SDC, Material and Methods

Luminex Multiplex Assay

Plasma samples collected during the experiment were used to measure the concentration of inflammatory cytokines present. A Luminex 8-plex magnetic bead kit (EMD Millipore, Billerica, MA) was designed to contain eight key cytokines/chemokines (TNF-α, IL-6, IL-8, IP-10, MCP-1, IL-1β, interferon γ, granulocyte colony-stimulating factor) that mark the presence of an active inflammatory reaction. Triplicate samples were analyzed for each condition treated.

ELISA

An enzyme-linked immunosorbent assay (ELISA) was performed on frozen plasma samples. Plasma levels of TAT, which is a marker for activation of coagulation, were determined using Enzygnost TAT Micro (Siemens Healthcare, Newark, DE). Islet damage was evaluated by measuring secreted C-peptide and proinsulin in the plasma using human C-peptide ELISA and proinsulin ELISA (Mercodia, Uppsala, Sweden).

Viability Determination by Propidium Iodide

Islets were incubated with Hoechst 33342 (10 μg/mL and propidium iodide (20 μg/mL) (Sigma Aldrich, St. Louis, MO) for 10 minutes at 37°C before imaging via fluorescent microscopy (1). Fluorescent micrographs were merged in Image J (National Institutes of Health, Bethesda, MD), and the propidium iodide–positive area (red) was divided by the Hoechst 33342–positive area (blue) to calculate islet viability.

Reference

1. Itoh T, Takita M, Sorelle JA, et al. Correlation of Released HMGB1 Levels with the Degree of Islet Damage in Mice and Humans and with the Outcomes of Islet Transplantation in Mice. *Cell Transplant* 2012.