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Review article

Regulatory mechanisms of immune tolerance in type 1 diabetes and their failures

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ABSTRACT

In this brief review we propose to discuss salient data showing the importance of immune regulatory mechanisms, and in particular of Treg, for the control of pathogenic anti-β-cell response in autoimmune diabetes. Disease progression that culminates with the massive destruction of insulin-secreting β-cells and advent of hyperglycemia and glycosuria tightly correlates with a functional deficit in immune regulation. Better dissection of the cellular and molecular mechanisms through which the immune system normally sustains tolerance to "self", and which become defective when autoimmune aggression is overt, is the only direct and robust way to learn how to harness these effectively, so as to restore immune tolerance in patients with insulin-dependent type 1 diabetes. No doubt that regulatory T cells are a privileged mechanism underlying this self-tolerance in the periphery. The discovery of the key role of the transcription factor FoxP3, represented the cornerstone leading to the great advances in the field we are witnessing today.

Type 1 diabetes is certainly one of the prototypic T cell-mediated autoimmune diseases where immune regulatory mechanisms relying on specialized subsets of T cells have been the most thoroughly analyzed from the fundamental point of view and also largely exploited in a translational therapeutic perspective.

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1. Introduction

It is now well established that during T lymphocyte differentiation in the thymus the filter of central tolerance is incomplete, thus opening the way to the periphery for potentially harmful

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autoreactive T cells. Hence the importance of the immune mechanisms that mediate peripheral tolerance and which, in normal individuals, participate to maintain the delicate balance of physiological self-tolerance, thus avoiding pathogenic autoreactivity. In recent years major progress has been made in our understanding of one important mechanism underlying peripheral tolerance namely, specialized regulatory T cells differentiating as a distinct thymic lineage autoreactive by essence; T cells termed Treg. The road was long and fraught with pitfalls to reach a precise characterization of markers to detect and monitor Treg cells and to dissect the molecular mechanisms underlying their regulatory function. Regulatory T cells that differentiate at the periphery, related to thymic Treg, with which they share markers and functional capacities, have been identified. Initially termed adaptive Treg cells they may be induced, at least in theory, by a wide variety of antigens presented by adequate antigen-presenting cells (APCs) in a suitable cytokine environment. As such they appear as the teleological attempt to expand the T cell repertoire dedicated to immunoregulation while compensating the progressive deficit in thymic Treg, due to the involution of this organ in the adult.

2. Few words of history

The concept of suppression was initially proposed by Gershon and Kondo in 1970 and coined "infectious tolerance" [1,2]. This was based on adoptive transfer experiments showing that immune paralysis, unresponsiveness or tolerance to sheep red blood cells could be transferred to naïve hosts upon the infusion of T cells from the tolerant mice. The idea spread to various models of cell-mediated immunity including organ transplantation, tumor immunology and delayed-type hypersensitivity, to propose the existence of a dedicated subset of T lymphocytes capable of regulating a variety of cellular and humoral immune responses.

However, in the light of current knowledge, it is clear that in these old days, due to the nascent technologies, it was impossible to dissect such a complex phenomenon. This explains that the situation became very problematic when in the 80s, despite the availability of monoclonal antibodies to reliably characterize lymphocyte subsets such as $CD4^+$ and $CD8^+$ T cells no marker unique to suppressor T cells could be identified. Thus, the whole concept of suppression was discredited in an abusive and passionate way. Depicting well to what extent the situation in those days had become confused there remains still today a certain reluctance to use the word "suppression".

3. Immunoregulation in autoimmune diabetes in the "pre-*FOXP3*" era

When the concept of T cell-mediated suppression was dropped most of the immunological community concentrated on other peripheral tolerance mechanisms, seemingly more engaging, such as lymphocyte anergy, or more easy to approach as was the case for immune deviation [3,4]. From the description of Th1 and Th2 cells by T. Mosmann and R. Coffman and for several years the vast majority of past experiments linked with suppression were revisited through the prism of the Th1/Th2 immune deviation paradigm i.e. IL-12, IFNγ/IL-4, IL-10 balance [5,6].

