Serum-free production of a chimeric E-selectin-IgG protein from 1 to 100 l scale: Repeated batch cultivation versus continuous spin filter perfusion

Andreas Werner^{1*}, Dirk Lütkemeyer¹, Iris Poggendorf¹, Jürgen Lehmann¹ & Johannes Müthing²

¹ Institute of Cell Culture Technology, University of Bielefeld, Bielefeld, Germany; ² Institute for Medical Physics and Biophysics, University of Münster, Münster, Germany

(* Author for correspondence; E-mail: awe@zellkult.techfak.uni-bielefeld.de; Fax: +49 521 106 6328)

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Abstract

On inflamed endothelium the cell surface protein E-selectin is expressed which supports the initial process of attachment – capturing and rolling of leukocytes. A recombinant CHO cell line secreting a soluble E-selectin-IgG chimera was cultivated competitively under serum free conditions in three different bioreactor systems: a 1 l Super-Spinner, a 2 l stirred tank bioreactor equipped with a spinfilter, and a 100 l stirred tank bioreactor. In the smallest system 25.4 mg E-selectin-IgG were produced in 62 days using a repeated batch process while achieving a maximal viable cell density of 3.7×10^6 cells ml⁻¹. Using continuous perfusion mode a total amount of 35.2 mg were produced with a maximal viable cell density of 1.65×10^7 cells ml⁻¹ in the 2 l bioreactor within 29 days. Large scale cultivation in a 100 l stirred tank bioreactor yielded 105.6 mg in three batches with a maximal viable cell density of 9.7×10^5 cells ml⁻¹ within 15 days. After removal of the cells by continuous centrifugation and a depth filter clearance step, the supernatants were concentrated via ultra filtration. Purification was performed by affinity chromatography with rProtein A. Integrity of the E-selectin-IgG protein was checked with SDS PAGE. Its activity was verified in a cellular adhesion assay performed with HL-60 cells and a recombinant CHO cell line expressing membrane-anchored E-selectin constitutively, and E-selectin expressing HUVECs, respectively. Soluble E-selectin-IgG was used to block adhesion to these cell layers competitively. A concentration of 18.8 and 37.5 µg ml⁻¹ was sufficient to reduce the amount of adhering HL-60 cells to 50% on CHO and HUVEC layers, respectively.

Introduction

In mammals a bacterial infection causes the critical body defense reaction of inflammation (Vestweber and Blanks, 1999). This state is characterized by a complex coordination of a variety of cell types. Leukocytes have to be guided out of the bloodstream to the site of infection in the tissue (McEver, 1997). After activation endothelial cells express E-selectin (Tedder et al., 1995) on their cell surface which interacts with the sugar epitope sialyl-Lewis^x on leukocytes (Varki, 1994; Feizi, 2001). The expression of E-selectin requires de novo protein synthesis and can be elicited by

a 4 h treatment of cultured endothelial cells with certain Gram-negative endotoxins and cytokines such as IL-1 and tumor necrosis factor α (TNF- α) (Bevilacqua et al., 1987; Murakami et al., 2001). The interaction of E-selectin with the tetrasaccharide leads to tethering and rolling of the leukocytes on the endothelial cell layer and to static binding which is a prerequisite for the following transmigration step through the blood vessel wall (Konstantopoulos and McIntire, 1996). But also adhesion and migration of tumor cells on and through the vascular endothelium, both critical steps of the metastatic invasion, are dependent on E-selectin interactions (Laferriere et al., 2001).

Soluble E-selectin can be used to study the influence of the endothelial E-selectin for the leukocyte-endothelial cell adhesion as well as tumor-endothelial cell adhesion. By its application in a competitive assay the cell-cell binding can be blocked. Furthermore, it can serve for the detection of E-selectin ligands on leukocyte and tumor cell membranes. Therefore, there is a need to produce this protein in a soluble form.

