Journal of Autoimmunity 71 (2016) 35–43

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Review article

# Analysis of antigen specific T cells in diabetes – Lessons from pre-clinical studies and early clinical trials

Balasubramanian Krishnamurthy [a, b], Claudia Selck [a, b], Jonathan Chee [a, b], Guarang Jhala [a, b], Thomas W.H. Kay [a, b, *]

[a] St. Vincent's Institute, 41 Victoria Parade, Fitzroy, 3065, Victoria, Australia
[b] The University of Melbourne Department of Medicine, St Vincent's Hospital, Fitzroy, 3065, Victoria, Australia

ARTICLE INFO

*Article history:*
Received 12 March 2016
Received in revised form
31 March 2016
Accepted 31 March 2016
Available online 12 April 2016

*Keywords:*
Autoantigens
Proinsulin
NOD mice

ABSTRACT

Antigen-specific immune tolerance promises to provide safe and effective therapies to prevent type 1 diabetes (T1D). Antigen-specific therapy requires two components: well-defined, clinically relevant autoantigens; and safe approaches to inducing tolerance in T cells specific for these antigens. Proinsulin is a critical autoantigen in both NOD mice, based on knockout mouse studies and induction of immune tolerance to proinsulin preventing disease whereas most antigens cannot, and also in human T1D based on proinsulin-specific T cells being found in the islets of affected individuals and the early appearance of insulin autoantibodies. Effective antigen-specific therapies that prevent T1D in humans have not yet been developed although doubt remains about the best molecular form of the antigen, the dose and the route of administration. Preclinical studies suggest that antigen specific therapy is most useful when administered before onset of autoimmunity but this time-window has not been tested in humans until the recent "pre-point" study. There may be a 'window of opportunity' during the neonatal period when 'vaccine' like administration of proinsulin for a short period may be sufficient to prevent diabetes. After the onset of autoimmunity, naive antigen-specific T cells have differentiated into antigen-experienced memory cells and the immune responses have spread to multiple antigens. Induction of tolerance at this stage becomes more difficult although recent studies have suggested generation of antigen-specific TR1 cells can inhibit memory T cells. Preclinical studies are required to identify additional 'help' that is required to induce tolerance to memory T cells and develop protocols for effective therapy in individuals with established autoimmunity.



**Contents**



* Corresponding author. St Vincent's Institute, 41 Victoria Parade, Fitzroy, VIC, 3065, Australia.
*E-mail address:* tkay@svi.edu.au (T.W.H. Kay).

http://dx.doi.org/10.1016/j.jaut.2016.03.018
0896-8411/

## 1. Introduction

T1D is a complex disease with underlying genetic and environmental components resulting in autoimmune destruction of pancreatic beta cells and loss of insulin production over a long period before and after the clinical diagnosis is made [1]. Once an individual develops two or more islet autoantibodies progression to diabetes is virtually certain and it is in this pre-clinical period that intervention is potentially attractive. A major conundrum is how people progress from genetic susceptibility with evidence of T cell autoreactivity, that may be no different to control subjects, to autoimmune disease.

There is a strong genetic basis for the disease. Overall the risk of disease for siblings of patients with T1D is approximately 6%, which is about 15-fold higher than in the general population. The risk for identical twins was reported to be as low as 30% but more recent data have suggested that concordance rate increases with a longer observation period of follow up. The most important susceptibility alleles are within the major histocompatibility complex (MHC). The MHC complex has an odds ratio for disease of close to 7. There are many non-MHC T1D loci, all with odds ratios of less than 2. These include *IDDM2* which is in the insulin promoter and affect level of insulin expression in the thymus and thus negative selection. In addition to the genetic contribution, environmental factors are also important in disease onset and progression. The increased incidence of T1D in youth suggests that children are being exposed to environmental factors at a very early age or even *in utero*. Currently, large studies such as TEDDY and ENDIA are designed to identify different environmental triggers linked to the disease development [2–4].

