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Tolerogenic dendritic cells induce antigen-specific hyporesponsiveness in insulin- and glutamic acid decarboxylase 65-autoreactive T lymphocytes from type 1 diabetic patients

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Abstract Tolerogenic dendritic cells (tDC) constitute a promising therapy for autoimmune diseases, since they can anergize T lymphocytes recognizing self-antigens. Patients with type 1 diabetes mellitus (T1D) have autoreactive T cells against pancreatic islet antigens (insulin, glutamic acid decarboxylase 65 -GAD65-). We aimed to determine the ability of tDC derived from T1D patients to inactivate their insulin- and GAD65-reactive T cells. CD14+ monocytes and CD4+CD45RA- effector/memory lymphocytes were isolated from 25 patients. Monocyte-derived DC were generated in the absence (control, cDC) or presence of IL-10 and TGF-β1 (tDC), and loaded with insulin or GAD65. DC were cultured with T lymphocytes (primary culture), and cell

Abbreviations: Ab, antibody; Ag, antigen; AGE, advanced glycation end products; APC, antigen presenting cells; cDC, control dendritic cells; CFSE, carboxy-fluorescein diacetate succinimidyl ester; cTL, T cells cultured with cDC; FCS, foetal calf serum; GAD, glutamic acid decarboxylase; GM-CSF, granulocyte/macrophage colony-stimulating factor; Hb, haemoglobin; LPS, lipopolysaccharide; mAb, monoclonal antibody; NOD, non-obese diabetic; T1D, type 1 diabetes mellitus; tDC, tolerogenic DC; Th, T helper; Treg, regulatory T cells; tTL, T cells cultured with tDC.

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proliferation and cytokine secretion were determined. These lymphocytes were rechallenged with insulin-, GAD65- or candidin-pulsed cDC (secondary culture) to assess whether tDC rendered T cells hyporesponsive to further stimulation. In the primary cultures, tDC induced significant lower lymphocyte proliferation and IL-2 and IFN-γ secretion than cDC; in contrast, tDC induced higher IL-10 production. Lymphocytes from 60% of patients proliferated specifically against insulin or GAD65 (group 1), whereas 40% did not (group 2). Most patients from group 1 had controlled glycemia. The secondary cultures showed tolerance induction to insulin or GAD65 in 14 and 10 patients, respectively. A high percentage of these patients (70–80%) belonged to group 1. Importantly, tDC induced antigen-specific T-cell hyporesponsiveness, since the responses against unrelated antigens were unaffected. These results suggest that tDC therapy against multiple antigens might be useful in a subset of T1D patients.

1. Introduction

Type 1 diabetes mellitus (T1D) is an autoimmune disease that results in selective damage of pancreatic islet beta cells and leads to total insulin deficiency [1]. Once T1D is diagnosed, the existing treatments are insulin replacement and islet-cell transplantation or regeneration. However, insulin administration does not completely protect against long-term complications of the disease, and pancreas or islet transplantation is not suitable for the majority of patients due to the lack of donor organs. Furthermore, unless immunological tolerance to pancreatic autoantigens is re-established before islet-cell replacement, diabetes would remain a chronic disease.

The immune-mediated beta-cell death is believed to be caused by a T helper (Th) 1-dominant response (and perhaps Th17) against several autoantigens, including insulin, glutamic acid decarboxylase 65 (GAD65), and tyrosine phosphatase IA-2 [2–6]. A further degree of complexity in the development of T1D comes from abnormalities in the immunosuppressive abilities or frequency of regulatory T cells (Treg), which down-modulate undesirable exacerbated T-cell responses [7,8]. The development of Th1 effectors and inducible regulatory T cells is determined by professional antigen presenting cells (APC) such as dendritic cells (DC). These APC have the ability to internalize, process, and present self and non-self antigens (Ag) to T cells. DC were originally characterized by their capability to evoke efficient T-cell responses [9]. However, DC are also essential for the generation and maintenance of peripheral T-cell tolerance [10] through mechanisms that involve induction of anergy or apoptosis in self-reactive T cells, or development of Treg [11]. These activities are carried out by tolerogenic DC (tDC), that have low antigen-presentation and co-stimulatory abilities, and additionally could produce immunosuppressive cytokines [12]. The tolerogenic function of DC is determined by microenvironmental signals such as immunosuppressive factors (e.g. vitamin D3, corticosteroids) or anti-inflammatory cytokines (IL-10, TGF-β1) [12–15], as well as by the absence of "danger signals" (some pathogen-associated molecular patterns, inflammatory factors) that drive the activation and maturation of resident tissue DC [9].

Several immunotherapies are currently investigated to accomplish long-term tolerance to self-Ag. Dendritic cells are effective in the modulation of immune responses, and their feasibility for cancer immunotherapy in humans has been demonstrated [16]. More recently, the use of tDC in transplantation to prolong the graft survival [17] and in autoimmune diseases is being explored.

In the non-obese diabetic (NOD) mouse, a model to study T1D, several types of DC prevent and even reverse the disease through various mechanisms: (i) induction of Treg with DC isolated from pancreatic lymph nodes [18], DC derived from bone marrow precursors with IL-10 [19], or DC generated in the presence of CD40-, CD80-, and CD86-targeting antisense DNA [20], (ii) immunological bias to Th2 responses with conventional DC derived from bone marrow precursors [21], or with splenic DC pulsed with γ-globulin [22], and (iii) induction of insulin-specific tolerance with immature DC [23]. In humans, phase I clinical trials with tDC have been conducted in patients with T1D and rheumatoid arthritis [24,25]. For T1D, patients were injected with autologous monocyte-derived DC treated ex vivo with anti-sense oligonucleotides targeting CD40, CD80 and CD86 co-stimulatory molecules. These tDC have poor co-stimulatory ability and secrete low levels of inflammatory cytokines [20]. The trial demonstrated the safety of the treatment. The autoimmune response of the patients was not enhanced, and the injection with tDC did not result in systemic immunosuppression [24]. The increase of a B220+CD11c- regulatory B cell subset was observed after tDC treatment, but whether they have a beneficial role remains unknown.

In a previous study, we showed that monocyte-derived tDC generated with IL-10 and TGF-β1 from healthy donors were able to induce Ag-specific tolerance to tetanus toxoid [15]. To our knowledge, there is a single study that evaluated the induction of Ag-specific tolerance in lymphocytes from T1D patients in vitro, by using the same tDC [26]. In this study, the induction of insulin-specific T-cell tolerance was verified in 5 out of 8 patients evaluated. In view of these results, we aimed to expand these previous findings by examining a larger cohort of T1D patients and by evaluating the tolerance induction with tDC in GAD65-specific T cells in addition to insulin-reactive T cells, since GAD65 is one of the major auto-Ag in T1D.

2. Materials and methods

2.1. Patients

Blood samples (30–40 ml) were obtained from 25 patients diagnosed with T1D (Table 1). None of them had any active infection, neoplasia, or chronic complications such as