

Correspondence

A20 deficiency sensitizes pancreatic beta cells to cytokine-induced apoptosis *in vitro* but does not influence type 1 diabetes development *in vivo*L. Catrysse^{1,2}, M. Fukaya³, M. Sze^{1,2}, K. Meyerovich³, R. Beyaert^{1,2}, A.K. Cardozo³ and G. van Loo^{*1,2}*Cell Death and Disease* (2015) 6, e1918; doi:10.1038/cddis.2015.301; published online 15 October 2015

Dear Editor,

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by the infiltration of inflammatory cells into the pancreatic islets of Langerhans, followed by the selective destruction of insulin-producing β -cells, resulting in hyperglycemia. One of the mechanisms causing β -cell death is the intra-islet release of inflammatory mediators such as interleukin-1 β (IL-1 β), tumor necrosis factor (TNF) and interferon- γ (IFN- γ) by activated immune cells.¹ Hence, the transcription factor NF- κ B promotes pro-inflammatory and pro-apoptotic responses in β -cells on cytokine exposure. A transgenic mouse line in which NF- κ B activation is attenuated specifically in β -cells conferred nearly complete protection against multiple low dose streptozotocin (MLDSTZ)-induced T1D.² Contrary, mice with constitutively active NF- κ B signaling in β -cells spontaneously develop full-blown immune-mediated diabetes.³

The ubiquitin-editing enzyme A20 is a critical negative regulator of NF- κ B signaling in response to multiple stimuli, including TNF and IL-1. Moreover, A20 can also act as a strong anti-apoptotic protein in specific cell types.⁴ A20 has been identified as the most highly upregulated anti-apoptotic protein in cytokine-stimulated primary islets and insulinoma cell lines.⁵ Consistent with this, overexpression of A20 in islets confers resistance to cytokine-mediated activation of NF- κ B, protecting them from apoptosis in the early post-transplantation period.⁶ Interestingly, not only have NF- κ B polymorphisms been identified in patients with T1D,⁷ also A20/*TNFAIP3* has been identified as a T1D susceptibility locus in humans.⁸ Together, these data suggest an important role for A20 in β -cell function and T1D. Therefore, we generated and characterized A20-deficient mice which lack expression of A20 specifically in β -cells (Supplementary Figure 1A).

We first confirmed the anti-apoptotic function of A20 in β -cells, as primary islets isolated from β -cell-specific A20 knockout (A20 ^{β -KO}) mice were more susceptible to cytokine-induced cell death compared with wild-type islets (Supplementary Figure 1A). As A20 has a crucial role in

β -cell survival *in vitro*, we next investigated whether A20 ^{β -KO} mice would be more susceptible to diabetes development when compared with wild-type littermates. A20 ^{β -KO} mice aged normally without any evidence of metabolic defects. Phenotypic analysis of A20 ^{β -KO} mice up to the age of 12 months revealed no pathological signs in the pancreas. A20 ^{β -KO} mice and control littermates were subjected to a model of T1D induced by MLDSTZ, however, both control and A20 ^{β -KO} mice developed a similar hyperglycemia, which was confirmed in a glucose tolerance test (ipGTT) performed 5 weeks after the first STZ injection (Supplementary Figure 1B). Next, we crossed A20 ^{β -KO} mice with C57BL6-*Ins2*^{Akita}/J mice, which carry a mutation in the insulin *Ins2* gene that prevents normal folding and secretion and induces endoplasmic reticulum stress leading to β -cell death. Mice carrying the *Ins2*^{Akita} mutation become hyperglycemic very early in life, however, no differences could be observed in conditions of A20 deficiency in β cells. In agreement, ipGTT shows severe and similar defects in insulin secretion in both *Ins2*^{Akita} and A20 ^{β -KO/Akita} mice (Supplementary Figure 1C). Finally, A20 ^{β -KO} mice were backcrossed into a non-obese diabetic genetic background, and glucose levels were measured every week in order to follow diabetes development. Although only 40% of all mice developed diabetes, no differences could be detected between control and A20 ^{β -KO} mice (Supplementary Figure 1D). In conclusion, A20 deficiency in β cells does not affect β -cell apoptosis nor disease development *in vivo*.

Conflict of Interest

The authors declare no conflict of interest.

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