diabetes patients (T1D). Alternatively activated dendritic cells (aaDCs) have been regarded as promising tools for clinical application in autoimmune diseases (ADs), although their ability to induce antigen-specific tolerance in T cells derived from ADs has yet to be determined.

**Methods** Monocyte-derived dendritic cells (DCs) were produced utilizing GM-CSF and IL-4, and aaDCs by

adding IL-10 and TGF- $\beta$  (10/TGF-DC) during differentiation. Both cell groups were insulin-loaded, matured with lipopolysaccharide, and cocultured with autologous effector/memory T cells derived from T1D individuals, in order to evaluate the induction of insulin-specific tolerance.

**Results and Discussion** In five of eight T1D patients analyzed in vitro, 10/TGF-DC were able to induce insulin-specific tolerance in effector/memory CD4+ T cells (50.4% $\pm$  13.2 less proliferation), without affecting the proliferative response to an unrelated antigen (candidin). Tolerance induction was dependent on the current activation state of CD4+ T cells in each patient. 10/TGF-DC-stimulated T cells acquired an IL-2<sup>low</sup>IFN- $\gamma$ <sup>low</sup>IL-10<sup>high</sup> cytokine profile, and their hyporesponsiveness could be reverted upon exposure to IL-2. This study shows a perspective about the in vitro ability of monocyte-derived 10/TGF-DC to induce antigen-specific tolerance in effector/memory T cells generated during the course of an autoimmune disease.

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**Keywords** Dendritic cells · tolerance · effector/memory T cells · type 1 diabetes

## Abbreviations

ADs	autoimmune diseases
cTC	control T lymphocytes
iDCs	immature DC
LPS	lipopolysaccharide
mDCs	mature DC
MFI	mean fluorescence intensity
rTC	T cells with no prior stimulation in vitro
T1D	type 1 diabetes
tDCs	tolerogenic DC
10/TGF-DC	DC generated with IL-10/TGF- $\beta$ 1
tTC	tolerant T lymphocytes

## Introduction

Type 1 diabetes (T1D) results from autoimmune destruction of insulin-producing  $\beta$  cells located in the islets of Langerhans within the pancreas [1]. It is believed that the mechanism by which the disease is initiated depends on a complex interaction between genetic and environmental factors [2, 3]. Although some autoantibodies serve as biomarkers of the disease progress, evidences indicate that B lymphocytes do not play a major role [4], and a Th2 polarization indicates a good prognosis [5, 6]. In humans,  $\beta$  cell destruction is dependent on autoreactive T CD4+ helper and CD8+ cytotoxic T cells that respond to several antigens synthesized by pancreatic  $\beta$  cells (i.e., insulin derivatives, glutamic acid decarboxylase, and tyrosine phosphatase IA-2) [7, 8]. Although  $\beta$  cell-specific naïve T cells may gain access to the islets, their activation occurs after encountering antigen presenting cells (APC) displaying  $\beta$  cell antigens in the draining lymph nodes, where naïve T cell/APC cross-talking induces their clonal expansion and differentiation to effector and memory cells. Then, activated T cells migrate either to B cell areas in order to assist in antibody production or outside the lymphoid tissue to sites with active inflammation [9].

T1D patients and healthy individuals have diabetogenic autoreactive T cells; nevertheless, in the former, they are preferentially effector/memory cells with an activated phenotype [10–12]. These evidences suggest that a successful tolerance induction directed during the prodromal phase or at the onset of type 1 diabetes would avoid the activation of diabetogenic T cells, albeit once the disease is already developed, tolerization must also be focused on effector/memory T cells.

Under steady-state, DCs avoid unwanted responses against self-antigens and commensal organisms in vivo [13–15], therefore they have been regarded as possible tools for clinical application in ADs. Tolerogenic capacities were initially attributed to DC in an immature state due to antigen presentation in the absence of costimulation [16–18]. However, in vivo permanence of the immature state cannot be warranted in environments with active inflammation, such as ADs. Hence, mature DCs exposed to several immunomodulatory agents are being studied in the design of strategies for tolerance induction [19]. Recently, we demonstrated that human-monocyte-derived DCs treated with IL-10 and TGF- $\beta$ 1 (10/TGF-DC) are able to induce tetanus toxoid-specific tolerance and regulatory T cells in memory T lymphocytes of healthy volunteers vaccinated with the toxoid [20]. Thus, in the present work, we aimed to analyze this capability on effector/memory T cells generated during an autoimmune response. Our results showed that 10/TGF-DC can induce insulin-specific tolerance in effector/memory T cells of T1D patients in vitro and

that this tolerance induction is dependent on the current activation state of CD4+ T cells in each patient. These results suggest that an initial analysis of the activation state of CD4+ T cells could improve the efficacy of 10/TGF-DC in strategies for tolerance induction in autoimmune patients.

## Patients and Methods

### T1D Patients

Blood samples (20 mL) were obtained from eight T1D adults patients (23–45 years old) diagnosed with diabetes for at least 6 years and without severe complications, elevated titers of anti-insulin Ab-positive, and without active infection or neoplasia. Informed consent was obtained according to the Helsinki Declaration.

