TOLL-LIKE RECEPTOR 4: A NOVEL STRESS MARKER IN PANCREATIC ISLET BETA CELLS

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Introduction: Toll-like receptors (TLRs) are widely recognized as essential elements in triggering of innate immunity, binding pathogen-associated molecules, initiating a cascade of pro-inflammatory events and activating the immune response. Non-immune cells can express TLRs, including a report that TLR2, 3 and 4 are expressed on pancreatic beta cells. In addition, endogenous ligands such as heme, HSP60, or HMGB1 are capable of triggering TLR4. These products are present in situations such as type1 diabetes, brain death and pancreas harvesting for transplantation, and human pancreatic islet isolation procedures, but the relationship between expression of TLRs on beta cells and stress-mediated pro-inflammatory responses has not been yet established. **Objective:** Here, we evaluated TLR4 expression in beta cells purified from freshly isolated human islets and in the MIN6 mouse beta cell/insulinoma cell line. **Methods and Results:** TLR4 expression was analyzed in 5 samples of freshly isolated human islets obtained from brain-dead donors. The expression varied from 5 to 20% in beta cells, measured by fluorescence-activated cytometry (FACS) using double staining with specific anti-TLR4 antibody and insulin granule-specific Newport Green, and was further increased in beta cells from islets cultured under nutrient-deprived conditions. A significant portion of these cells was negative for TMRE staining indicating they were in the process of cell death. Tracking cultured islet cell preparations (n=10) at times 0, 24 and 48 hours by real-time PCR showed a typical molecular signature for inflammatory genes (IL1-R, MCP-1, Fas, Casp-1) correlating positively with expression of TLR4, mainly in islets derived from low yield isolation procedures. Monitoring gene expression in beta cells cultured for 5-10 days and exposed to the prototypical TLR4 ligand LPS for 48h showed a dose-dependent increase in TLR4 and CD14 mRNA, loss of cell viability and decreased insulin content and secretion. These effects were only observed in beta cells positive for TLR4 by FACS analysis. TLR4-positive MIN6 cells were also LPS-responsive, increasing mRNA levels for TLR4 and CD14, and decreasing FACS-evaluated cell viability and insulin content. **Conclusion:** Taken together, the data indicate that TLR4 may have an important role in the homeostasis of the pancreatic beta cell, integrating exogenous signaling through TLR4 with insulin production and/or secretion, and beta cell viability. Support - CNPq, FAPESP, FINEP