

## 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<sup>1,2</sup>, M. Fukaya<sup>3</sup>, M. Sze<sup>1,2</sup>, K. Meyerovich<sup>3</sup>, R. Beyaert<sup>1,2</sup>, A.K. Cardozo<sup>3</sup> and G. van Loo<sup>\*1,2</sup>*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  $\beta$ -cells, resulting in hyperglycemia. One of the mechanisms causing  $\beta$ -cell death is the intra-islet release of inflammatory mediators such as interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor (TNF) and interferon- $\gamma$  (IFN- $\gamma$ ) by activated immune cells.<sup>1</sup> Hence, the transcription factor NF- $\kappa$ B promotes pro-inflammatory and pro-apoptotic responses in  $\beta$ -cells on cytokine exposure. A transgenic mouse line in which NF- $\kappa$ B activation is attenuated specifically in  $\beta$ -cells conferred nearly complete protection against multiple low dose streptozotocin (MLDSTZ)-induced T1D.<sup>2</sup> Contrary, mice with constitutively active NF- $\kappa$ B signaling in  $\beta$ -cells spontaneously develop full-blown immune-mediated diabetes.<sup>3</sup>

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

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

$\beta$ -cell survival *in vitro*, we next investigated whether A20 <sup>$\beta$ -KO</sup> mice would be more susceptible to diabetes development when compared with wild-type littermates. A20 <sup>$\beta$ -KO</sup> mice aged normally without any evidence of metabolic defects. Phenotypic analysis of A20 <sup>$\beta$ -KO</sup> mice up to the age of 12 months revealed no pathological signs in the pancreas. A20 <sup>$\beta$ -KO</sup> mice and control littermates were subjected to a model of T1D induced by MLDSTZ, however, both control and A20 <sup>$\beta$ -KO</sup> 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 <sup>$\beta$ -KO</sup> mice with C57BL6-*Ins2*<sup>Akita</sup>/J mice, which carry a mutation in the insulin *Ins2* gene that prevents normal folding and secretion and induces endoplasmic reticulum stress leading to  $\beta$ -cell death. Mice carrying the *Ins2*<sup>Akita</sup> mutation become hyperglycemic very early in life, however, no differences could be observed in conditions of A20 deficiency in  $\beta$  cells. In agreement, ipGTT shows severe and similar defects in insulin secretion in both *Ins2*<sup>Akita</sup> and A20 <sup>$\beta$ -KO/Akita</sup> mice (Supplementary Figure 1C). Finally, A20 <sup>$\beta$ -KO</sup> 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 <sup>$\beta$ -KO</sup> mice (Supplementary Figure 1D). In conclusion, A20 deficiency in  $\beta$  cells does not affect  $\beta$ -cell apoptosis nor disease development *in vivo*.

**Conflict of Interest**

The authors declare no conflict of interest.

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