### Media and Reagents

Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, and 50  $\mu$ M 2-ME. The following reagents were used: human recombinant GM-CSF (Prospect-Tany TechnoGene Ltd), IL-4, IL-10, TGF- $\beta$ 1, IL-2 (R&D Systems), carboxyfluorescein succinimidyl ester (CFSE) (Molecular Probes), candidin (antigen-derived from *Candida albicans*, supplied by the Department of Basic Mycology, UNAM, Mexico), insulin, and lipopolysaccharide (LPS; Sigma-Aldrich).

### Cell Separation

Peripheral blood mononuclear cells were obtained by Ficoll-Hypaque gradient from total blood samples. Cells were purified by magnetic cell sorting using MACS isolation kits. CD14+ monocytes were separated by using CD14+ microbeads and CD4+CD45RA- effector/memory T cells by negative selection using CD4+ T cell isolation kit and anti-CD45RA microbeads (Miltenyi Biotec).

### Differentiation of Monocytes to Control DC and 10/TGF-DC

Monocytes were cultured at  $1 \times 10^6$  cell/mL and treated with GM-CSF (20 ng/mL) and IL-4 (20 ng/mL) to obtain control DC or GM-CSF and IL-4 plus IL-10 (20 ng/mL) and TGF- $\beta$ 1 (40 ng/mL) to obtain 10/TGF-DC. Cultures were fed with fresh medium and cytokines every 2 days. At day 6, insulin, candidin (1.0  $\mu$ g/ml), or no Ag were added to iDC, and 8 h later cells were treated with LPS (0.5  $\mu$ g/ml) for two more days to obtain mDC.

## Flow Cytometry Analysis and Ab used for Staining

Purified FITC-conjugated mAb to CD83, CD40, CD86, and PE-conjugated mAb to HLA-DR, CD1a, and CD14 (BD Biosciences) were utilized. Fluorescence staining was analyzed with a FACSCalibur (BD Biosciences) and Cell Quest software. Data were expressed as MFI.

## Insulin-Specific Proliferation Assay

CD4+CD45RA-lymphocytes were labeled with CFSE and co-cultured with insulin-loaded or unloaded autologous control DC or 10/TGF-DC at 10:1 (T cell/DC) ratio for 5 days. Then lymphocytes were harvested, and proliferation was assessed by the CFSE dilution method by flow cytometry.

## Tolerance Assay

Unlabeled CD4+CD45RA-lymphocytes were cocultured with insulin-loaded autologous control DC [referred as control T cells (cTC)] or 10/TGF-DC [referred as “tolerant T cells” (tTC)] at 10:1 T cell/DC ratio for 5 days. Additionally, CD4+CD45RA-T cells were maintained in culture without antigenic stimulus and used as control, termed “rested” T cells (rTC). After the 5-day period, lymphocytes were harvested, resuspended in fresh medium, and rested for four additional days. Then, each lymphocyte population was labeled with CFSE and recalled with autologous insulin-loaded control DC (with or without exogenous IL-2) or candidin-loaded control DC at 10:1 T cell/DC ratio for 5 days. Lymphocytes were harvested, and proliferation was assessed by the CFSE dilution method by flow cytometry.

## Cytokine Detection in Culture Supernatants

Production of IL-10 and IL-12p70 in supernatants of iDC matured with LPS for 24 h and production of IL-2, IFN- $\gamma$ , and IL-10 in supernatants collected at day 5 from co-cultures of CD4+CD45RA-T cells with control DC or 10/TGF-DC were quantified by ELISA kits (BD Biosciences, R&D Systems).

## Statistical Analysis

Data are expressed as the mean values $\pm$ SD of individual experiments. The Student's two-tailed paired *t* test was performed to assess statistical differences between two experimental groups, assuming equal variances; a *p* value < 0.05 was considered significant.