Materials and methods

Cells, cultivation mode and product

A recombinant CHO cell line producing the E-selectin-IgG-chimera (Hahne et al., 1993) was cultivated in a 1 l Super-Spinner (Heidemann et al. 1994), a 2 l stirred tank bioreactor equipped with a spinfilter and a 100 l scale stirred tank bioreactor (all B. Braun Biotech, Melsungen, Germany). Cell numbers were determined in duplicate by trypan blue exclusion with a hemocytometer. Repeated batch mode was exerted for the smallest and largest vessels in contrast to perfusion mode for the 2 l bioreactor. The temperature was set to 37 °C, pH to 7.2 and pO₂ to 40% air saturation. The pH-value was kept constant through a variable CO₂ gas stream and the pO₂-value was adjusted with a gas-mixture of nitrogen, air and oxygen. Bubble-free aeration was obtained with a membrane stirrer consisting of 2.5 m polypropylene hollow fiber membrane tubing (Lehmann et al., 1987, 1988) in case of the Super-Spinner run and a silicone tubing for the experiments in the the larger vessels, respectively.

The secreted protein is composed of a murine E-selectin including the lectin domain, the EGF-repeat, and the first two complement binding domains and also of a human IgG-part containing the hinge region and the CH2 and CH3 domains.

Medium

The CHO cells were cultivated in DMEM/Ham's F12 (1:1) (Gibco, Paisley, Scotland) supplemented with 5 mg l⁻¹ human transferrin (Serological Proteins, USA), 5 mg l⁻¹ bovine insulin (Sigma, Deisenhofen, Germany), and 500 mg l⁻¹ bovine serum albumin (Serva, Heidelberg, Germany). In addition 3 g l⁻¹ glucose was supplemented. Also the amino acid concentrations of glutamine (+4 mM), tryptophan (+50 µM), asparagine (+50 µM), methionine (+30 µM), serine (+30 µM), and proline (+170 µM) were raised. The medium and the supplements were

sterilized by 0.2 µm-membrane filtration (Sartorius, Göttingen, Germany).

E-selectin-IgG quantification

The chimera was quantified using a sandwich-ELISA. Rabbit F(ab)₂ fragments (Dianova, Hamburg, Germany) directed against human IgG were coated on the ELISA plates. After capturing for one hour the chimera was detected with peroxidase conjugated goat F(ab)₂ anti-human IgG (Dianova). Human IgG (Sigma) was used for generating a reference standard curve.

E-selectin-IgG purification

The cells were removed from the supernatant by continuous centrifugation with 3543 g in a Biofuge 17RS (Heraeus Sepatech, Osterode, Germany) and depth filtration through a Sartopure PP2 3 µm filter (Sartorius, Göttingen, Germany). The chimera was concentrated using a S10Y10 10 kDa ultrafiltration cartridge (Amicon, Frankfurt, Germany).

Purification was performed with a 40 ml protein A-sepharose column (Amersham Pharmacia Biotech, Uppsala, Sweden) by FPLC and subsequent superdex 200 gel filtration. Elution conditions were evaluated before with a 4 ml bed volume of recombinant protein A. For elution 0.1 M glycine HCl⁻¹ pH 2.5 + 10% glycerol was chosen.

Protein integrity was tested with SDS-PAGE. Gradient gels (8–25%, gelbuffer: 0.112 M acetate, 0.112 M Tris, pH 6.4) with a separation range of 6–300 kDa and SDS-bufferstrips (3% agarose, 0.2 M Tricine, 0.2 M Tris, 0.55% SDS, pH 8.1) were used. The samples were diluted in non-reducing sample buffer (0.063 M Tris/HCl, 2% SDS, 0.1% Bromphenol blue, pH 6.8) and 1 µl sample/sample well was applied, respectively. The low molecular weight standard (LMW) was used as reference (all Amersham Pharmacia Biotech).

