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Blocking adhesion of cancer cells to endothelial cell types by *S. agalactiae* type-specific polysaccharides

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Abstract

By using immortalized and normal endothelial cells, we were able to detect inhibitory effects of type specific polysaccharides from *Streptococcus agalactiae* on adhesion of cancer cells to endothelial cells, which is an essential step of cancer metastasis. The inhibition was probably due to specific structures of the bacterial polysaccharides, since the structures of the saccharides are very similar to those of cancer specific sialyl Lewis carbohydrates (sialyl Le^a and Le^x) which bind to ELAM-1 of endothelial cells. This result indicated that the bacterial polysaccharides from *S. agalactiae* could be very useful and hopeful as cancer metastasis inhibitors.

Abbreviations: HUVECs – human umbilical cord vein endothelial cells; ELAM-1 – endothelial leukocyte adhesion molecule- 1

Introduction

The most fearsome aspect of cancer is metastasis and the invasion of cancer cells into surrounding tissues and various organs, which often results in death. Therefore, it is very important to develop effective cancer metastasis inhibitors. The process of cancer metastasis consists of several steps; detachment of cancer cells from the original tumor, invasion into a blood vessel, spreading to various and distant places through the blood stream and invasion to various organs from blood vessel. In the last step, sialyl Lewis carbohydrates (sialyl Le^a and Le^x) of cancer cells interact with ELAM-1 of endothelial cells (rolling), then tight binding of cells is formed through other attachment factors (Takada *et al.*, 1993). Therefore, sialyl Le^a and Le^x carbohydrates play a key role in metastasis by mediating cell-cell interaction between cancer and endothelial cells (Takada *et al.*, 1993).

Type specific polysaccharides from group B streptococci, *Streptococcus agalactiae* Ia and Ib, have very similar structures to those of sialyl Lewis carbohydrates (Jennings *et al.*, 1983). These polysaccharides consist of a NeuNAc-Gal-GIcNAc trisaccharide unit, which is a structure specific to sialyl Lewis carbohydrates, but do not contain a branched fucose residue observed in the cancer specific carbohydrates. The trisaccharides branch from a main chain which has Glc-Gal repeating structure.

In this paper, we investigated inhibitory effects of these bacterial polysaccharides on the adhesion between endothelial cells and cancer cells by using immortalized human endothelial cells, which express ELAM-1, and normal endothelial cells to confirm the usefulness of the polysaccharides as cancer metastasis inhibitors.

Materials and methods

Preparation of type specific polysaccharides

Group B streptococci, *S. agalactiae* Ia and Ib were kindly supplied by Dr. Michio Ohta, Nagoya University. These strains were inoculated into flasks containing 100 ml of Todd-Hewitt broth (BRL) supplemented with 2% glucose and 1.5% Na_2HPO_4 and grown at 37°C (Hunolstein *et al.*, 1993). We partially purified the bacterial polysaccharides from culture media, since these saccharides were known to be released from cells through the exponential phase and the onset of the stationary phase. The bacteria were killed with 2% formaldehyde at the end of the logarithmic growth phase and then were allowed to stand at 4°C overnight prior to centrifugation.

After centrifugation, the supernate of the formalin fixed culture was extracted with 30% ethanol solution. The precipitate was removed by centrifugation and ethanol was added to 80%. After centrifugation, the precipitate which contained the extracellular polysaccharides was suspended in 0.01 M Tris-HCl buffer, pH 7.3 containing 0.001 M $MgCl_2$ and $CaCl_2$. The solution was treated with DNase, RNase and then Proteinase K. The enzyme treated supernatant was applied to a 1.5 × 90 cm column of Sepharose 6B equilibrated in 0.05 M Tris-HCI buffer, pH 7.4 and eluted with the same buffer at a flow rate 12 ml h^{-1}. The polysaccharide peaks were identified by phenol-sulfuric acid assay (Dubois *et al.*, 1965) and an ELISA method using the type specific antisera (Ohbayashi *et al.*, 1987). The fractions containing type specific sugars were pooled and dialyzed against distilled water. The final samples were lyophilized.