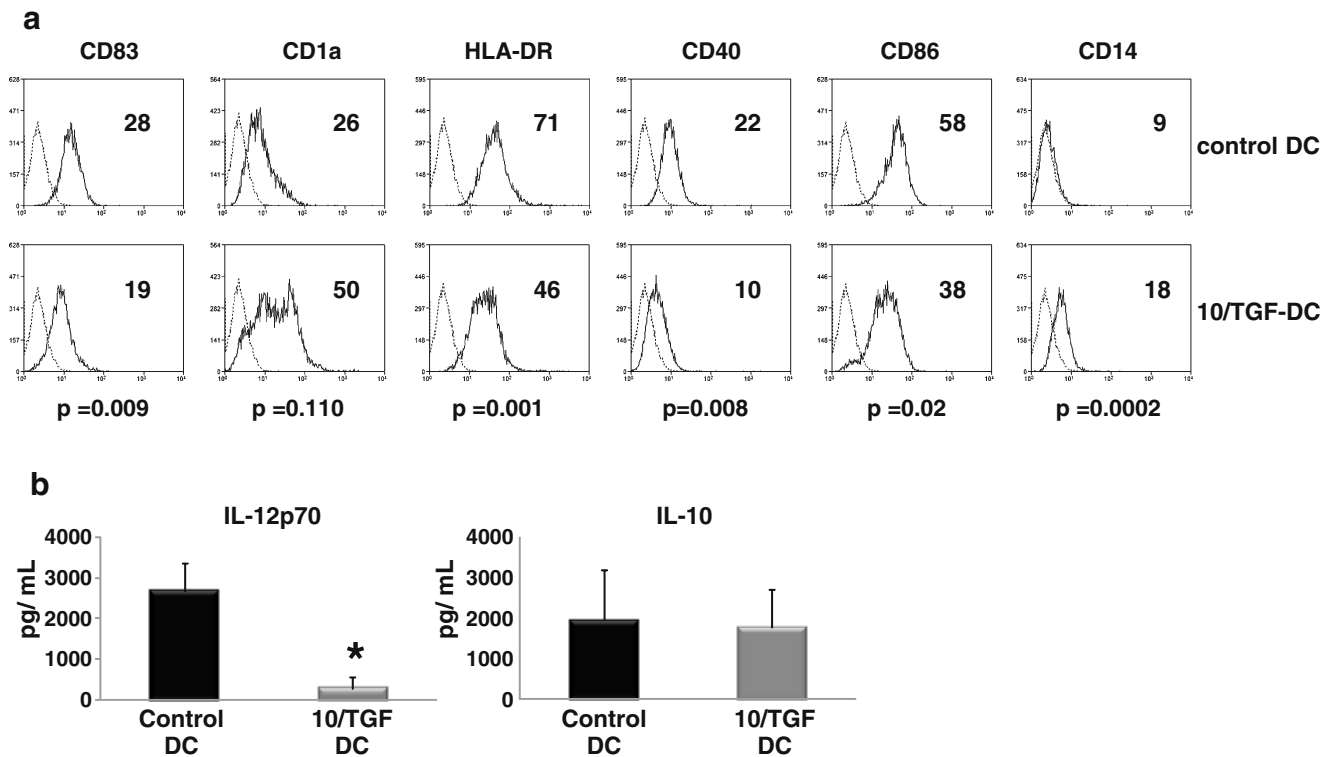
## Results

### IL-10/TGF- $\beta$ Treatment Affects Dendritic Cells Maturation

Control DC showed a classical phenotype of mature DC; they expressed high levels of DC differentiation markers such as CD83 and CD1a, MHC II, and costimulatory molecules (CD40 and CD86) with null or low expression of the monocyte/macrophage marker CD14. In contrast, even though 10/TGF-DC expressed marginal higher levels of CD1a than control DC, there were no statistically significant differences (*p*=0.1). In addition, 10/TGF-DC showed lower levels of HLA-DR (*p*=0.001), CD40 (*p*=0.008), and CD86 (*p*=0.02) molecules and of the differentiation marker CD83 (*p*=0.009) but maintained higher expression of CD14 (*p*=0.0002) compared with control DC (Fig. 1a, Table I). Functionally, 10/TGF-DC produced tenfold lower levels of the inflammatory cytokine IL-12 p70 than control DC (*p*=0.00004), although IL-10 secretion was similar in both types of DC (Fig. 1b).

### Differences in Insulin-specific Stimulation Ability between Control DC and 10/TGF-DC Were Observed only without Active Proliferation of Effector/Memory T Cells

Tolerogenic DCs (tDC) regularly induce poor allogeneic and/or antigen-specific proliferation and effector cytokine secretion when stimulating naïve or memory T cells [19–21]. Thereby, in order to analyze the ability of 10/TGF-DC to induce insulin-specific proliferation, we co-cultured CFSE-labeled CD4+CD45RA-T cells with insulin-pulsed or non-pulsed control DC and 10/TGF-DC. In six of eight T1D patients, priming with control DC resulted in a significantly stronger insulin-specific proliferation than that induced with 10/TGF-DC (*p*=0.002, Fig. 2a, left panel, and c). The basal proliferation in the absence of insulin was low and similar for both types of DC (insulin-; Fig. 2a, right panel, and c). Cytokine quantification in supernatants of co-cultures showed lower IL-2 (*p*=0.007) and IFN- $\gamma$  (*p*=0.001) production by T cells stimulated with 10/TGF-DC and higher levels of IL-10 (*p*=0.003) than in co-cultures with control DC (Fig. 3a). Phenotype analysis after 5 days stimulation did not show differential expression of regulatory T cells markers (CD25, CTLA-4, CD39, and Foxp3) in T cells stimulated with control DC or 10/TGF-DC (data not shown). Conversely, in two T1D patients, there were no differences between insulin-specific proliferation induced by control DC and 10/TGF-DC (Fig. 2b, left panel and d). In these patients, we purified elevated numbers of CD4+CD45RA-(effector/memory) T cells (Table II), while we observed high basal proliferation (Fig. 2b, right panel, and d), and cytokine quantification in co-culture supernatants revealed no



