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Biochemical Engineering Journal 35 (2007) 301–308

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Modulation of hybridoma cell growth and antibody production by coating cell culture material with extracellular matrix proteins

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Received 18 May 2006; received in revised form 21 December 2006; accepted 16 January 2007

Abstract

The influence of coating polystyrene tissue culture plates with different proteins on murine hybridoma cell growth and antibody production was investigated. Fibronectin, collagen I, bovine serum albumin and laminin were used to coat NUNC® and COSTAR® cell culture plates. Cell number and antibody concentration in culture fluids were quantified as indicators for cell viability, proliferation and productivity. Adhesive behaviour, morphology, expression of surface receptors of hybridoma cells and the presence of tyrosine-phosphorylated proteins in cell lysates were characterized by cell adhesion experiments, microscopy, flow cytometry and Western Blot analysis.

It was shown that coatings with fibronectin (0.2 μg/ml) lead to a substantial improvement of cell growth by 50–70% and an increase of monoclonal antibody production by 100–120%.

Collagen I coatings showed an improvement in cell growth by 30–70% and by 60% for the production of monoclonal antibodies. Coatings with BSA and laminin had minor effects on these parameters. It was found that the hybridoma cell lines used in this study did not express the α_2-chain of the $\alpha_2\beta_1$-integrin, which is responsible for binding to collagen and laminin.

However, the presence of β_1-integrin on the cell surface was shown, which should enable hybridoma cells to bind fibronectin. We propose, therefore, that fibronectin adsorption to cell culture materials may be a promising approach to enhance the production of monoclonal antibodies by cultivated hybridoma cells.

Keywords: Hybridoma cells; Tissue culture plates; Cell growth; Antibody production; Extracellular matrix; Fibronectin adsorption

1. Introduction

Morphology, growth and function of most cells are strongly dependent on the adhesive interactions with neighbouring cells and extracellular matrices [1]. Therefore adhesive interactions of cells with foreign substrata used in biomedical and biotechnological applications may have a significant impact on their growth and function [2]. Since, protein adsorption occurs rapidly if materials contact protein fluids, such as serum-containing culture media, the cellular behaviour may be controlled by the composition of the adsorption layer [3]. In particular, the adsorption of adhesive proteins, such as fibronectin as a component of the extracellular matrix on biomaterials, has been shown to promote adhesion, growth and function of cells greatly [4]. Extravasation, homing and programming of B lymphocytes are processes that require adhesive interactions with ECM components and different cell types [5].

Integrins are involved in these processes controlling cell-matrix and cell–cell adhesion by specific ligand binding [6]. Integrins represent a family of αβ-heterodimeric adhesion receptors and are constitutively expressed on lymphocytes or can be activated by antigens, chemokines and phorbol esters [7].

The ligation of integrins by its specific ligand induces a signal transfer called outside-in-signalling, which triggers signal cascades involved in expression of genes important for survival, growth and differentiation of cells [8]. The major integrins on B cells are lymphocyte function-associated-antigen-1 (LFA-1 or $\alpha_L\beta_2$-integrin), very late antigen-4 (VLA-4 or

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doi:10.1016/j.bej.2007.01.035

$\alpha_4\beta_1$-integrin) and $\alpha_4\beta_7$-integrin. LFA-1 binds to intercellular adhesion molecule-1 (ICAM-1 or CD54).

For example, $\alpha_4\beta_7$-integrin binds to fibronectin which is expressed on the luminal surface of endothelial as one of the first steps during extravasation of lymphocytes [9]. The subsequent migration of lymphocytes to lymphoid organs is crucial for encountering antigens and differentiation of cells [10]. Differentiation of B lymphocytes results in the secretion of monoconal antibodies (mAb) to label invading pathogens and guide the activity of other leukocytes.

Such antibodies can now be produced in large quantities by cultivating hybridoma cells *in vitro*. Since hybridoma cells are fusion products of B lymphocytes (plasma cells) and myeloma cells the knowledge about their adhesive phenotype and expression of surface receptors may be useful to control their growth and antibody production. Myeloma cells represent malignant plasma cells located in the bone marrow [11]. Adhesion molecules expressed by myeloma cells are CD44 (HCAM), CD49e (VLA-5 or α_5-integrin), CD50/54 (ICAM1) and CD56 [12]. While plasma cells may circulate in the blood and lymph vessels, myeloma cells remain located in the bone marrow. Therefore, a fusion of both cell types may combine different adhesive behaviour and functions. It should be noted that a fusion of a plasma cell with a myeloma cell may not simply result in a receptor repertoire of both cell types. Some chromosomes may get lost after fusion and the expression of surface integrins may also depend on specific extracellular signals [13].

