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Review

Immune intervention with T regulatory cells: Past lessons and future perspectives for type 1 diabetes

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ABSTRACT

In type 1 diabetes (T1D), insulin-producing pancreatic β-cells are attacked and destroyed by the immune system. Although man-made insulin is life-saving, it is not a cure and it cannot prevent long-term complications. In addition, most T1D patients would do almost anything to achieve release from the burden of daily glucose monitoring and insulin injection. Despite the formation of very large and promising clinical trials, a means to prevent/cure T1D in humans remains elusive. This has led to an increasing interest in the possibility of using T cells with regulatory properties (Treg cells) as a biological therapy to preserve and restore tolerance to self-antigens. In the present review we will attempt to consolidate learning from the past and to describe what we now believe could in the future become a successful Treg-cell based immune intervention in T1D.

1. Background

This section briefly introduces the basic concepts that lie at the core of this review.

1.1. Immune intervention

The term "immune intervention" refers to any therapeutic action that alters the immune system and that, if successful, cures a given immune-mediated disease. An immune intervention can be chronic, as it needs to be administered constantly and without interruption in order to be operational. The various immunosuppressive drugs exemplify this type of intervention: they actively keep the immune system under control for as long as they are administered. Alternatively, immune intervention can be momentary and can lead to a stable resetting of the immune system. Immunologically speaking, this latter intervention implies the rearrangement of the immune system to a state of tolerance in which aggressive and regulatory mechanisms are finely balanced: effective protective immune responses properly co-exist with suppression of excessive responses, which can otherwise lead to the recognition of self-antigens and the development of autoimmune diseases (reviewed in [1]).

One weapon the immune system disposes of, to induce and maintain peripheral tolerance, is the occurrence of lymphocytes that are endowed with constitutive or induced regulatory properties. Several cells with regulatory activity circulate in the immune system, and the $CD4^+$ T regulatory (Treg) cells play a particularly essential role in maintaining immune tolerance, as well as in preventing autoimmunity and chronic inflammation.

1.2. Treg cells

Several types of $CD4^+$ Treg cells have been described on the basis of their origin, generation and mechanism of action. In a simplistic manner, these cells can be categorized as endogenous and inducible Treg cells, which respectively arise in the thymus and in the periphery (reviewed in [2]). Here we will focus on $FOXP3^+$-Treg and Tr1 cells, which have been the subjects of our investigation over the last 10–15 years.

1.2.1. $FOXP3^+$-Treg cells

The expression of the transcription factor forkhead box P3 (FOXP3) defines two subsets of Treg cells: naturally occurring Treg (nTreg) cells, which are generated in the thymus, and induced $FOXP3^+$-Treg cells (iTreg), whose differentiation from naive T helper cells in the periphery is driven by TGF-β (reviewed in [3]).

nTreg cells are defined on the basis of their constitutive expression of high levels of CD25 and FOXP3, and of their dual inability to produce interleukin-2 (IL-2) and to proliferate *in vitro* (reviewed in [4]). nTreg cells are considered to be stable as regards the

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retention of regulatory function and of FOXP3 expression in the periphery. The DNA methylation status of a defined region within the FOXP3 locus, termed the Treg-specific demethylated region (TSDR), is found to be constantly demethylated exclusively in nTreg cells but not in TGF-β-induced FOXP3$^+$ Treg cells nor in recently activated FOXP3-expressing effector T cells. This marker is accordingly considered to be nTreg cell-specific [5]. In addition, the use of CD45RA/RO is instrumental in the appropriate distinction between bona fide human nTreg cells and FOXP3-expressing cells that lack regulatory function. The group of Sakaguchi refined the categorization of human nTreg cells so that it distinguished CD25^{hi}CD45RA$^+$FOXP3^{hi} (*i.e.,* naive) and CD25^{hi}CD45RA$^-$FOXP3^{hi} (*i.e.,* activated) regulatory cells, from the non-regulatory CD25^{hi}CD45RA$^-$FOXP3^{low} cells. These latter produce inflammatory cytokines, do not possess regulatory function, and are not demethylated at the FOXP3 locus [6,7]. Although the mechanism/s of action of nTreg cells is not fully elucidated it is clear that they potently suppress activation, proliferation, and/or effector functions of both CD4$^+$ and CD8$^+$ T cells and of possibly natural killer, natural killer T, B, and dendritic cells. Their ability to control such a range of target cells in varying phases of the immune response presumably derives from the implementation of multiple modes of suppression in a multi-step manner. Said modes include cell contact-dependent suppression, functional modification or killing of antigen-presenting cells (APC), and secretion of immunosuppressive cytokines (reviewed in [3]).

FOXP3$^+$-iTreg cells can be generated by antigenic stimulation of CD4$^+$ naïve T cells in the presence of TGF-β and IL-2, the latter being indispensable for cell-survival. In addition, the vitamin A metabolite retinoic acid (RA) facilitates the differentiation of naive T cells into Treg cells in the presence of TGF-β (reviewed in [8]). FOXP3$^+$-iTreg cells express the same cell surface markers as do nTreg cells, and suppress immune responses through cytokines and contact-dependent mechanisms. As mentioned above, iTreg cells can be distinguished from nTreg cells on the basis of FOXP3 DNA methylation patterns [5]. Because this distinction is not always feasible, the present review refers to FOXP3$^+$-Treg cells as comprising both FOXP3$^+$-nTreg and -iTreg cells.