**Fig. 1** IL-10/TGF- $\beta$  treatment inhibits dendritic cells maturation. Immature monocyte derived-DCs generated in absence (*control DC*) or presence of IL-10/TGF- $\beta$  (*10/TGF-DC*) were stimulated with LPS for 2 days to induce DC activation/maturation. **a** Expression of the indicated molecules evaluated with the corresponding mAbs (*solid lines*) or isotype-matched mAbs (*dotted lines*), analyzed by flow cytometry. The values shown within histograms correspond to the

mean channel fluorescence of each marker, and the results are representative of eight experiments with similar results. **b** Cytokine quantification in supernatants of 24-h stimulated DC. Supernatants of LPS-stimulated DC were collected and frozen at  $-70^{\circ}\text{C}$  until ELISA assays (IL-12 p70 and IL-10) were performed. Results are expressed as average $\pm$ SD of eight individual experiments. Statistical analysis (10/TGF-DC vs control DC): \* $p < 0.05$

differences by employing control DC or 10/TGF-DC as stimulators (Fig. 3b).

#### In Some Patients, IL-10/TGF- $\beta$ 1 treated DC-Induced Insulin-Specific Tolerance in Effector/Memory T Cells

In order to evaluate whether 10/TGF-DC-stimulated CD4+CD45RA-T cells reduced their capability to proliferate

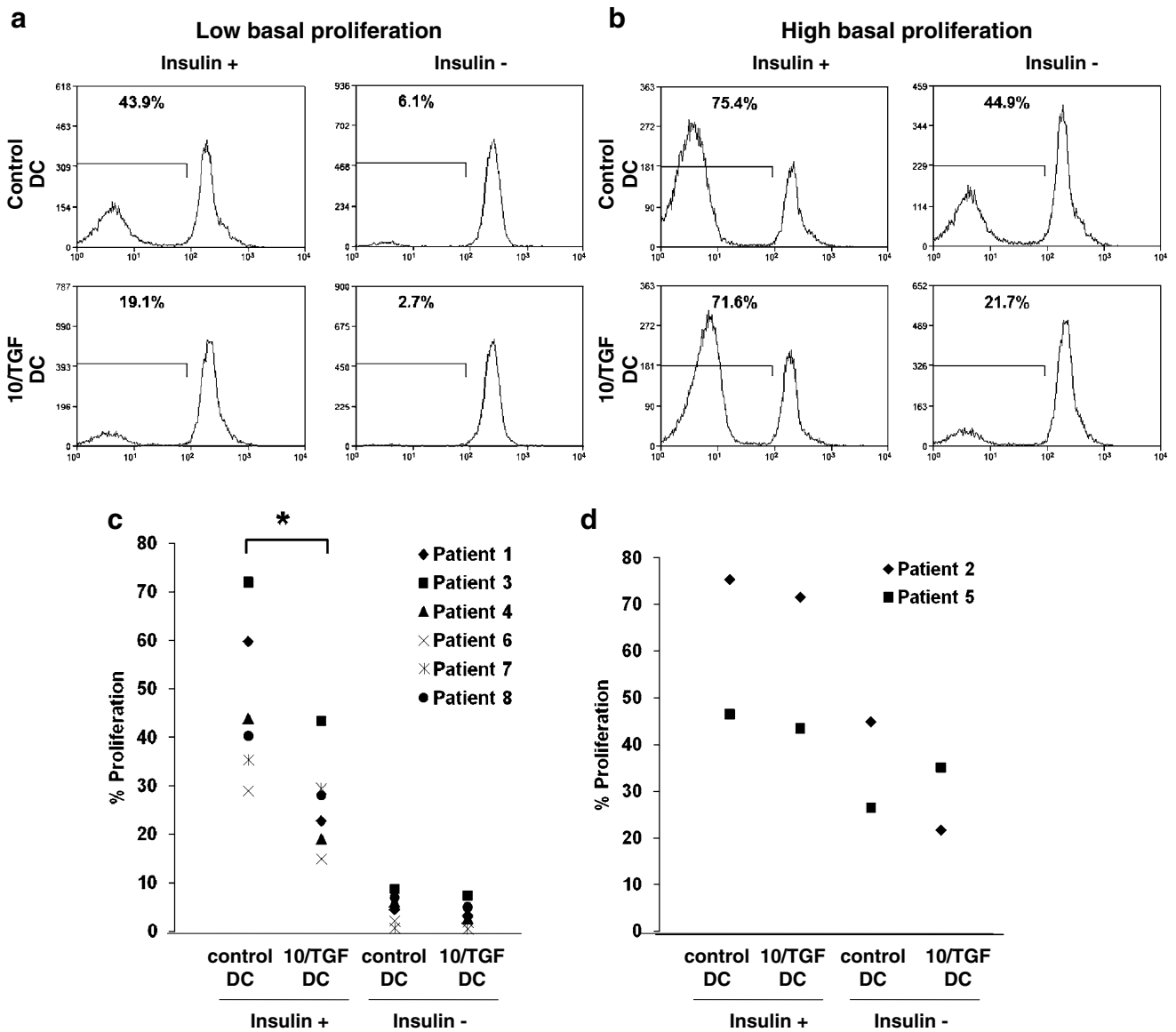
**Table I** Phenotypic Profile of Control and 10/TGF-DC