In non-obese diabetic (NOD) mice, that spontaneously develop the autoimmune disease, most of the data reported implicating the balance of pathogenic and protective cytokines in regulating disease development related to immune intervention using recombinant cytokines, anti-cytokine antibodies or β-cell autoantigens delivered by several routes, with no evidence for the involvement of β-cell-specific cytokine-producing T cells, and in particular Th2 cells in the control of the spontaneous disease [7–9].

Results showed that blockade of Th1 T cells through IFNγ neutralization prevented disease development [10] just as did some manipulations, in particular autoantigen treatment, favoring Th2 development and IL-4 production [11,12]. Regulation by these "protective" Th2 cells was not restricted to the autoantigen delivered but also spread, through "bystander suppression", to responses specific for other locally expressed β-cell antigens [12]. Furthermore, the protective effect was not seen in IL-4-deficient NOD mice ($IL\text{-}4^{-/-}$) [11]. Conversely, disease incidence and severity was increased by treatment with cytokines such as IL-12 favoring Th1 cell differentiation and, in turn, inhibiting Th2 development [13].

Support for a role for Th1/Th2 balance in the control of type 1 diabetes (T1D) came from initial results in NOD mice with invalidated CD28 gene expression and disruption of the CD28/B7 pathway. In these mice where IFNγ production was normal contrasting with a defect in Th2 cytokine production disease was accelerated and more severe [14]. Since these initial observations it has been well established that it is not the Th1/Th2 imbalance but rather the absence of thymic-derived Tregs which is responsible in $CD28^{-/-}$NOD mice for the acceleration of disease [15].

It is fair to admit that, although attractive, the Th1/Th2 model of immune regulation did not fit the data when confronted to the situation of the spontaneous disease. Thus, gene invalidated IL-4 and IL-10-deficient NOD mice did not show accelerated disease [16,17]. Moreover, no inhibition of the regulation was observed in $IL\text{-}4^{-/-}$ mice or in mice treated with neutralizing antibodies to IL-4 and/or IL-10 [18]. Treatment with Complete Freunds Adjuvant (CFA) protects NOD mice from disease, an effect that was initially interpreted as being mediated by the induction of protective Th2 cells [19,20]. This conclusion was challenged by D. Serreze et al. showing that IL-4 and IL-10-deficient NOD mice were still sensitive to the CFA-mediated protection whereas IFNγ deficient NOD mice were not [21].

Already in the "pre-FoxP3" era, the NOD mouse was certainly one of the experimental model where numerous data had been accumulated strongly suggesting the existence of dedicated $CD4^+$ T cell subsets mediating immune regulation. It is interesting to recall here the main results. Autoimmune diabetes with overt hyperglycemia (i.e., meaning that about 70% of the insulin-secreting β-cell mass has been destroyed) develops in NOD mice by 3 and 5 months of age, much later than the onset of islet infiltration by mononuclear cells or insulitis, beginning at 3 weeks of age and that is the hallmark of breakdown of self-tolerance. Initial evidence to suggest that this delayed occurrence of overt β-cell destruction and disease relied on a specialized subset of T suppressor cells stemmed from experiments showing that adoptive transfer of diabetes by pathogenic polyclonal $CD4^+$ and $CD8^+$ T cells from the spleen of diabetic NOD mice was only observed in syngeneic immune deficient recipients, i.e. neonates (before 3 weeks of age), sublethally irradiated adult mice, adults thymectomized animals treated with depleting antibodies to CD4, NOD *Scid* or NOD $Rag^{-/-}$ recipients [8,22–24]. Moreover, cyclophosphamide, an alkylating agent that has been shown to selectively affect T cell-dependent regulation triggered acute diabetes within 2 weeks when injected into young pre-diabetic NOD mice [8]. More direct evidence was provided by adoptive co-transfer experiments. Thus, mature $CD4^+$ thymocytes and splenocytes from young pre-diabetic mice fully prevented disease transfer by diabetogenic cells into immune incompetent recipients; an activity that was enhanced when $CD4^+CD62L^{hi}$ T cells were enriched [18,22,25]. These protective T cells could be evidenced earlier in the thymus (by 2 weeks of age) than in the spleen, where they were detectable by 3–4 weeks of age [22,26]. The thymic dependency of splenic regulatory T cells was initially confirmed by the observation that they were no longer detectable in adult mice