CONTROLLING ENDOTHELIAL CELL BIOSECRETORY FUNCTION THROUGH SURFACE MODIFICATION

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INTRODUCTION

The adhesion of cells is a process that is essential to organogenesis, development, wound healing, angiogenesis, and tissue remodeling. This initial cell/surface interaction is therefore critical to biomedical, pharmacological and biotechnological applications and very often requires a define architecture to ensure appropriate cell function [1]. As certain proteins control cell adhesion, it has been hoped that cell transplantation and tissue engineering could be augmented by pre-adsorption of specific proteins to biological or synthetic surfaces [2]. The questions that remain, however, are whether such proteins can affect cell biosecretory function as well as adhesion, and if so in a protein-specific manner.

MATERIALS AND METHODS

Protein adsorption studies

TCPS plates were incubated for 2h at room temperature with solutions of fibronectin (Fn), laminin (Ln), and gelatin (Sigma-Aldrich, MO) in phosphate buffered saline (PBS, pH 7.4, Gibco BRL Products, NY). Proteins radiolabeled with 2mCi of ^{125}I (NEN Life Science Products, MA) following the iodo-bead method (Pierce, IL) and purified by gel filtration, were used as tracers in the adsorption studies. After incubation, the coating solution was rinsed and the TCPS discs placed in a gamma counter for quantification.

Cell culture and function

After isolation bovine aortic endothelial cells (BAEC) were cultured to passage 5 in Dulbecco's modified Eagle medium (DMEM, Gibco BRL Products) containing 10% fetal bovine serum (FBS, HyClone, UT) and 1% of penicillin-streptomycin (PS) and 1% L-glutamine (G) (Gibco BRL Products). For adhesion studies cells were seeded in serum-free medium at a density of $4x10^5$ cells/well. Cells were detached by trypsinization and cell number measured using a Coulter counter (Beckman, FL).

Cell function was evaluated three days before and after confluency based on the production of total protein, glycosaminoglycan (GAG), and prostacyclin (PGI_2) in the conditioned medium collected after 24h incubation with serum-free DMEM. Total protein production was determined by means of the Bio-Rad protein assay (Bio-Rad laboratories, CA). Total GAG production was quantified by means of the dimethylmethylene blue (DMB) assay. Heparan sulfate levels were also determined by the DMB assay by pre-treating the conditioned medium with chondroitinase ABC (Seikagaku America, MA) to eliminate chondroitin sulfate and dermatin sulfate. Prostacyclin (PGI_2) present in the conditioned medium was determined by a 6-keto-prostaglandin $F1_\alpha$ enzymeimmunoassay (EIA) system (Amersham, MA). Purified mouse anti-human monoclonal antibody CD49e, and purified mouse IgG2a monoclonal immunoglobulin as the isotype control, were purchased from Pharmingen (CA). Blockage of the Fn receptor was carried out in suspension, at 37°C for 20min. Samples were centrifuged and the excess of antibody was aspirated off before cell seeding.

RESULTS AND DISCUSSION

Fn and Ln adsorption isotherms appeared to follow a Langmuir isotherm [3] with *plateau* values of 950±9ng/cm^2, and 625±8 ng/cm^2, respectively. Gelatin, however, presented a very different adsorption isotherm than Fn and Ln and no *plateau* was achieved within the concentration range examined. The amount of adsorbed protein reached 1.8µg/cm^2 for a solution concentration of 2000µg/ml.

Fn, Ln, and gelatin are well-known cell adhesion mediators and their ability to increase cell attachment has been previously described [3]. A preliminary cell-attachment experiment was performed to validate our method and to investigate the degree of specificity of the proteins for cell adhesion. Solutions of 50µg/ml of Fn, 100µg/ml of Ln, and 500µg/ml of gelatin were used to coat TCPS plates. These solution concentrations resulted in a surface density of adsorbed protein of 500ng/cm^2. Uncoated TCPS plates, hydrated with PBS, were carried out in parallel as negative ($PBS^{(-)}$) and positive ($PBS^{(+)}$) controls, for BAEC being seeded in serum-free or in 10%FBS-containing medium, respectively. Cell number was increased by 25%, 19%, and 20% for Fn-, Ln-, and gelatin-coated TCPS, respectively compared to $PBS^{(-)}$.