	Control DC	10/TGF-DC
CD1a	46.9 $\pm$ 16.3	68.0 $\pm$ 21.4
CD14	9.1 $\pm$ 3.5	19.6 $\pm$ 5.6*
CD40	79.0 $\pm$ 37.9	23.2 $\pm$ 7.7*
CD83	24.6 $\pm$ 6.2	16.5 $\pm$ 2.7*
CD86	54.4 $\pm$ 22.0	28.6 $\pm$ 6.6*
HLA-DR	76.2 $\pm$ 33.9	40.6 $\pm$ 26.1*

Data are expressed as mean fluorescence intensity (MFI) of eight independent patients, mean $\pm$ SD

\* $p < 0.05$  (10/TGF-DC versus control DC)

against a second insulin-specific stimulus with competent DC, T cells previously stimulated with control DC (cTC) or 10/TGF-DC (tTC) were labeled with CFSE and rechallenged with insulin-loaded autologous control DC. Under these conditions, in five of eight T1D patients (all of them with previous low basal proliferation), cTC showed strong insulin-specific proliferation (Fig. 4a, left upper panel, and c), with elevated levels of IL-2 and IFN- $\gamma$  and low levels of IL-10 in co-cultures (Fig. 5a). In contrast, tTC proliferation was significantly lower than observed in cTC ( $p = 0.002$ ; Fig. 4a, left middle panel, and c), with lower levels of IL-2 ( $p = 0.0009$ ) and IFN- $\gamma$  ( $p = 0.001$ ), and elevated IL-10 ( $p = 0.0005$ ; Fig. 5a). As a survival control, we stimulated CD4+CD45RA-T cells that were maintained in the absence of stimulus (rTC), and these cells had a similar proliferation profile as cTC (Fig. 4a, left bottom panel, and c). Additionally, with the aim of investigating whether the response against an unrelated antigen was not altered, cTC, tTC, and rTC were rechallenged with candidin-loaded autologous control DC. Our results showed that lymphocytes responded similarly against candidin irrespective of their prior stimulation, suggesting an insulin-specific tolerance induction



**Fig. 2** Stimulatory capability of control DC and 10/TGF-DC on effector/memory T cells in autologous insulin-specific responses. CFSE-labeled autologous CD4+CD45-T cells were co-cultured with control DC or 10/TGF-DC at 1:10 (DC/T) cell ratio. After 5 days, lymphocytes were harvested, and the percentage of proliferating cells was calculated by CFSE dilution by flow cytometry. Histograms show

representative donors with low (a) or high basal proliferation (b) of lymphocytes challenged with insulin-loaded (insulin+) or unloaded (insulin-) DC. (c, d) Percentage of proliferating T cells challenged with insulin as in (a, b) in patients with low (c) or high basal proliferation (d), where each symbol represents an individual patient

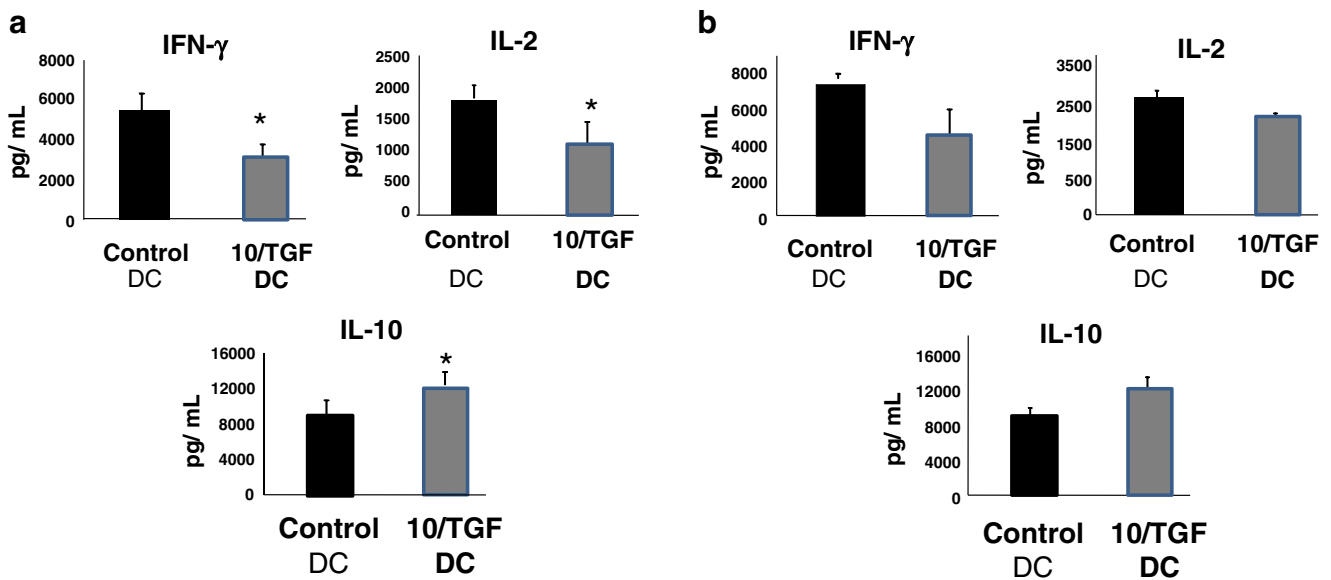
(Fig. 4a, middle panel, and c). Moreover, tTC stimulation with insulin-loaded control DC in the presence of exogenous IL-2 induced comparable proliferation to the observed with cTC and rTC, breaking down the tolerance state in tTC (Fig. 4a, right panel, and c). These results reaffirm the tolerogenic properties of IL-10/TGF-β-treated DC and demonstrate their ability to induce insulin-specific tolerance in effector/memory T cells generated in vivo in T1D patients.