Immunological methods

For the detection of ELAM-1, cells were cultured on Culture Chamber (Nunc, Naperville, Illinois) and interleukin-1β (Becton Dickinson Labware, Bedford, MA) was added 4 h before cell fixing. Endothelial cells were fixed with ethanol. ELAM-1 was detected with mouse anti-ELAM-1 antibody (Genzyme, Cambridge, MA) and FITC-labeled rabbit anti-mouse IgG (Kappel, Organon Teknika Co, West Chester, PA) using a fluorescence microscope.

Cell adhesion assay using HUVECs

Colo201 colon cancer cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). #5-1 human immortalized endothelial cells and normal HUVECs were maintained in TCM199 medium supplemented with 10stimulated with 1.0 ng ml^{-1} of interleukin-1β (IL-1β) for 4 h in 96-well plates (Takada *et al.* 1993). To the plates, ^{35} S-labeled cancer cells (1×10^5 cells/well) were added in the presence and the absence of the bacterial polysaccharides and incubated for 30 min at room temperature. After washing with phosphate buffered saline (PBS), remaining radioactivity was measured with a liquid scintillation counter. As a control, binding of labeled cells to IL-1 β stimulated immortalized and normal HUVECs without the saccharides was measured and results were normalized with this control.

Results and discussion

Recently, we have established a human endothelial cell line by using an origin-defective SV40 DNA (Kirinaka *et al.*, 1995). We investigated the expression of ELAM-1 on immortalized endothelial cells #5-1, since this molecule is reported to be very important for cell adhesion between cancer cells and endothelial cells in metastasis (Takada *et al.*, 1993). IL-1β is known to induce ELAM-1 on HUVECs (Bevilacqua *et al.*, 1989). As shown in Figure 1, cell line #5-1 expressed ELAM-1 depending on IL-1β and the level of the expression was higher than that of normal endothelial cells. We also examined the ability of the #5-1 cells to adhere to cancer cells. Colo201 colon cancer cells adhered only to IL-1β stimulated normal and immortalized HUVECs (Figure 2).

Structures of type Ia and Ib specific polysaccharides are very similar to that of sialyl Le^x and Le^a, respectively (Figure 3). Therefore, these polysaccharides were expected to show competitive inhibitory effects on adhesion between cancer cells and HUVECs, and to hamper cancer metastasis. Group B streptococci, *S. agalactiae* usually produces two classes of capsular polysaccharides (Tai *et al.*, 1979). One is a type specific polysaccharide which is similar to sialyl Lewis carbohydrates and the other is a group B specific polysaccharide produced by any type of group B streptococci. To detect these polysaccharides, type specific and group specific antisera were applied to fractions from gel filtration chromatography. On the Sepharose

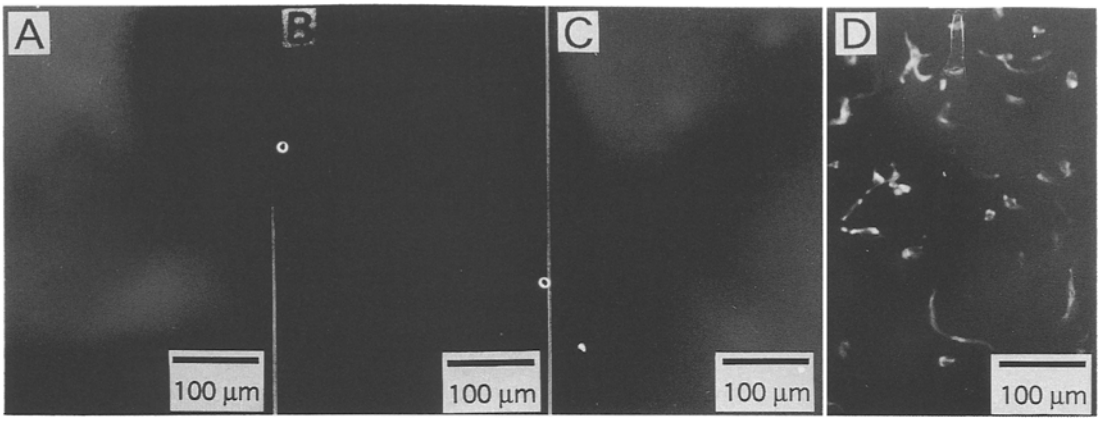

Figure 1. Expression of ELAM-1. Normal cells with (B) and without (A) interleukin β. #5–1 with (D) and without (C) interleukin β.