Because of their growth in suspension, hybridoma cells do not require adhesion to substrata to survive and to produce antibodies [14]. This also fits with the observation that plasma cells, which represent differentiated B cells, do not require adhesion to extracellular matrices (ECM) or intercellular adhesion to survive and function [15]. However, we have recently shown that adhesion of hybridoma cells to certain polymer membranes may result in a stimulation of their growth and in an inhibition of antibody production [16]. Similar results were also obtained by Luo and Yang [17], who showed an inverse relation between adhesion and specific mAb production of hybridoma cells. On the other hand, our previous investigation has also demonstrated that a membrane provoking moderate adhesion and growth of hybridoma cells stimulated antibody secretion to an extend that greatly exceeded that of suspended cells [16].

Since polymer membranes may adsorb different quantities of proteins dependent on their chemical composition [18], we were interested to know whether proteins, which can be adsorbed from tissue culture media or represent components of the ECM have an impact on behaviour of hybridoma cells. Therefore, we studied the effect of different proteins adsorbed to two different types of polystyrene tissue culture material (TCPS) on hybridoma growth and antibody production. The proteins fibronectin, laminin and collagen I were selected, as components of the extracellular matrix, while bovine serum albumin (BSA) was chosen as a major component of serum-containing culture media. A major finding of this study was that coating TCPS with low quantities of fibronectin leads to a substantial improvement of hybridoma growth and monoclonal antibody production.

2. Materials and methods

2.1. Cell culture

The hybridoma cell line H10 (mouse IgG2b, anti-rat-FcεRI), kindly provided by I.Pecht, Weizman Institut of Science, Rehovot, Israel [19], was grown in RPMI 1640 complete medium (Biochrom AG, Berlin, Germany) supplemented with 2 mM L-glutamine (Biochrom AG, Berlin, Germany), 50 μM 2-mercaptoethanol (Roth, Karlsruhe, Germany), 10% heat-inactivated fetal calf serum (Biochrom AG, Berlin, Germany) at 37 °C and 5% CO_2. All cell culture experiments were performed using this medium.

2.2. Cell growth experiments

We compared NUNC® and COSTAR® cell culture plates, which were coated with protein solutions of fibronectin (0.2–20 μg/ml), collagen I (1–80 μg/ml), BSA (10–800 μg/ml) and laminin (0.4–20 μg/ml) prior to cell culture experiments. Uncoated TCPS from the same manufacturers were used as controls. Dilutions of protein solutions were prepared with phosphate-buffered saline without calcium and magnesium (PBS, 150 mM NaCl, 5.8 mM NaH_2PO_4, 5.8 mM Na_2HPO_4, pH = 7.4). After coating the plates at 37 °C for 2 h, the wells were washed with PBS and the cells were placed in the wells (100 μl/well) with a starting density of 4×10^4 cells/ml and incubated at 37 °C and 5% CO_2. The cells were completely harvested after 3 days. The cell numbers were quantified after complete cell lysis with 1% Triton X-100 using a cytotoxicity detection kit based on the release of lactate dehydrogenase (LDH, Roche Applied Science, Penzberg, Germany). The activity of the cytosolic enzyme LDH can be used to estimate the amount of cells [20]. Standard curves were established by counting cells and comparing the numbers with LDH activity. Each experiment was repeated eight times. Mean values and standard deviations were calculated.

BSA, collagen I (from rat tail) and laminin (from human placenta) were purchased from Sigma. Fibronectin (from human plasma) was supplied from Roche Diagnostics, Mannheim, Germany.

2.3. Quantification of antibody production

To determine the concentration of monoclonal antibodies (mAb) produced by the hybridoma cells, 96-well plates (Greiner bio-one GmbH, Frickenhausen, Germany) were coated with purified goat-anti-mouse-Ig antibodies (produced in our laboratory—50 μl/well at a concentration of 8 μg/ml in PBS) for 2 h at room temperature (RT) or overnight at 4 °C. The wells were washed and blocked with PBS containing 5% newborn calf serum (NCS, Biochrom AG, Berlin, Germany) for 30 min at RT. After washing, 50 μl cell-free culture supernatant was added per well and incubated at RT for 1 h. Mouse antibody binding was revealed by incubation with a peroxidase-conjugated anti-mouse-Ig antibody (dilution 1:5000, Sigma) for 1 h at RT. After each incubation step the wells were washed