Cellular adhesion assay

HUVECs and CHO-E cells were grown in 96 well microtiter plates (Maxisorp, Nunc, Wiesbaden, Germany) in DMEM/F12 medium supplemented with 10 and 5% FCS (PAA, Austria), respectively, until confluency. The culture medium was sucked off and the cells were overlaid with 1.35 × 10⁵ HL-60 cells per well. Cell-cell contacts were enhanced by centrifugation of the microtiter plate at 32 g for 2 min. Unbound cells

were removed by sixfold automatical washing with HEPES buffer (10 mM HEPES, 150 mM NaCl, 5 mM CaCl_2 , pH 7.0) in an automated ELP-40 microplate strip washer (Bio-Tek Instruments, Germany).

Cell counting

Adhered HL-60 cells were quantified using the Adhex cell counter system (Innovatis, Bielefeld, Germany). The Adhex equipment is composed of a IX50 inversal microscope (Olympus, Germany) with a motor driven frame for microtiter plates (Märzhäuser, Germany), a CCD camera eliciting black and white screen pictures (Jai, Sweden), and a personal computer (Pentium II, 266 MHz) with the imaging software. Counting areas and pictures correspond to squares of $1 \text{ mm} \times 1 \text{ mm}$.

Inhibition of cell-cell adhesion

E-selectin-IgG-chimeric protein ($300 \mu\text{g ml}^{-1}$) was diluted with medium in a microtiter plate in 1:2 series in $100 \mu\text{l}$ volumes. 2.7×10^5 HL-60 cells ($100 \mu\text{l}$ volumes) were added per well and incubated for 1 h at 37°C and 5% CO_2 . 1.35×10^5 cells ($100 \mu\text{l}$ cell suspensions) were then transferred to the HUVEC and CHO-E cell layers (adhesion assay plate). Centrifugation, removal of unbound HL-60 cells, and cell counting was performed as described above.

Results and discussion

Growth of CHO cells and production of E-selectin-IgG in Super-Spinners

The recombinant CHO cell line secreting a soluble E-selectin-IgG was initially cultivated in a Super-Spinner flask starting with a volume of 300 ml and a viable cell density of $7.5 \times 10^4 \text{ cells ml}^{-1}$ (Figure 1). After 4 days the Super-Spinner was topped up in two steps beginning with a volume of 600 ml until day 7 and thenceforward with a maximal level of 1 l. From day 7 to day 26 overall 4 batches were repeated each time by replacing 85% of the culture medium by fresh medium. As the viable cell density reached a value of $2 \times 10^6 \text{ cells ml}^{-1}$ the amount of dead cells began to rise on days 20 and 24, respectively. To minimize the number of dead cells the time interval for medium replacements was reduced from 5 to 3 days beginning at day 26. The average viability increased from 78.1% (sd 14.4) in the first 26 days to 96.5% (sd 2.0) for the period from day 26 to day 62. Overall, 18 batches were

performed within 62 days. From day 26 to day 43 the viable cell density increased to $3.4 \times 10^6 \text{ cells ml}^{-1}$ followed by a plateau phase with an average viable cell density of $2.6 \times 10^6 \text{ cells ml}^{-1}$ and maximal viable cell density of $3.7 \times 10^6 \text{ cells ml}^{-1}$ at day 56.

E-selectin-IgG product concentration continuously rose within each batch. In the period from day 26 to day 44 the top concentrations nearly paralleled the viable cell concentrations. The maximal product concentration of $3660 \mu\text{g l}^{-1}$ was obtained at day 59. Overall, 25.4 mg E-selectin-IgG were produced within 62 days. At the end of the last batch 2.7×10^8 recombinant CHO cells were transferred into the 2 l perfusion bioreactor.

