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Brief communication

Inhibition of LPS-induced proinflammatory responses of J774.2 macrophages by immobilized enzymatically tailored pectins

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Abstract

The surface of an implant device can be modified by immobilizing biological molecules on it to improve its integration into the host tissue. We have previously demonstrated that enzymatically tailored plant pectins are promising nanocoatings for biomaterials. This study investigates whether a coating of modified hairy region (rhamnogalacturonan-I) from apple pectin (MHR-α) which has anti-adhesive properties can inhibit the generation of inflammatory mediators by lipopolysaccharide (LPS)-activated macrophages. For that purpose, J774.2 murine macrophages were cultured for 24 h on MHR-α-coated Petri dishes and tissue culture polystyrene controls, with and without LPS. Cell morphology, cell growth, nitrite and TNF-α secretion were studied. The results indicate that MHR-α coating inhibits the LPS-induced activation of macrophages.

Keywords: Pectin-modified hairy region; Pectin-grafted biomaterials

1. Introduction

The surface properties of biomaterials trigger extracellular signals that modulate the behaviour of adherent cells. The integration of an implant into host tissue can be improved by modifying the surface of the implanted device by immobilizing biological molecules on it [1]. We have previously shown [2] that enzymatically tailored plant pectins (differing in charge, molecular weight, degree of branching and acetylation) can be used as nanocoatings to improve the biocompatibility of biomaterials and medical devices.

The pectins are complex polysaccharides that have "smooth" and "hairy" regions. The smooth region is made up of homogalacturonan (HGA) and optionally xylogalacturonan. They both have a (1→4)-α-D-Gal*p*A backbone and can be methyl-esterified. In addition, HGA may be partially *O*-acetylated at the *O*-3 or *O*-2 position of the GalA moieties [3,4]. The hairy region (HR) consists of rhamnogalacturonan-I (RG-I), which has a backbone of →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→ repeats branched at the *O*-4 position of the rhamnose moiety [5,6]. Techniques for tailoring side chain structures in vitro by specific enzymes are well established [7]. Commercial pectinase preparations can be used to liquefy fruit and vegetable tissues and then isolate RG-I-rich fractions on a pilot scale [8,9]. The enzymes degrade the HGA part (smooth regions) of the pectin, leaving the HR intact. We have previously shown that immobilized modified hairy regions (MHRs) of pectin influence the morphology, adhesion, cell cycle progression and survival of fibroblasts [10]. Numerous studies have shown that pectic polysaccharides are potentially important immunomodulatory agents that may, for example, increase the

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synthesis of Fc-receptors by macrophages [11], enhance the capacity of phagocytes [12], increase the adhesion of macrophages and production of oxygen radicals [13,14], or influence complement fixing activities, proinflammatory cytokine secretion and chemotaxis of macrophages [15–18].

We have also shown that immobilized MHRs modulate bone-cell and macrophage behaviour in vitro [19]. An immobilized MHR from apple, MHR-α, is a non-inflammatory molecule that could be used to reduce the adhesion of medical devices [10,19]. The present study examines the capacity of MHR-α to inhibit the generation of secreted inflammatory mediators by lipopolysaccharide (LPS)-activated J774.2 murine macrophages. This could lead to the development of tailored pectin-grafted surfaces to prevent inflammation and improve the biocompatibility of implants.

2. Materials and methods

2.1. Pectic substratum

MHR-α was prepared as described by Schols et al. [8,10] by treating apple pulp with the commercial enzyme preparation Rapidase C600 from DSM Food Specialities, Delft, The Netherlands. The resulting suspension was centrifuged, the juice clarified by ultrafiltration and the ultrafiltration retentate lyophilized to yield the MHR. The polymer was characterized as described [20].

