Role of danger signals from the enveloped cells in immune responses against microencapsulated human islets.

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INTRODUCTION AND OBJECTIVE

Transplantation of islets is proposed as a therapy to cure diabetes type 1. However, the outcomes for using this treatment are variable and it still requires the use of immunosuppressant (de Vos et al., 2006). Encapsulation of pancreatic islets is a solution for this issue (de Vos et al., 2007). This technique is employed to protect the cells from the immune response of the host. Graft survival however is limited (Stokes et al., 2013) which can be attributed to factors related to the capsules and the enveloped islets.

The role of the latter, *i.e.* the islets, has received not more than minor attention. Immune responses against encapsulated islets can occur after binding of islet-derived molecules to pattern recognition receptors (PRRs). These PRRs are able to bind to proinflammatory islet-derived molecules, such as damage-associated molecular patterns (DAMPs), that can be released when islets in the capsules are under stress. Toll-like receptors (TLRs) are the most commonly known PRRs. We hypothesize that the islets release proinflammatory islet-derived components that can elicit inflammatory responses via TLRs.

The present study is intended to investigate the role of components derived from the islets as triggering factors of the immune response against islets in the first weeks after implantation.

MATERIALS AND METHODS

Cell stimulation

Two cell-lines were stimulated using supernatant from human islets cultured in starving conditions at 20% and 1% O_2 for detection of proinflammatory components. The islets were analyzed using live/dead staining for fluorescent confocal microscopy. Samples were collected during days 1, 3, 5, and 7. We applied the following cell-lines: THP1-MD2-CD14 (MyD88(+)), a human cell-line carrying all TLRs with a reporter plasmid under control of the NF-κB, expressing a secreted embryonic alkaline phosphatase (SEAP) gene that can be measured. The THP1-defMyD (MyD88(-)) cell-line has the same construction as MyD88(+) but it has a non-functional MyD88 coupling protein and it can be used to prove activation via TLRs in a MyD88 dependent manner.

Confocal analysis

LIVE/DEAD Viability/Citotoxicity Kit from InvitroGen was used. Collected islets were incubated with Calcein AM (1 mM) and Ethidium Bromide (EB) (2 mM) at room temperature avoiding light. The islets were washed with KRH. Fluorescent confocal microscopy was measured at an emission wavelength of 517 nm (Calcein AM) and 617 nm (EB).

RESULTS AND DISCUSSION

As encapsulated islets are not revascularized after transplantation they face low oxygen condition and nutrient deprivation after transplantation. We study the effect of this on immune activation. We mimicked in vitro conditions at which human islets are exposed to low oxygen tensions and to lower concentrations of nutrients than in the vascularized condition. This was done by culturing islets in control medium (20% oxygen and 10% FCS) and comparing their activation of NF-kB on the monocyte line THP-1 with that of islets cultured on hypoxic conditions (1% oxygen) and nutrient deprivation (0% FCS).

As shown in Figure 1, islets produce under all in vitro conditions components that activate NF-kB in THP-1 cells, including the normal control conditions of 20% oxygen and 10% FCS. This increases during prolonged periods of culture illustrating progressive damage of the islets.

The production of proinflammatory molecules increased significantly under hypoxic conditions (Figure 1a). This increase in activation was twofold higher. This was different when nutrient deprivation was applied. In this case we found a strong release of proinflammatory islet-derived molecules in the first days but decreased fast thereafter. This can be explained by pronounced cell-death due to nutrient deprivation in the first days as will be explained below.

We next questioned whether the immune response was Toll-like receptor (TLR) dependent or also dependent on other pattern recognition receptors. We therefore compared the responses of THP-1 cells containing functional and non-functional MyD88, *i.e.* the main signaling molecule for TLR. As shown in Figure 2, the responses are two to fourfold lower in the THP1 MyD88(-) implying that the islet derived factors are mainly signaling via TLRs but that also via other families of pattern recognition receptors. With

nutrient deprivation the effects were even more extreme. There was an approximate tenfold decrease in activation. The effects of other PRRs are minimal but still there.

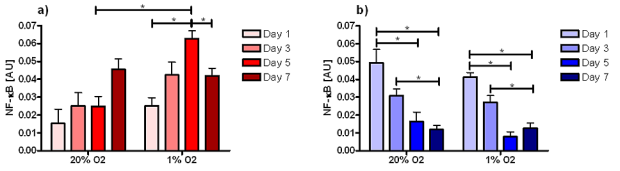

Figure 1: Effects of control (a) and nutrient deprivation model (b) at 20 and 1% O_2 of islets in activation of NF-kB in MyD88(+) cell-line. Values are presented as mean±SEM (n=4); p<0.05(*)

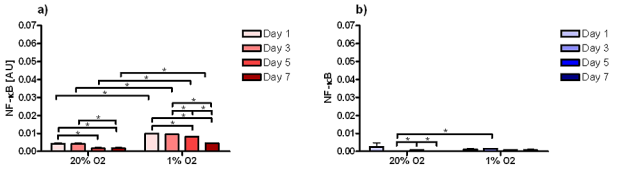

Figure 2: NF-kB activation dependency on MyD88(-) cell-line of control (a) and nutrient deprivation model (b) at 20 and 1% O_2 of islets. Values are presented as mean±SEM (n=4); p<0.05(*)

Confocal analysis was used to assess the viability of the islets under different conditions using live/dead staining with Calcein AM (green, live) and EB (red, dead). As shown in Figure 3 control islets do not present an obvious change in the viability of its cells from day 1 to 7. They remain viable as illustrated by the predominant green staining. Under hypoxic conditions more cells are in the active process of dying (red stain) or are necrotic which results in transparent ghost cells. This is even worse and associated with more cells in the process of dying when hypoxia and nutrient deprivation are combined.

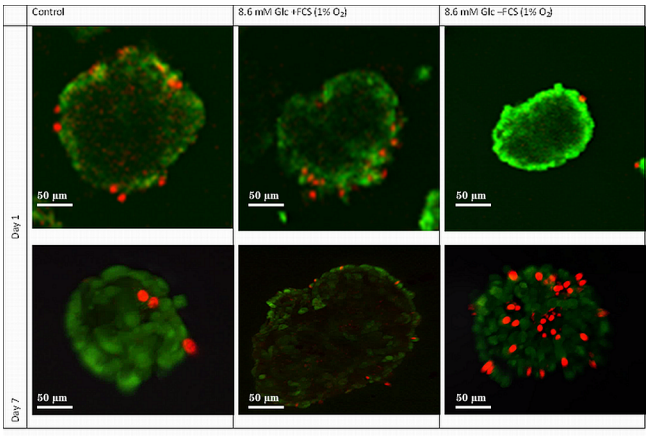

Figure 3. Microscope confocal images of islets stained with Calcein AM (green, live cells) and Ethidium Bromide (red, dead cells)

CONCLUSIONS

Human islets exposed to conditions they face after transplantation in encapsulated cells result in release of islet-derived factors that stimulate immune cells in a NF-κB dependent fashion. This NF-κB activation is mainly TLR dependent. Our data suggest that inhibition of TLRs should be applied in, at least, the first week after transplantation when encapsulated cells face a novel microenvironment with hypoxia and nutrient deprivation.

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ACKNOWLEDGMENTS

To CONACYT for scholarship number 310053 and scholarship granted by the Secretary of Public Education and the Government of Mexico granted to GAPJ.