Growth of CHO cells and production of E-selectin-IgG in a 2 l perfusion bioreactor

The production of large quantities of E-selectin-IgG was performed by up-scaling of the reactor volume to 2 l and by retention of the grown cells through a spin filter. The continuous working perfusion bioreactor was inoculated with a viable cell density of $1.3 \times 10^5 \text{ cells ml}^{-1}$ (Figure 2).

For the first 5 days the culture was kept in batch mode. Then perfusion was started at a cell density of $6.8 \times 10^5 \text{ cells ml}^{-1}$ with a dilution rate of 0.5 reactor volumes per day. At day 9 the dilution rate was raised to 1.0 reactor volumes per day to keep the glucose concentration at a level of 5 mM (data not shown). The retention rate caused by the rotating spin filter (120 rpm) was determined to be 99% as the concentration in the effluent medium was $2.8 \times 10^4 \text{ cells ml}^{-1}$ at reactor cell concentrations of $2.8 \times 10^6 \text{ ml}^{-1}$. At day 18 1.3×10^{10} cells were transferred into the 100 l bioreactor for further up-scaling of the process. For this reason the viable cell density decreased from $8.1 \times 10^6 \text{ cells ml}^{-1}$ to $2.5 \times 10^6 \text{ cells ml}^{-1}$. After the cell harvesting step the CHO cells grew to a maximal concentration of $1.65 \times 10^7 \text{ cells ml}^{-1}$ at day 28. To prevent any limitation of nutrients in the culture medium the dilution rate was set to 1.5 reactor volumes per day at day 27. The viability was stable at 85.7% (sd 3.2) throughout the whole process except for the first three days after inoculation (54.2%, sd 10.2). Neither limitation of glucose nor limitation of any amino acid occurred during cultivation (data not shown). But the viability was approximately 10% lower than the value of the Super-Spinner culture. This was possibly caused by higher shear forces through stirring with 120 rpm compared to 40 rpm for the

Figure 1. Growth of recombinant CHO cells and E-selectin-IgG product concentration in a 1 l Super-Spinner flask.

Figure 2. Growth of recombinant CHO cells and E-selectin-IgG product concentration in a 2 l bioreactor equipped with a rotating spin filter.

Super-Spinner. At day 29 the cell density stabilized but rapid blocking of the spin filter occurred so that the process was stopped. The E-selectin-IgG product concentration continuously increased to a maximal value of $1810 \mu\text{g l}^{-1}$ at day 27 and then lowered caused by the higher dilution rate. In the last 3 days the culture produced $3.6 \text{ mg E-selectin-IgG per day}$, whereas the Super-Spinner culture produced maximal $1 \text{ mg E-selectin-IgG per day}$ at the end of the cultivation. Using continuous perfusion mode in total an amount of 35.2 mg was produced in the 2 l bioreactor within 29 days.

Growth of CHO cells and production of E-selectin-IgG in a 100 l bioreactor

The next strategy of up-scaling the process was to raise the bioreactor volume to 100 l . A repeated batch mode process was chosen for production in the large vessel. Overall, three batches were performed in the 100 l bioreactor (Figure 3). The first one was started with a volume of 35 l and an inoculation density of $3.4 \times 10^5 \text{ ml}^{-1}$. When the cell concentration reached a value of $5.2 \times 10^5 \text{ cells ml}^{-1}$ after three days of cultivation the second batch was initiated as a fed batch by filling up the volume to 107 l . The CHO cells grew to a density of $9.3 \times 10^5 \text{ viable cells ml}^{-1}$ at day nine. Although the 100 l bioreactor culture was cultivated with bubble-free aeration in batch mode as the Super-Spinner culture, the maximal cell density was approximately three times lower. Maybe the shear forces in the big tank had a growth limiting effect, because the 100 l bioreactor was equipped with a steel impeller which generated a greater convective flow than the tumbling membrane wrapped stirrer in the Super-Spinner. On the other hand the maximal cell density of the Super-Spinner culture did not exceed $1.4 \times 10^6 \text{ cells ml}^{-1}$ within the first 15 days of cultivation. The maximal cell densities in the Super-Spinner were enabled because the CHO cells tend to form spheroids which were sedimented at each medium replacement. For this reason the cell density peaked at a value of $3.7 \times 10^6 \text{ cells ml}^{-1}$ in the small system. If the culture in the 100 l bioreactor was kept for a longer period, maybe higher cell densities were able due to spheroid formation of the cells. However, as the viable cell number declined at day ten, 77 l cell culture broth were harvested at a E-selectin-IgG peak concentration of $650 \mu\text{g l}^{-1}$. With adding 72 l fresh medium the third batch was started. Within five days a viable cell density of $9.7 \times 10^5 \text{ cells ml}^{-1}$ and a total cell density of