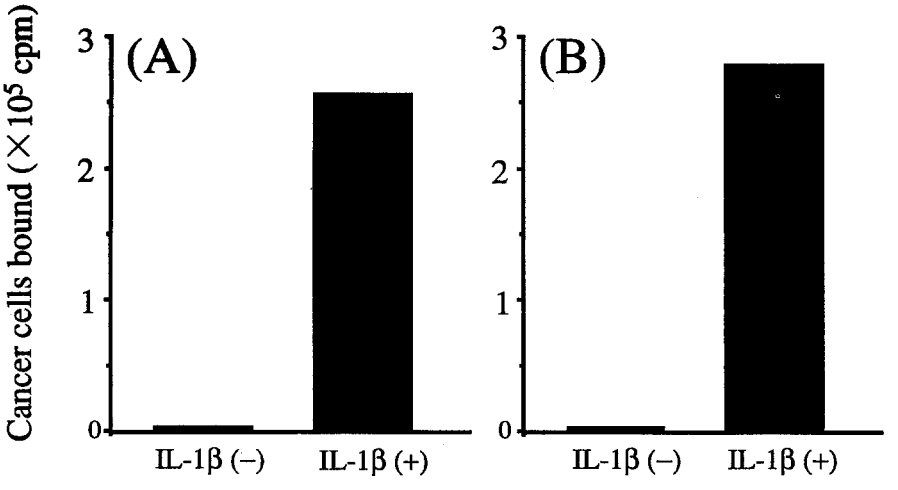

Figure 2. Adhesion of Colo201 human cancer cells to interleukin-1β activated normal (A) and immortalized (B) endothelial cells.

6B column, type specific polysaccharides were eluted in high molecular weight fractions (Figure 4) and these fractions showed little reactivity with group B specific antiserum (data not shown). According to elution profiles from the gel filtration, the molecular weight of the type specific polysaccharides ranged from about 400 to 700 kDa which means at least 400 NeuNAc-Gal-GlcNAc repeats are included in one polysaccharide molecule. The final yield was about 1–3 mg purified type specific polysaccharides per 500 ml culture. These polysaccharides were used in the cell-cell adhesion assay to demonstrate the inhibitory effects.

As shown in Figure 5, the type specific polysaccharides exhibited inhibitory effects on cell adhesion. The adhesion of Colo201 human cancer cells to IL-1β stimulated normal HUVECs was clearly inhibited by both type Ia and Ib specific polysaccharides. We were able to observe the same inhibitory effects when #5-1 immortalized endothelial cells were used (Figure 5). The adhesion was reduced to 10–40% of control and the type specific Ia and Ib polysaccharides showed the inhibitory effects at 10 μg ml^{-1}. This inhibition was probably due to specific structures of the type specific polysaccharides because the other type of polysaccharide, for instance, colominic acid (polysialic acid) showed only a little inhibitory effect at the same concentration (Figure 5). Furthermore, these polysaccharides did not show any cytotoxic effect on human endothelial cells (data not shown).

In this study, we were able to detect the ability of type specific polysaccharides from *S. agalactiae* Ia and Ib to inhibit adhesion of cancer cells to human endothelial cells. But the experiments do not conclusively prove that the blocking is due to binding of

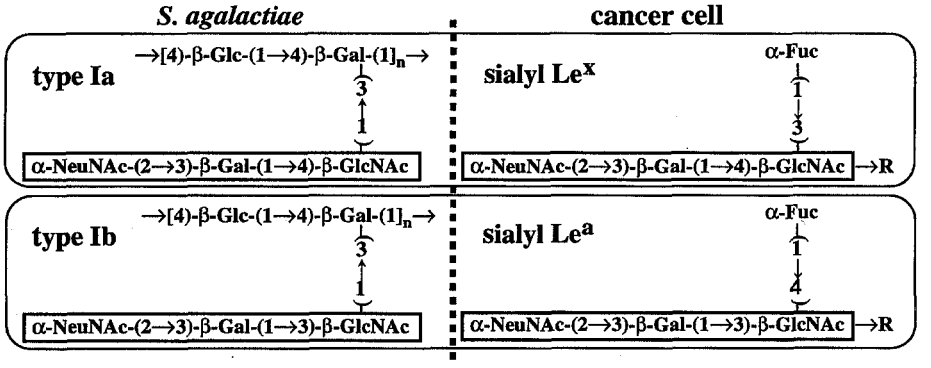

Figure 3. Comparison of cell surface polysaccharides from *S. agalactiae* with cancer specific carbohydrates