Despite the above results, in three patients out of eight (two of them with previous high basal proliferation), we observed no differences either in proliferation (Fig. 4b,

right panel, and d) or in cytokine production (Fig. 5b) between cTC and tTC rechallenged with insulin- (with or without IL-2) or candidin-loaded control DC.

**Discussion**

Antigen-specific tolerance induction in T cell-mediated autoimmune diseases including T1D has been an elusive achievement for immunologist [22]. In diverse experimental assays, tolerogenic strategies applied in young predia-



**Fig. 3** Cytokine quantification in co-cultures with control and 10/TGF-DC. IFN- $\gamma$ , IL-2, and IL-10 were quantified in supernatants of co-cultures established as in Fig. 2 of patients with low (a) or high basal proliferation (b). They were measured in 5-day super-

natants by ELISA assays. Results are expressed as average $\pm$ SD of eight individual experiments. Statistical analysis (10/TGF-DC vs control DC): \* $p$ <0.05

betic NOD mice can prevent spontaneous diabetes [22–28], while when an autoimmune reaction is already in progress; the presence of effector/memory T cells complicates tolerance induction [29–31].

This work showed that, in five of eight T1D patients, CD4+CD45RA-T cells purified from peripheral blood and stimulated with autologous IL-10/TGF- $\beta$ -treated, insulin-pulsed DC had a significantly decreased proliferation against a subsequent challenge with fully competent DC pulsed with the same antigen. This effect was associated with a reduction of IL-2 and IFN- $\gamma$  secretion by T cells, which might be due to their low proliferation. Nevertheless, although differences in the expression of regulatory T cells

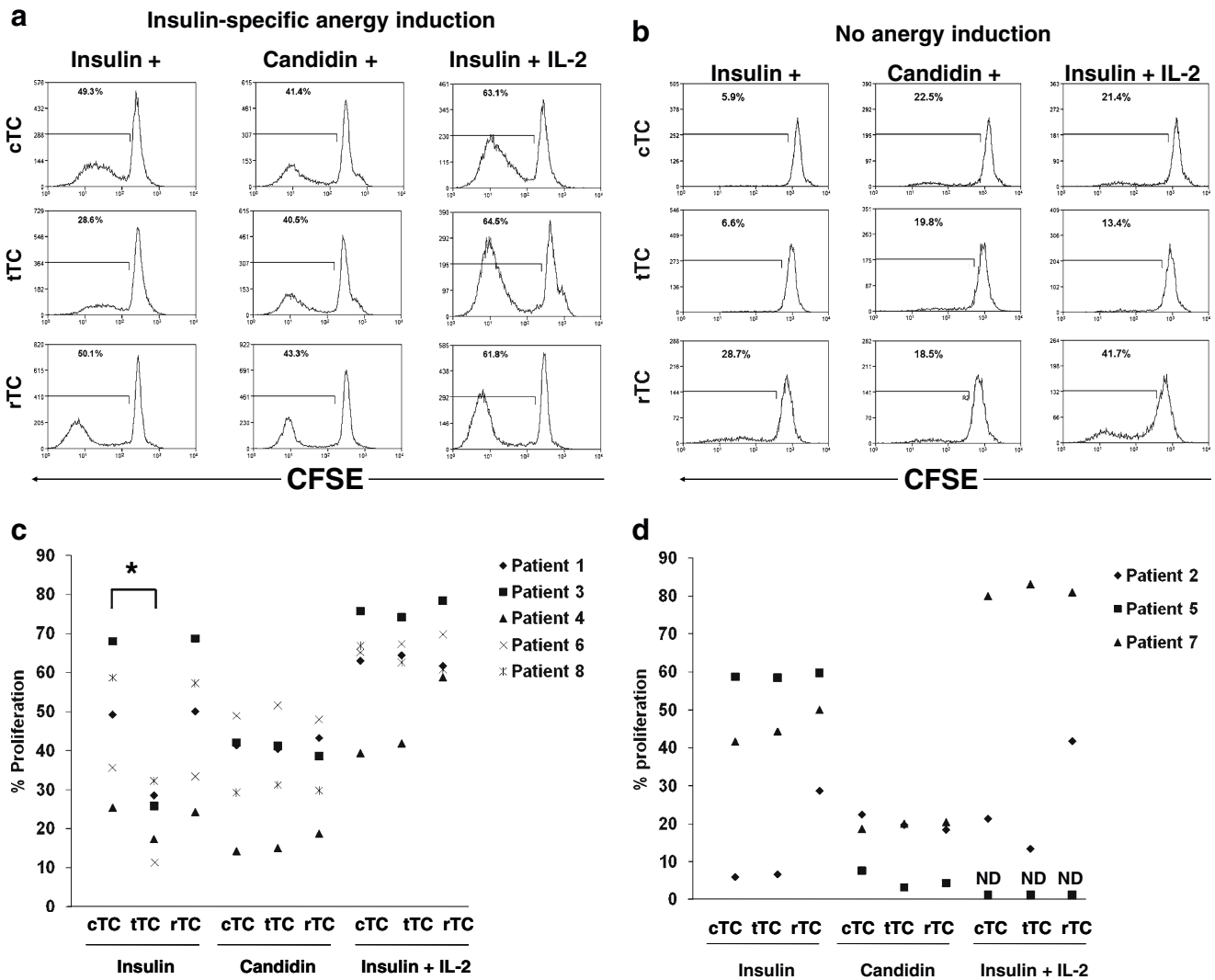
markers after 5 days of stimulation were not found, higher levels of IL-10 were also detected in these cultures, suggesting the possibility of regulatory T cell induction, as it was previously demonstrated in naïve [21] and memory T cells [20, 32] by stimulating with IL-10 and TGF- $\beta$ -treated DC. We demonstrated that the decreased T cell proliferation was not due to a survival deficiency or polyclonal affectation because lymphocytes that were maintained in culture with no DC stimulus (rTC) had similar proliferation than cTC. Additionally, tTC proliferation could be recovered by exogenous addition of IL-2 with a comparable proliferative response against the stimulus with an unrelated antigen (candidin) than cTC. Since clonal anergy is defined as a hyporesponsive state affecting IL-2 production and proliferation on restimulation [31, 38], these results indicate an insulin-specific anergy induction on effector/memory T cells by IL-10/TGF-DC.