To study the influence of pre-adsorbed Fn, Ln, and gelatin on cell behavior, BAEC on pre-coated TCPS were incubated for 24h in serum-free medium. The collected conditioned medium was analyzed

for total protein, soluble GAG, and PGI_2 production. The total amount of protein and GAG produced by BAEC on the hydrated TCPS remained below 50% of the production of both sub-confluent and post-confluent BAEC seeded on TCPS pre-coated with Fn, Ln, or gelatin. There was no significant difference among the three coatings. In contrast, PGI_2 levels produced by BAEC seeded on TCPS pre-coated with Fn and Ln, but not gelatin, were 3-fold and 3.8-fold, respectively, higher than those produced by BAEC on uncoated TCPS (see Fig.1).

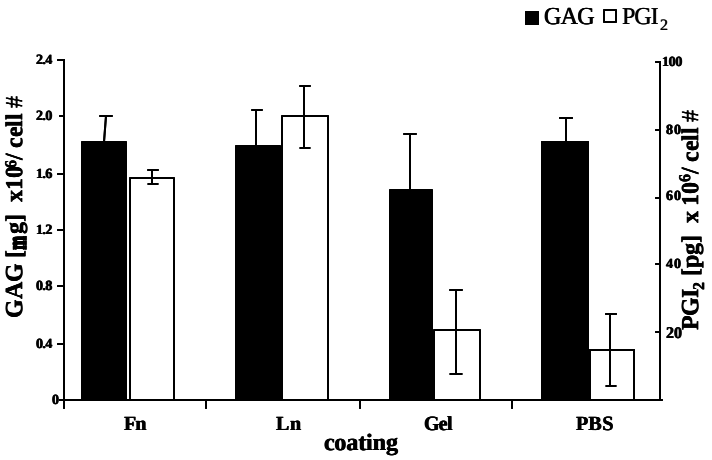

Figure 1. GAG and PGI_2 production by confluent EC seeded on coated TCPS

To investigate the mechanism behind of the observed stimulation of PGI_2 production and to test the hypothesis that the adhesion of BAEC to Fn or Ln leads to the activation of different signaling pathways than the adhesion of cells to gelatin, the following three-steps strategy was carried out: 1) incubation of EC with different concentrations of a specific monoclonal antibody to achieve complete saturation of the Fn-receptor; 2) study the effect of the blockage on cell attachment to Fn-coated TCPS; and 3) determination of PGI_2 production by the selectively blocked cells. A monoclonal antibody (CD 49e), which reacts with the α_5 integrin, a dimer of 135/25 kD, that associates with β_1 integrin (CD 29) to form VLA-5, a well-established Fn-receptor, was used to block this receptor. Subsequently, Fn receptor-blocked were allowed to attach and proliferate till confluence. The analysis of the conditioned medium of post-confluent cells revealed that PGI_2 production by the Fn receptor-blocked cells was decreased by 43% in comparison with the unblocked ones.

CONCLUSIONS

Adsorption studies with radiolabeled proteins were carried out to establish the adsorption isotherms of Fn, Ln, and gelatin on TCPS. Subsequently, the adhesion and the biochemical secretion of BAEC seeded on TCPS discs coated with the same amount of Fn, Ln or gelatin were examined. The three coating proteins non-specifically promote sub-confluent and post-confluent endothelial cell production of total protein and GAG up to 2.5-fold of the reference value. In contrast, Ln and Fn, not gelatin, drastically enhanced post-confluent BAEC production of PGI_2. However, specific antibody-blockage of the α_5 integrin, constituent of the Fn-receptor in BAEC, appeared to inhibit the up-regulation of PGI_2 production observed on Fn-coated surfaces. The results indicate that the cell adhesion mediators used as coating agents dictate cell biochemical production as well as adhesion and growth. The possibility of controlling the levels of PGI_2 produced by BAEC using surfaces pre-coated with Ln or Fn is of crucial relevance for future applications in tissue-engineered constructs. The controlled released of PGI_2, a powerful vasodilator, is key in the inhibition of events such as smooth muscle cell proliferation and platelet aggregation, leading causes of pathologies such as restenosis and acute thrombosis.

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