Figure 5. Specific productivity of the different bioreactor cultures is given in mass E-selectin-IgG per 10^6 recombinant CHO cells and process time for the 1 l Super-Spinner, the 2 l perfusion bioreactor, and the 100 l bioreactor.

$1.25 \times 10^6 \text{ cells ml}^{-1}$ was gained. The average viability was 83.9% (sd 7.7) for the whole process period. At day 15 the third batch was harvested containing an E-selectin-IgG concentration of $560 \mu\text{g l}^{-1}$. Although the maximal product concentration compared to the Super-Spinner and the 2 l perfusion bioreactor cultures was 3–5 times lower in total $105.6 \text{ mg E-selectin-IgG}$ were produced within 15 days.

Comparison of the productivity of the three bioreactor systems

An optimal process delivers maximal product yield in minimal time and bioreactor volume. The accumulated product yield of the three processes is shown in Figure 4.

The production of $25 \text{ mg E-selectin-IgG}$ needs 60 days with the Super-Spinner system, 26 days with the 2 l perfusion bioreactor, and 7 days with the 100 l bioreactor. Therefore the biggest vessel seems to be superior concerning the time aspect. But this is qualified by the period of additional 18 days for the generation of a cell mass for inoculation of the 100 l bioreactor. In fact for the production of $25 \text{ mg E-selectin-IgG}$ the 2 l perfusion bioreactor is on a par with the big vessel, because in both cases 25 days are necessary to generate the quantity of the desired protein. For production of larger amounts the 100 l bioreactor is most suitable because the process time of the perfusion bioreactor is limited until blocking of the spin filter occurs.

Although the specific productivity was highest in the Super-Spinner system ($243 \text{ ng}/(1 \times 10^{-6} \text{ cells d})$, sd 117) (Figure 5) the bioreactor volume is limiting the product yield. Additionally with every started batch

Figure 3. Growth of recombinant CHO cells and E-selectin-IgG product concentration in a 100 l bioreactor.

Figure 4. Accumulated E-selectin-IgG product of the 1 l Super-Spinner, 2 l perfusion bioreactor, and 100 l bioreactor cultures.

Figure 6. Productivity of the different bioreactor cultures is given in mass E-selectin-IgG per reactor volume and process time for the 1 l Super-Spinner, the 2 l perfusion bioreactor, the 100 l bioreactor, and the whole expanding process consisting of 18 days cultivation in the 2 l perfusion system and 15 days in the 100 l bioreactor.

cells were taken out of the Super-Spinner due to the product harvesting. The specific productivity of the 2 l perfusion bioreactor culture was $135 \text{ ng}/(1 \times 10^{-6} \text{ cells d})$ (sd 42). This means that the specific productivity is almost half as high as in the Super-Spinner, but the maximal cell concentration was five times higher which is vital.