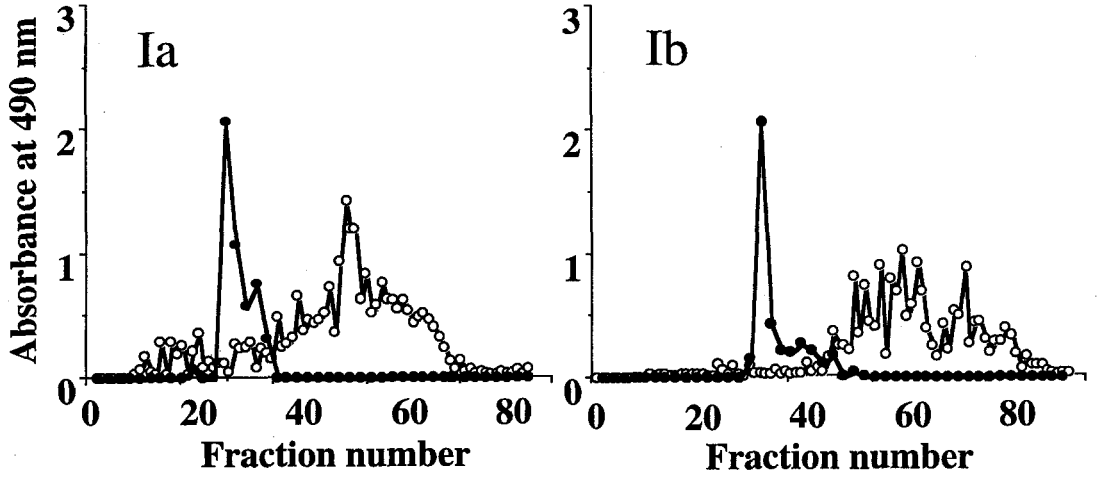

Figure 4. Gel filtration of polysaccharides from *S. agalactiae*. Total polysaccharides (○) were analyzed by phenol-sulfuric acid method and type specific polysaccharides (●) were detected by ELISA using type specific antisera.

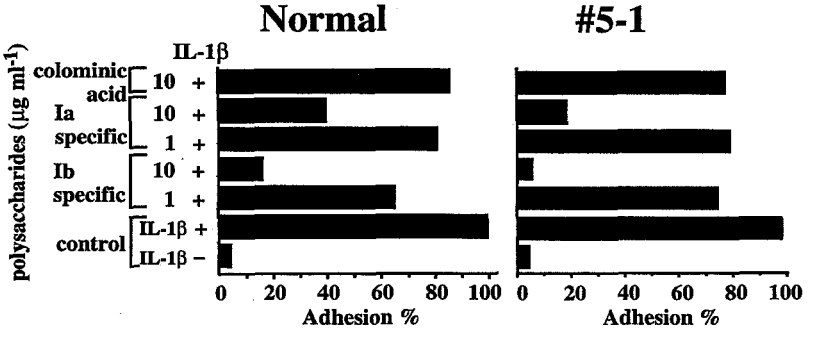

Figure 5. Inhibitory effects of type specific polysaccharides from *S. agalactiae* on adhesion of Colo201 human cancer cells to interleukin-1β activated normal and immortalized HUVECs.

polysaccharide to ELAM-1; additional controls are needed to probe this interaction specifically. We also found that the #5-1 immortalized endothelial cell line was very effective to investigate the inhibitory effects. Although we need to investigate these effects *in vivo* so as to develop these polysaccharides and their derivatives as cancer metastasis inhibitors, the present results suggest that the streptococcal polysaccharides may be very useful and hopeful for development of new cancer metastasis inhibitors.

Acknowledgments

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