Genetic factors along with environmental factors break tolerance of T and B cells to islet antigens in T1D. In normal individuals, the immune system has sophisticated mechanisms to preserve tolerance to self-antigens while allowing robust responses against pathogens. In the thymus, developing naive self-reactive T cells are exposed to short peptides, derived from self-proteins, expressed on antigen presenting cells (APCs). T cells with high reactivity to self antigen/MHC are deleted from the immune repertoire – a process known as negative selection [5]. As an additional layer of protection, some high/intermediate reactive T cells are converted to exert regulatory functions [called T regulatory cells (Treg)]. Widespread autoimmunity ensues when the expression of self-antigens is decreased in the thymus [6,7]. Conversely, overexpression of autoantigens by professional antigen presenting cells enhances negative selection of antigen-specific T cells and prevents autoimmune diseases including gastritis and diabetes [8,9]. This tolerance strategy does not involve Treg generation. The process of thymic negative selection is not perfect. Even in healthy individuals some auto-reactive T cells escape into the periphery. These T cells can be physiologically regulated and neutralised by so-called peripheral tolerance mechanisms. These mechanisms are: anergy, phenotypic polarisation (also called immune deviation), activation-induced apoptosis, and suppression by Treg. In T1D, and other autoimmune diseases, as yet unknown events cause the mechanisms fail and immune tolerance is broken leading to activation of autoreactive T cells. Loss of immune tolerance is more likely in those at increased genetic risk.

### 1.1. The non-obese diabetic mouse model

The NOD mouse is a valuable experimental model for the investigation of pathogenesis of T1D, but it must be used judiciously and not simply equated with human T1D. It exhibits many features of the human clinical disease including shared genetic susceptibility most notably in the HLA/MHC region and the pathological hallmark of infiltration of the islets with immune cells [10] albeit quantitatively different to humans. Very likely because of the HLA similarity, there is overlapping auto-antigen-specificity at the cellular level and to a less extent the humoral level [11]. The major targets of autoantibodies in humans (insulin, glutamate decarboxylase 65, IA2/IA2β and ZnT8) are documented targets of T cells in the NOD mouse [12]. However, in the NOD mouse humoral autoimmunity appears predominantly restricted to insulin [13].

### 1.2. Proinsulin-the essential antigen

The autoreactive T cells and antibodies that develop during the course of NOD diabetes target several proteins expressed by beta cells, including proinsulin, islet cell antigen 512 (IA-2), GAD, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), zinc transporter 8, and chromogranin A. Of these proteins, only two are solely expressed in β-cells: insulin and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) [14,15]. Cytotoxic T cells specific for either antigen were isolated from naïve NOD mice, and were able to transfer T1D to NOD–*scid* mice. T cell reactivity to multiple antigens in the NOD mouse and multiple islet autoantibodies in humans may indicate parallel, unrelated targets contributing independently to disease pathogenesis or a primary initiating antigen that causes responses to other autoantigens once islet inflammation occurs. There is considerable evidence to suggest that of the several beta-cell autoantigens recognised by T cells in NOD mice, proinsulin is recognised first, and this initiates beta-cell autoimmunity [8,16–18].

### 1.3. Proinsulin 1 and 2 knockouts

Gene knockout experiments have also indicated a central role for insulin in diabetes. Insulin is encoded by two genes in mice: *Ins1* and *Ins2*, with the latter being the dominant form expressed in the thymus. Knockouts of the *Ins1* or *Ins2* genes respectively retard or accelerate diabetes [19,20]. Knockout of both *Ins1* and *Ins2*, with restoration of hormonal function by transgenic expression of insulin, with an inactivated CD4 and CD8 epitope, completely prevented the development of anti-insulin antibodies, insulitis, and diabetes [17]. Knockouts of GAD65 or IA-2 expression did not alter diabetes onset in NOD mice [21–23]. Interestingly, even in mouse lines with either C57BL/6 or 129:C57BL/6 mixed backgrounds, all of which carried the H-$2^b$ MHC haplotype, which would normally convey resistance to islet autoimmunity, systemic deletion of *Ins1*