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A20 deficiency sensitizes pancreatic beta cells to cytokine-induced apoptosis *in vitro* but does not influence type 1 diabetes development *in vivo*

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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-1β (IL-1β), tumor necrosis factor (TNF) and interferon-γ (IFN-γ) by activated immune cells.[1] Hence, the transcription factor NF-κB promotes pro-inflammatory and pro-apoptotic responses in β-cells on cytokine exposure. A transgenic mouse line in which NF-κB 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-κB signaling in β-cells spontaneously develop full-blown immune-mediated diabetes.[3]

The ubiquitin-editing enzyme A20 is a critical negative regulator of NF-κB 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-κB, protecting them from apoptosis in the early post-transplantation period.[6] Interestingly, not only have NF-κB 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 ($A20^{\beta-KO}$) 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 $A20^{\beta\text{-KO}}$ mice would be more susceptible to diabetes development when compared with wild-type littermates. $A20^{\beta\text{-KO}}$ mice aged normally without any evidence of metabolic defects. Phenotypic analysis of $A20^{\beta\text{-KO}}$ mice up to the age of 12 months revealed no pathological signs in the pancreas. $A20^{\beta\text{-KO}}$ mice and control littermates were subjected to a model of T1D induced by MLDSTZ, however, both control and $A20^{\beta\text{-KO}}$ 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 $A20^{\beta\text{-KO}}$ 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 $A20^{\beta-KO/Akita}$ mice (Supplementary Figure 1C). Finally, $A20^{\beta\text{-KO}}$ 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 $A20^{\beta\text{-KO}}$ 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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