Type I (insulin-dependent) diabetes is a well described disease resulting from an autoimmune reaction against the pancreatic islet β-cells. Various factors play a role in the induction of this autoreactive phenomenon, however the primum movens and the precise sequence of events remain unclear. Human leukocyte antigens (DR3 and DR4), viral infection and dietary factors are among the genetic and non-genetic factors predisposing to the development of diabetes (1–3). Humoral and cellular immune responses are altered with an increased prevalence of autoreactive antibodies (against pancreatic islet β-cell proteins) and T-cells.

Over the last years several β-cell antigens have been studied in detail and seem to play a role in initiating the immune response against the pancreatic islets. One of these proteins, the glutamic acid decarboxylase (GAD), was a compelling candidate in the non-obese diabetic (NOD) mouse. This murine model is the most commonly used animal model of type I diabetes. Spontaneous loss of T-cell tolerance to GAD has been shown to correlate with the onset of insulitis and was preventable by tolerization to this autoantigen (4, 5).

Yoon et al. recently looked at the causative role of GAD on autoimmune diabetes in NOD mice (6). To do so they suppressed the expression of GAD in pancreatic β-cells by creating various lines of transgenic NOD mice expressing antisense GAD. Antisense transgenes are RNA molecules that are complementary to the mRNA of the gene that is to be suppressed. When an antisense is expressed in a cell, it specifically targets its complementary sequence on the mRNA, thus preventing the translation of the mRNA into protein (reviewed in (7) and (8)).

Yoon et al. cloned the antisense GAD transgenes under the control of the rat insulin promoter, thus ensuring tissue-specific expression in the pancreatic islet β-cells. They obtained three lines of transgenic NOD mice with high (H-AS), medium (M-AS) and low (L-AS) expression of antisense GAD. In the H-AS NOD mice they observed a total suppression of GAD protein in western blots, whereas in the mice with medium and low expression of antisense GAD, suppression was moderate and low respectively.

After a 40-week follow-up none (0 of 15) of the H-AS NOD mice developed diabetes. However 67% (12 of 18) of the M-AS and 75% (12 of 16) of the L-AS developed diabetes. Histological examination revealed peri-insulitis in only 20% of the H-AS NOD mice, whereas most of the M-AS and L-AS NOD mice islets had signs of insulitis.

These results clearly show that GAD plays a role in the development of type I diabetes in NOD mice. Hence, Yoon and his colleagues examined whether suppression of GAD expression in the islets hindered the generation of β-cell-specific diabetogenic T-cells. Splenocytes from 20-week-old non-diabetic H-AS NOD and non-transgenic NOD mice were transfused into NOD-severe combined immunodeficient mice (NOD.scid). None of the NOD.scid recipients that received splenocytes from the H-AS NOD mice developed diabetes, whereas 90% of the NOD.scid mice that received splenocytes from non-transgenic NOD mice developed diabetes. These elegant transfusion experiments show that GAD expression is necessary for the generation of diabetogenic T-cells. They also found that in the absence of GAD T-cell proliferation in response to other β-cell autoantigens (HSP60 and insulin) was diminished. To examine whether the GAD-suppressed islets were prone to attack by diabetogenic T-cells, islets from H-AS NOD and non-transgenic NOD mice were transplanted into acutely diabetic NOD mice. Mice transplanted with GAD-expressing islets, after a brief period of normoglycaemia, showed a recurrence of diabetes with a massive infiltration and destruction of the islets within 2 weeks. However, the H-AS GAD islet recipients remained free of diabetes up to 40 days after the transplantation. When splenocytes from acutely diabetic NOD mice were transfused into 6-week-old, irradiated, H-AS NOD and non-transgenic mice, this resulted in the occurrence of diabetes in 70% of the non-transgenic mice, whereas none of the H-AS GAD mice developed diabetes up to 4 weeks after transfer. All of these experiments demonstrate that in NOD mice the expression of GAD is necessary for the autoimmune destruction of β-cells.

This study clearly shows that GAD expression is an essential element in the pathogenesis of type I diabetes in NOD mice. The absence of this autoantigen blocks the development of autoreactive diabetogenic T-cells, and weakens the immune response to other islet autoantigens. Diabetogenic T-cells are also incapable of provoking diabetes in NOD mice in the absence of GAD, thus underlining the key role of this protein in this murine model. Whether these findings are of clinical relevance to humans remains to be proven, and the impact of antisense therapy also remains a limiting
factor since techniques capable of specifically delivering vectors to the islets have to be developed. However, the modulation of the expression of GAD and other proteins remains an appealing target for preventive strategies.

References
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