TLR4 signals through islet macrophages to alter cytokine secretion during diabetes

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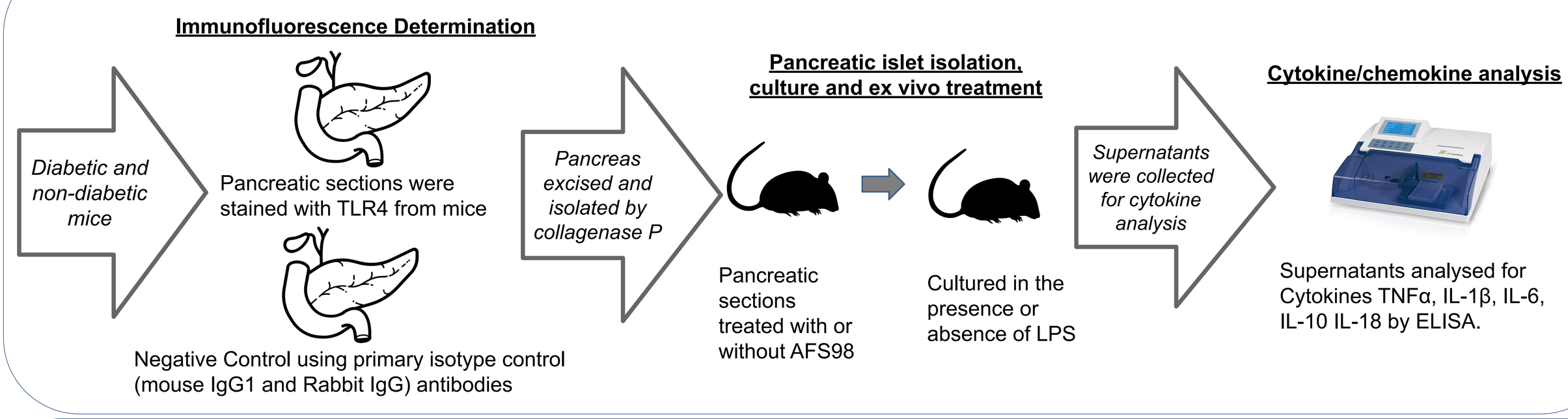
Introduction

Toll-like receptors (TLRs), particularly TLR4, may act as immune sensors in tissues to regulate innate immunity. Pancreatic islets have been shown to respond to TLR signalling, but the source of signalling within the islet remains unclear. It was previously thought that TLR4 colocalized with β-cells however NRLP3 inflammasome complex activation, downstream of TLR4 signalling increased IL-1β production and secretion in whole islets but not in isolated β-cells. Hence this suggests that islet TLR4 signalling, while important, may not take place in β-cells.

Analysing human islets also reveal that macrophages were an important source of islet TLRs. Uncovering the specific cell source and how it mediates TLR signalling, especially within type 2 diabetes (T2D) islets will yield new targets to tackle islet inflammation, hormone secretion dysregulation and ultimately diabetes. Hence, this study serves to suggest that islet macrophages are a dominant source of TLR-4 mediated signalling in both healthy and diabetic islets.

Aims To determine the source of TLR4 signaling in healthy and diabetic islets.

Methods



Results and Discussion

Findings

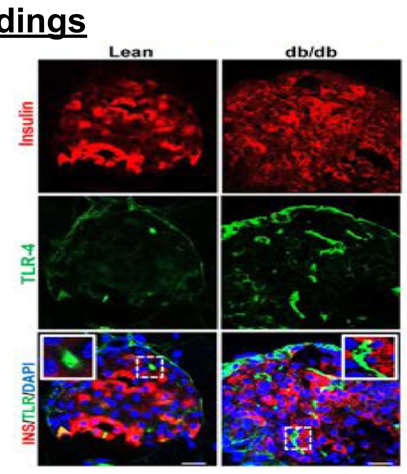


Figure 1. TLR4 is higher in diabetic islets but are not expressed on beta-cells. Pancreatic sections from healthy and diabetic mice (Db/Db) were stained for insulin (Red), TLR4 (Green) and all nuclei (blue). Scale bar = 20um