All chemicals used in surface modification were from Sigma–Aldrich (Saint Quentin Fallavier, France). Surface modification of polystyrene (PS) was carried out as follows: PS was first surface-functionalized by the introduction of amino groups via deposition from allylamine plasma, as described by Morra et al. [2]. MHR was then covalently coupled to the surface amino groups of the PS. In brief, 5 ml of an unbuffered 0.5% solution of MHR-α was placed into the aminated PS and coupled by carbodiimide-mediated condensation between carboxyl groups present in the MHR and amino groups of the surface [2].

The endotoxin-free status of the MHR-α coated PS was checked by Cambrex Bio-Science, Verviers, Belgium.

2.2. Macrophage culture and assays

The murine macrophage cell line J774.2 (European Collection of Cell Cultures) was maintained in Dulbecco's modified Eagle's medium (Gibco Invitrogen, Cergy-Pontoise, France) supplemented with 10% fetal bovine serum, penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}) and 4 mM L-glutamine. Cells were grown in a 100% humidified incubator at 37 °C with 10% CO_2 and passaged 2–3 days before use. The J774.2 cells (2×10^4 ml^{-1}) were seeded on tissue culture polystyrene (TCPS) Nunclon® 12-well multi-plates or on MHR-α-coated Petri dishes and activated with bacterial LPS (10 ng ml^{-1}; Sigma–Aldrich, Saint Quentin Fallavier, France).

Cell proliferation was assessed by mechanically removing a sample 24 h post-seeding and counting viable cells (N) by trypan blue exclusion (Sigma–Aldrich, Saint Quentin Fallavier, France). Results are presented as the proliferation index: N/N_0 (N_0 = number of cells seeded).

The accumulation of nitrite in J774.2 cell culture supernatants was measured using the Griess reagent system (Promega, Charbonnieres, France). An aliquot of cell culture medium (50 μl) was incubated with 50 μl of 1% sulfanilamide for 10 min, followed by incubation with 50 μl of 0.1% N-1 naphthylethylenediamine dihydrochloride solution for 10 min in the dark. The absorbance at 550 nm was then measured. Micromolar concentrations of nitrite were calculated from a standard curve. The nitrite content was normalized to 10^5 cells.

TNF-α secretion into culture supernatants was measured 24 h post-seeding. A murine TNF-α development kit (Interchim, Montluçon, France) was used according to the manufacturer's instructions. Standard curves were prepared from serial dilutions of the recombinant murine TNF-α included in the kit. Results were normalized to 10^5 cells.

2.3. Statistical evaluation

Statistical evaluations were performed using GraphPad InStat software and all the reported values are expressed as means ± standard error of the mean. The Mann–Whitney non-parametric test was used.

3. Results and discussion

Pectin polysaccharides are components of dietary fiber and possess immunomodulating activity, acting mainly on neutrophils and macrophages. For example, the RG-I type structure has complement fixing activity and modulates the secretion of proinflammatory mediators [7]. We examined the potential biomedical use of MHR-α, an enzymatically modified apple MHR that appears to prevent cell adhesion [10,19]. We assessed the capability of this modified pectin derivative to inhibit the proinflammatory responses of J774.2 macrophage murine cells induced by LPS, a bacterial endotoxin that is a powerful macrophage activator [21]. The activation by LPS and cytokine release by J774.2 macrophages was studied after cells had been seeded on MHR-α-coated surfaces for 24 h. Control cells were cultured on TCPS with (positive control) or without (negative control) LPS (10 ng ml^{-1}).

The sugar and substituent composition of MHR-α is shown in Table 1. According to Schols et al. [9], arabinose is mainly derived from branched arabinan side chains. In general, the Rha/GalA ratio is a good indication of the amounts of RG-I and HGA present. MHR-α seems therefore to contain equal amounts of RG-I and HGA. It is quite highly methylated and acetylated: roughly one-third of the GalA residues are methyl-esterified.

We first examined the morphology of macrophages grown on the TCPS control substratum and on MHR-α-grafted Petri dishes, with and without LPS. J774.2 macro-

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