1.2.2. CD4$^+$ Tr1 cells

CD4$^+$ Tr1 cells arise in the periphery after encountering antigen (Ag) in the presence of a tolerogenic environment; IL-10 is a typical example. Their unique cytokine production profile (*i.e.,* IL-10^{++}IL-4$^-$TGFβ$^+$IFN-γ$^+$IL-2$^-$) distinguishes Tr1 cells from T helper 0 (Th0), Th1, Th2 and Th17 cells [9]. To date, no specific marker for Tr1 cells has been identified, but this cell subset has been classified in the peripheral blood of healthy individuals as CD4$^+$CD45RA$^-$CD25$^-$CD127$^-$ T cells [10]. Many different approaches to the induction of Tr1 cells both *ex vivo* and *in vivo* have been explored. Our group has delineated IL-10 as one of the indispensable Tr1-cell inducer factors (extensively reviewed in [11]), but other investigators have also described an important Tr1-cell-inducing role for TGF-β and IL-27 too [12,13]. Although Tr1 cells do not express FOXP3 [14], they have potent immunosuppressive properties and they produce IL-10 and IFN-γ. Tr1 cells suppress both naïve and memory T-cell responses, and down-regulate the expression of co-stimulatory molecules and the production of pro-inflammatory cytokines by APC. Tr1 cells need to be activated through their TCR in an Ag-dependent manner to exert their suppressive functions, but once activated, they mediate suppression in an Ag non-specific manner (reviewed in [11]).

1.3. Type 1 diabetes and critical time points for immune intervention

Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing beta cells are destroyed by the immune system. The disease is thought to be caused by the interplay of genetic and environmental factors. Proper glycemia levels can be maintained in T1D patients by exogenous insulin administration, but inadequate insulin replacement can lead to: loss of consciousness (hypo-glycemia) or high blood sugar levels (hyper-glycemia); ketoacidosis; atherosclerosis that can lead to poor circulation in the legs, to stroke and to heart conditions such as angina and heart attack; diabetic neuropathy and retinopathy; and susceptibility to infections.

T1D is a multi-step autoimmune disease. The initial stage, which is characterized by the development of islet autoimmunity and is marked by the presence of autoantibodies to insulin, GAD (GAD65), insulinoma-associated protein 2 (IA-2), and tyrosine phosphatase or zinc transporter 8 (ZnT8), is believed to be driven by environmental triggering [15]. Over the past 40 years, the incidence of childhood T1D worldwide has increased by 3–5% annually. Elimination of the environmental trigger(s) responsible for this epidemic would be the easiest and most efficient approach to *primary prevention* (*i.e.,* prior to any sign of metabolic decompensation). However, there is a lack of consensus on the role of environmental factor(s) in the initiation of islet autoimmunity [16]. After the onset of immunity against insulin-producing beta cells, most patients have a long preclinical period [17] that offers opportunity for *secondary prevention* (*i.e.,* after initiation of the disease but before its full-blown manifestation) and thus for halting progression to clinical diabetes. Large randomized trials initiated in the 1990s to target this stage of pre-T1D include the Diabetes Prevention Trial Type 1 (DPT-1), the European Nicotinamide Diabetes Intervention Trial (ENDIT), and the Diabetes Prediction and Prevention (DIPP) project (reviewed in [16]). These trials did not generate encouraging clinical results but did provided clear evidence that mild asymptomatic hyperglycemia may precede insulin dependence by months or years in individuals with islet autoantibodies. Thus, intervention at this "dysglycemic" stage of pre-T1D may theoretically preserve endogenous insulin secretion and prevent the acute and long-term complications of the disease [18]. However, the safety profiles of most of the newly tested immune interventions exclude provision of such interventions in children at high risk of developing T1D, for obvious ethical reasons. As a result, the preservation/restoration of insulin secretion through efficient immune intervention approaches after T1D diagnosis continues to be an attractive goal. Various therapeutic agents have been used in the resulting *tertiary prevention* (*i.e.,* within few weeks of the diagnosis) trials. These agents are often first tested first in adult patients with established T1D and, when proven safe, may be even applied to patients with dysglycemic pre-T1D and possibly to those with normoglycemic pre-T1D.

1.4. Treg cells and type 1 diabetes

Data generated in pre-clinical animal models clearly demonstrated that FOXP3$^+$-Treg cells are crucial for controlling T1D development. The seminal studies performed by Sakaguchi and colleagues demonstrated that transfer of CD4$^+$CD25$^+$-depleted T cells into neonatally thymectomized mice results in the onset of systemic autoimmune diseases, including insulin-dependent autoimmune diabetes [19]. Co-transfer of disease-inducing cells with nTreg cells prevented disease development. These studies were then confirmed and strengthened by many others (reviewed in [20]). Data on the role played by Tr1 cells in controlling T1D development in animal models are scanty, but they suggest that IL-