Role of pathogen-associated molecular patterns in immune responses against alginate based microcapsules.

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INTRODUCTION AND OBJECTIVES

The World Health Organization (WHO) defines type 1 diabetes as an autoimmune disease characterized by the absence or low production of insulin by the pancreas, or the inability of the body to use it properly. For their daily demand patients have to take insulin injections. Long-term use of insulin is associated with complications and frequent episodes of hypoglycemia (Kendal Jr. et al., 2004).

Transplantation of insulin producing pancreatic islets allows for a minute-to-minute regulation of the glucose levels and is not associated with complications (de Vos et al., 2010). Unfortunately, transplantation requires the application of immunosuppression which is at present not acceptable as alternative for insulin therapy. As a consequence islet-transplantation is not frequently applied. Encapsulation of islets may solve this issue (de Vos et al., 2007).

Encapsulation of pancreatic islets is a technique employed to protect the cells from the immune response of the host. The capsules can resist mechanical stress and allows appropriate diffusion of nutrients into the cells and release of metabolic products of interest (Antosiak-Iwańska et al., 2009).

One of the most widely materials used for encapsulation of islets is alginate. Alginate is a polysaccharide composed of different amounts of mannuronic acid (M-chains) and guluronic acid (G-chains) linked in blocks (MM-blocks, GG-blocks and MG-blocks). The proportion and configuration of blocks and the binding with multi-valent cations (i.e. Ca^{2+} and Ba^{2+}) when gelation occurs, give the alginate specific physical and chemical properties (Zimmermann et al., 2005). Alginates with varying MM-blocks, GG-blocks and MG-blocks have been applied. Reported success rates vary considerable (de Vos et al., 2010). Factors such as the presence of contaminations in the alginate have been hold responsible for these variations in success rates. The mechanisms of these responses have been poorly characterized but are essential if we wish to reproducibly make alginates with a predictable biocompatibility.

Immune responses are elicited after binding of undesired molecules to specialized receptors. These receptors are called pattern recognition receptors (PRRs). The PRRs bind the so-called pathogen-associated molecular patterns (PAMPs). PAMPs can be found on pathogens but it has been shown that more substances can contains molecules that bind to PAMPs. Toll-like receptors (TLRs) are the most commonly known PRRs. We hypothesize that alginates may contain PAMPs and elicit inflammatory responses via PRRs.

The present research is intended to study the role of PAMPs as triggering factors of the immune response against encapsulated islets in the first weeks after implantation.

MATERIALS AND METHODS

Cell stimulation

Two cell-lines were stimulated using alginates containing different amounts of guluronic acid (G) chains (Low G alginate (Mannucol DM), intermediate G (Keltone LV), and high G (Manugel DJB) sodium alginates) in their unpurified and purified form to screen for PAMPs effects of the alginate. All alginate types were used at 0.3% (w/v).

Cell-lines from InvivoGen were used, such as Thp1-XBlue™-MD2-CD14 (Thp1 MyD88 (+)), a human cell-line carrying all TLR's with a reporter plasmid under control of the NF-κB, expressing a secreted embryonic alkaline phosphatase (SEAP) gene that can be measured.

The second cell line is the Thp1-XBlue™-defMyD (Thp1 MyD88 (-)) which has the same construction of Thp1-XBlue™-MD2-CD14 but is deficient in MyD88 activity and it can be used to prove the activation via TLRs.

Alginate purification

For the purification of the alginates, the method by de Vos et al. (1997) was chosen, since it can reduce significantly the polyphenol in the alginate and maintain the levels of endotoxins below safety limits.

RESULTS AND DISCUSSION

Researchers use different kinds of alginate when preparing capsules, with varying degrees of success. We hypothesized that different degrees of PAMPS are responsible for this. We first tested three different types of alginate on Thp1 MyD88 (+) cells expressing all TLRs and coupled via the intracellular messenger Myd88 to NF-kB As shown in Figure 1 all alginates

activated NF-kB. The activation as higher however with low-G alginates than with intermediate-G and high-G alginates (Figure 1).

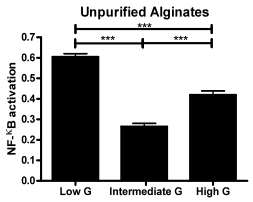

Figure 1 : Effects of alginate composition in activation of NF-kB. Values are presented as mean±SEM (n=20); p<0.001 (***)

To determine whether this activation is TLRs dependent we also tested the alginates on THP1 cells with a knock out on Myd88, i.e. the intracellular messenger for TLRs. A reduction in the production of NF-kB would indicate that molecules bind via toll-like receptors. In figure 2 we show only the results for intermediate-G alginate, but we found that with all alginates the activation was gone in the absence of Myd88. This made us conclude that activation occurs via TLRs and not via other pattern recognition receptors that signal via other routes.

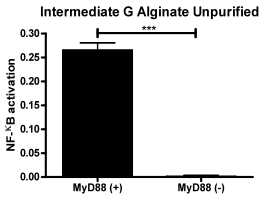

Figure 2 : NF-kB activation mediated via TLRs in intermediate G alginate unpurified. Values are presented as mean±SEM (n=20); p<0.001 (***)

Next we investigated whether impurities or alginate itself activates TLRs. We did this experiment by comparing the responses against purified and unpurified intermediate alginates (Data shown only for intermediate G alginate unpurified, Figure 3).

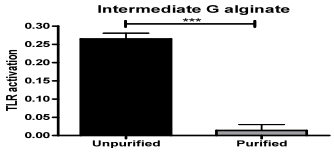

Figure 3 : Effects of purification of intermediate G alginate on NF-kB activation. Values are presented as mean±SEM (n=20); p<0.001 (***)

These results demonstrate the TLR activating components are contaminating PAMPs in crude alginates. We show for the first time the mechanism behind responses against alginates..

CONCLUSIONS

TLRs are involved in responses against alginates.
The activation of TLRs is alginate dependent.
Not alginate but contaminations present in crude alginate are activating TLRs.
The efficacy of purification procedures can be tested by testing for effect on TLR.

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