As mentioned above, 10/TGF-DC-stimulated T cells showed significant antigen-specific anergy rather than total absence of response to insulin. It is feasible that the use of autologous insulin-pulsed 10/TGF-DC on a polyclonal T cell population with a diversity of TCR affinities [33] could generate a heterogeneous population with distinct response degrees [34], since tolerance induction would depend on the array of signals received by each individual T cell during stimulation. Several studies have shown that the control of an autoimmune response could be achieved through reaching a low threshold of autoreactive T cells that are unable to establish an overt inflammatory response [35], as indicated by the existence of autoreactive clones with

**Table II** Cell Numbers of Naïve and Effector/Memory CD4+ T Cells

Patient	CD45RA+	CD45RA-	RA+/RA-
1	4.6	3.8	1.21
2	4.2	7.3	0.58
3	4.9	2.6	1.88
4	6.8	3.6	1.89
5	4.4	6.2	0.71
6	5.5	2.6	2.12
7	6.2	3.8	1.63
8	5.2	2.9	1.79

Numbers are the amount  $\times 10^6$  of CD4+CD45RA+ (naïve) and CD4+CD45RA- (effector/memory) T cells purified by magnetic cells sorting from 20 ml of blood and RA+/RA- represent the ratio of these cells in each patient



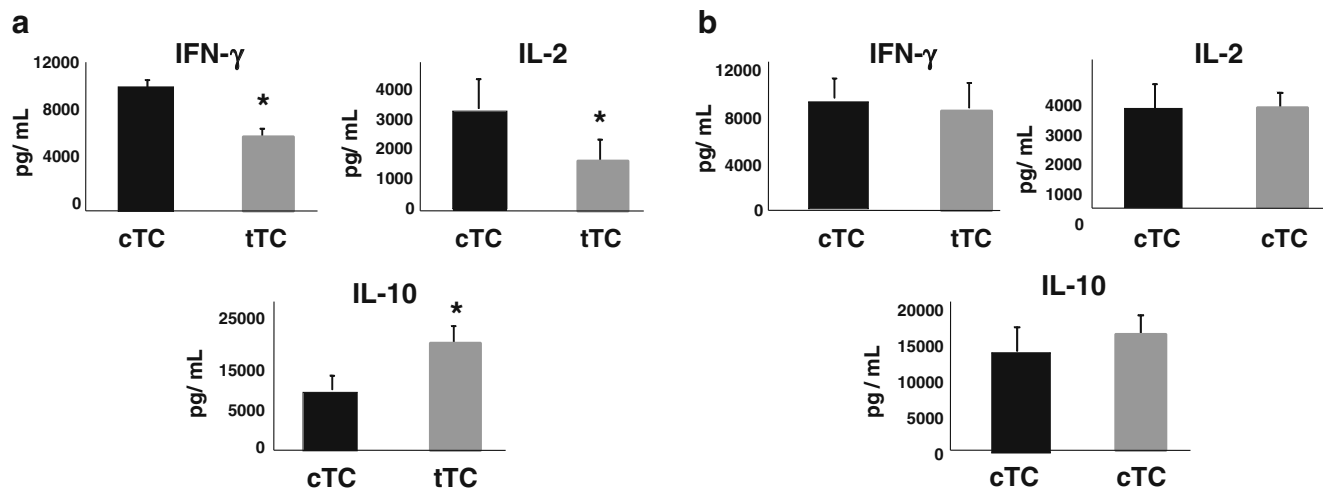
**Fig. 4** IL-10/TGF- $\beta$ 1-treated DC-induced insulin-specific anergy in effector/memory T cells. Lymphocytes previously stimulated with insulin-loaded control DC (cTC), 10/TGF-DC (tTC), or maintained in culture in absence of stimulus (rT) were harvested, resuspended in fresh medium, and rested for four additional days. Then, lymphocytes were labeled with CFSE and rechallenged with insulin-loaded control DC (insulin+), candidin-loaded control DC (candidin+) or insulin-

