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Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh

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Abstract

Water-soluble acidic polysaccharides from the cell walls of *Ulva rigida* are mainly composed of disaccharides that contain glucuronic acid and sulphated rhamnose. The structure of disaccharides resembles that of glycosaminoglycans (GAGs) as they both contain glucuronic acid and sulphated sugars. Glycosaminoglycans occur in the extracellular matrix of animal connective tissues but can also be produced by leucocytes at inflammatory sites. Certain types of GAGs can even activate macrophages and therefore the acidic polysaccharides from *U. rigida* probably modulate macrophage activity. In the present study, we evaluated the effects of *U. rigida* polysaccharides on several RAW264.7 murine macrophage activities, including expression of inflammatory cytokines and receptors, nitric oxide and prostaglandin E2 (PGE_2) production, and nitric oxide synthase 2 (NOS-2) and cyclooxygenase-2 (COX-2) gene expression. *U. rigida* acidic polysaccharides induced a more than two-fold increase in the expression of several chemokines (chemokine (C motif) ligand 1, chemokine (C-X-C motif) ligand 12, chemokine (C-C motif) ligand 22 and chemokine (C-X-C motif) ligand 14 (Cxcl14)) and in the expression of IL6 signal transducer and IL12 receptor beta 1. Incubation of macrophages with *U. rigida* polysaccharides also induced an increase in nitrite production, although this effect decreased considerably after desulphation of polysaccharides, suggesting that the sulphate group is important for the stimulatory capacity of these molecules. *U. rigida* polysaccharides also stimulated macrophage secretion of PGE_2 and induced an increase in COX-2 and NOS-2 expression. The results indicate that *U. rigida* acid polysaccharide can be used as an experimental immunostimulant for analysing inflammatory responses related to macrophage functions. In addition, these polysaccharides may also be of clinical interest for modifying certain macrophage activities in diseases where macrophage function is impaired or needs to be boosted.

Keywords: RAW264.7 macrophages; *Ulva rigida*; Sulphated polysaccharides; Cytokine expression; Nitric oxide; Prostaglandins

1. Introduction

Macrophages are multifunctional cells that have a central role in the innate and adaptive immune responses [1]. Macrophages protect the host by engulfing and killing pathogens, present antigens to lymphocytes and release numerous biologically active molecules that

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regulate the activity of other cells. They participate in inflammatory processes, which are important in removing pathogens and, depending on the stimuli, they may also participate in the resolution of inflammation by releasing anti-inflammatory cytokines and eliminating cell debris [2]. It is possible to modify the function of macrophages by inducing selective stimulation or suppression of cell activity. Some substances such as bacterial lipopolysaccharides, fungal β-glucans or certain types of carrageenans have been used to activate macrophages [3–6]. However, other substances such as some carrageenans, dextran sulphate or fucoidan (a marine sulphated polysaccharide obtained from seaweed) appear to inhibit macrophage functions [1,7]. Both groups of substances may have biomedical applications and may be of potential use in stimulating the immune system or in controlling macrophage activity to reduce associated negative effects.

The cell walls of seaweeds are rich in matrix polysaccharides of different shapes and with different biological properties. Some of those polysaccharides appear to exert immunomodulatory activities in mammals as they modify the activity of macrophages. Polysaccharides obtained from the two red algae *Porphyra yezoensis* and *Gracilaria verrucosa* stimulate phagocytosis and respiratory burst in mouse macrophages *in vitro* and *in vivo* [8–10]. Carrageenan, a sulphated polysaccharide obtained from red algae and known to be a potent inflammatory agent in rodents, primes mice leucocytes to produce TNF-α in response to bacterial lipopolysaccharide [11]. Some types of carrageenans induce potent macrophage activation [4,5,12]. However, other types appear to impair macrophage functions, indicating the importance of the molecular structure of these polysaccharides in their immunomodulatory activities. Seaweed polysaccharides also possess antitumour activity [13–15] and, in some cases, this activity was found to be associated with macrophages, the presence of which increased the release of certain cytokines such as tumour necrosis factor-α (TNF-α) [8,16,17].

Ulva rigida is rich in cell-wall polysaccharides, including cellulose and water-soluble polysaccharides that contain sulphate groups. The main type of water-soluble polysaccharide is ulvan, the main component of which is a disaccharide formed by β-D-glucuronic acid (1,4)-L-rhamnose 3 sulphate [18,19]. Interestingly, this disaccharide resembles glycosaminoglycans such as hyaluronan and chondroitin sulphate because they all contain glucuronic acid and sulphate. Human monocytes and macrophages produce proteoglycans – mainly chondroitin sulphate proteoglycans – at inflammatory sites [20,21] and certain types of chondroitin sulphates have been shown to activate monocytes and B-lymphocytes clones [22,23]. Furthermore, ulvan contains rhamnose, a sugar that is common in bacteria but not in animal tissues. All of these structural characteristics make ulvan worthy of investigation. The aim of the present study was to determine if acidic sulphated polysaccharides obtained from the seaweed *U. rigida* modulate the activity of murine macrophages.

2. Materials and methods

2.1. Preparation of the acid fractions from seaweed polysaccharides

U. rigida C. Agardh was collected from the coast of Galicia (NW Spain). Seaweeds were homogenized with distilled water (25 g in 100 ml) in a dounce homogenizer and maintained for 24 h at 4 °C. After centrifugation at 1500 ×*g* for 20 min, the supernatants were collected and filtered (8 to 0.2 μm). After precipitation of the proteins with 5% trichloroacetic acid (TCA) and dialysis of the supernatants against distilled water, polysaccharides were precipitated with ethanol and then fractionated into neutral and acid sugars, as previously described [24,25]. Briefly, polysaccharides were fractionated by anion exchange chromatography in a DEAE-Trisacryl column (3 cm × 30 cm) equilibrated with 5 mM phosphate buffer (pH 7.0). Non-adsorbed and adsorbed fractions were recovered, dialysed and concentrated to the initial volume by ultrafiltration (membranes of NMWL 10,000 Da). The molecular weight distribution of the acid fraction polymers was determined by gel permeation chromatography on a Sepharose CL-4B column (1.5 cm × 90 cm), with blue dextran (2000 kDa, Sigma) and glucose (Sigma) as standards.

Protein contents were determined colorimetrically by the Lowry method. Total sugar content of the extracts was determined colorimetrically by the phenol-sulphuric method, with glucose as standard [26]. The uronic acid content was determined by the m-hydroxydiphenyl method, with galacturonic acid as standard [27].

Polysaccharides were also analysed for monosaccharide composition by gas chromatography after 2N TFA hydrolysis and conversion of the hydrolysate into alditol acetates following a previously described method [28]. The alditol acetates obtained were analysed in a Hewlett Packard 5890 gas chromatograph system equipped with a SP-2330 capillary column (30 m), at an oven temperature of 240 °C. The temperature of the injector and detector was 300 °C.

Desulphation of the acid fraction was carried out as previously described [24,25]. The extracts (10 ml) were passed through a Dowex 50 W column (X-8, H^+), and the effluent was neutralized with pyridine and then lyophilised. The pyridinium salt obtained was desulphated with dimethyl sulphoxide containing 10% of distilled water. Aliquots of this solution were heated at 80 °C for 5 h. The reaction mixture was then diluted with distilled water, dialysed against distilled water for 4 days and concentrated to the initial volume to obtain the