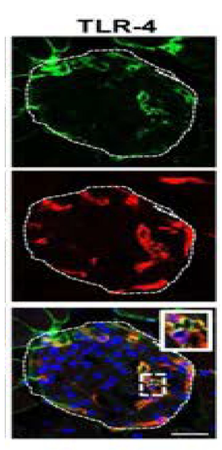


Figure 2. TLR4 presence in the diabetic islet is restricted to endothelial cells and islet macrophages
Pancreatic sections from diabetic mice (Db/Db) were stained for either CD31 (Left panel, Red) or CD68 (Right panel, Red), TLR4 (Green) and all nuclei (blue). Scale bar = 20um

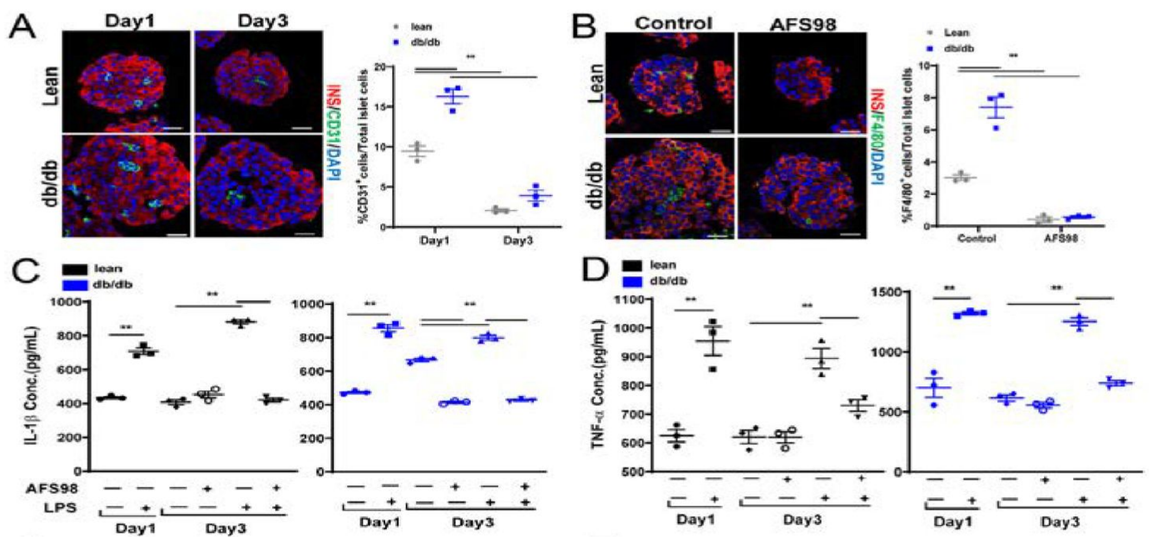


Figure 3: TLR4 activation by LPS is reduced in islets devoid of endothelial cells or macrophages
Islets were treated ex vivo, following cell-specific depletion (A: EC depletion; B: macrophage depletion), with LPS to stimulate TLR4 pro-inflammatory cytokine release (C: IL-1b; D: TNF-a). Scale bar = 20um.

Discussion

Upon analysing the islets, TLR4 was expressed but did not co-localise with insulin-producing β-cells. While TLR immunoreactivity in obese mice islets increased, this was driven mostly by increased islet endothelial cell and islet macrophage presence, in which TLR4 signalling through macrophages alters the islet cytokine secretome. TLR4 transcripts are restricted to human islet macrophages (data not shown).

The depletion of islet macrophages significantly downregulated the LPS-induced secretion of inflammatory cytokines and this was more pronounced in db/db islets suggesting that islet macrophages play a significant role in TLR4-mediated inflammatory cytokine secretion. We showed that a higher proportion of islet macrophages were TLR4+ in diabetic islets and hence contributing significantly to β-cell apoptosis.

Several limitations of this study are that it only involves RNA in human islets without the use of protein and that the uncertainty of the use of LPS, to stimulate TLR4 signalling, is able to reach the pancreatic islets.

Conclusion

In conclusion, our study provides a more complete picture for the source of TLR4 within islets. The diabetic islets is found to have an increased number of macrophages, which results in increased TLR4 signalling and hence increased inflammation. This finding will significantly impact therapy for mitigating pro-inflammatory mediated islet dysfunction and insulin insufficiency in Type 2 diabetes.

Acknowledgement and References

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