loaded control DC plus exogenous IL-2 (insulin+IL-2), and proliferation was analyzed after 5 days. Histograms show representative experiments in patients with anergy induction (a) or with no anergy induction (b). (c, d) Percentage of proliferating T cells in patients with anergy induction (c) or with no anergy induction (d); each symbol represents an individual patient. ND not determined

low affinity in healthy individuals with no autoimmunity development [36].

Anergy induction could not be achieved in three of the eight T1D patients, since cTC and tTC did not show differences in proliferation during the rechallenge with insulin-loaded control DC. Two of them (2 and 5) presented high basal non-insulin-specific proliferation and no differences between control DC and 10/TGF-DC stimulation. These data imply that those patients (despite selection criteria) might have a subclinical inflammatory state, as suggested for higher numbers of purified CD4+CD45RA-T cells, consequently, this population would contain superior

numbers of effector than memory T cells [37]. The behavior of T cells at rechallenge was different in the three patients. In patients 2 and 5, T cell proliferation during rechallenge was inversely correlated with their proliferation at priming, which might be due to antigen-induced cell death (AICD) of effector cells in patient 2 upon stimulation with control DC as well as 10/TGF-DC [36]. T cells from patient 5 responded well to the secondary stimulus, but their proliferation was abrogated in the presence of IL-2, a fact which could also be explained by the presence of a large number of death effectors as a consequence of IL-2-induced AICD [29].



**Fig. 5** Cytokine quantification in co-cultures of cTC and tTC stimulated with control DC. IFN- $\gamma$ , IL-2, and IL-10 were quantified in supernatants of co-cultures established as in Fig. 5 of patients with anergy induction (a) or lacking anergy induction (b). They were

measured in 5-day supernatants by ELISA assays. Results are expressed as average $\pm$ SD of eight individual experiments. Statistical analysis (10/TGF-DC vs control DC): \* $p$ <0.05

Lastly, T cells from patient 7 proliferated adequately upon recall, and their proliferation increased in the presence of IL-2. Although the reason why we could not induce insulin-specific anergy in this patient is unknown, it is feasible that some patients have intrinsic resistance to tolerance induction [28].

The IL-10/TGF- $\beta$  cytokine combination to produce 10/TGF-DC had already been described to induce tolerance in naive [21] and memory [20] T cells. Similar as reported using LPS as maturation stimuli and comparing with their non-treated counterparts, IL-10/TGF- $\beta$ -treated DC showed lower expression of CD83, HLA-DR, CD40 and CD86, and slightly retained the expression of CD14 as a marker related with the monocyte/macrophage lineage [21]. The ability of DC to activate or inactivate T cells and direct their final outcome depends on several factors, including their maturation/differentiation state, the balance in expression of costimulatory and inhibitory molecules, and the cytokine profile secretion among others [15]. The analysis of costimulatory molecules in different kinds of aaDC has shown a variable expression depending on the protocols used [19], and the need of some costimulatory molecules such as CD80 and CD86 has been involved in tolerance induction [38]. CD40 has is an essential costimulatory molecule for inducing activation and differentiation of effector T cells [39]. Different protocols used for induction of tDC concur in low expression of CD40; lack of costimulation through CD40 is associated with tolerance induction [40–43], and its effect is paramount in memory T lymphocytes to induce efficiently their differentiation to effector cells [44, 45]. Furthermore, low IL-12 secretion is directly associated with DC

endowed with tolerogenic functions as a result of the inhibition of signaling pathways involved in DC activation [15] and as inducers of Th2 responses [46, 47], and even though IL-10 production by aaDCs has been closely implicated in tolerance induction, it is not an absolute requisite for their tolerogenic function [15, 20]. In our study, 10/TGF-DC effectively showed low secretion of IL-12, while IL-10 production was comparable to control DC, analogously it has been reported elsewhere [20]. Despite that both IL-10 and TGF- $\beta$  are immunosuppressive cytokines with synergic effects on DC and T cells, TGF- $\beta$  can abrogate IL-10 production in DC [48]. In addition, we have previously demonstrated that IL-10 is not essential for memory T cell suppression induced by 10/TGF-DC; instead, other factors such as prostanoids and adenosine play important roles in their tolerogenic activity [20].

## Conclusion

In conclusion, this study shows the feasibility of monocyte-derived 10/TGF-DC to induce antigen-specific tolerance in effector/memory T cells generated during the course of an autoimmune disease. Broader and comprehensive studies are needed to investigate their possible application in therapeutic strategies.

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