The specific productivity of the 100 l bioreactor culture was $123 \text{ ng}/(1 \times 10^{-6} \text{ cells d})$ (sd 64). In fact the pO_2 - and pH-controlled cultures of the 2 l perfusion bioreactor and the 100 l bioreactor had lower specific productivities. Maybe the declining pO_2 - and pH-values during the several batches in the Super-Spinner system forced the cells to synthesize more E-selectin-IgG product per cell. The lower specific productivities of the two bigger vessels could also be caused by a slowly decreasing specific productivity of the CHO cells from the Super-Spinner culture over the cultivation period, because both systems were inoculated with cells which had been cultivated in the Super-Spinner for more than 60 days. But a slowly decreasing specific productivity did not occur since production rates at the beginning and at the end were the same.

The averaged productivities of the three bioreactor cultures for the whole processes are shown in Figure 6. Opposite to the repeated batch processes the perfusion process had the lowest productivity per volume and time ($24.6 \text{ µg/l} \cdot \text{d}$). After all, the Super-Spinner yields $30.1 \text{ µg/l} \cdot \text{d}$. The highest productivity was ascertained as $39.3 \text{ µg/l} \cdot \text{d}$ for the 100 l bioreactor. Therefore the 100 l bioreactor alone was in the long run the best system. But to generate the inoculation

cell mass for the 100 l bioreactor it took 18 days in the 2 l perfusion system. For this reason the total production period was 33 days long (18 + 15 days). In the first 18 days the 2 l perfusion culture produced 8.8 mg E-selectin-IgG while consuming 24 l of medium. In total the 100 l bioreactor culture yielded effectively only $17.1 \text{ µg/l} \cdot \text{d}$. The overall medium consumption was 203 l (24 + 179 l) compared to 13.6 l for the Super-Spinner and 49.3 l for the 2 l perfusion culture.

This means that three 2 l perfusion bioreactors running in parallel would produce an amount of 105.6 mg E-selectin-IgG which is exactly the quantity that was generated in the 100 l vessel alone. But regarding the whole process, the combination of 2 l perfusion and 100 l batch cultivation yielded 114.4 mg product within 33 days. Thus three perfusion bioreactors running in parallel could produce nearly the same amount in 29 days. Because each of these types of bioreactors needs 49.3 l of medium for the process the overall medium consumption would be 147.9 l. Compared to the big process 55.1 l medium could be saved. This means that three 2 l perfusion systems could be a real alternative to a 100 l bioreactor.

Purification of the E-selectin-IgG

The product had to be purified from the culture broth for further functional analysis. The cells were removed from the supernatant by continuous centrifugation with 3543 g in a Biofuge 17RS (Heraeus Sepatech) and depth filtration through a Sartopure PP2 3 µm filter (Sartorius). The E-selectin-IgG chimera was concentrated using a S10Y10 10 kDa ultrafiltration cartridge (Amicon). Purification was performed with a 40 ml protein A-sepharose column (Amersham Pharmacia Biotech) by FPLC and subsequent superdex 200 gelfiltration. For elution 0.1 M glycine/HCl pH 2.5 + 10% glycerol was exerted. Protein integrity was tested with SDS-PAGE (Figure 7). Gradient gels (8–25%, gel buffer: 0.112 M acetate, 0.112 M Tris, pH 6.4) with a separation range of 6–300 kDa and SDS-bufferstrips (3% agarose, 0.2 M Tricine, 0.2 M Tris, 0.55% SDS, pH 8.1) were used. The samples were diluted in non-reducing sample buffer (0.063 M Tris/HCl, 2% SDS, 0.1% Bromphenol blue, pH 6.8) and 1 µl sample/sample well was applied respectively. The low molecular weight standard (LMW) was used as reference (all Amersham Pharmacia Biotech).

The purification steps are exemplarily shown for the 100 l bioreactor harvest. The supernatant contained mostly BSA (67 kDa) (see Figure 7, lane 2).

Figure 7. SDS gelectrophoresis of E-selectin-IgG fractions. 1 + 8: LMW-protein-standard (Amersham Pharmacia Biotech, Uppsala, Sweden); 2: supernatant of the 100 l bioreactor harvest; 3: ultrafiltration concentrate of the 100 l harvest; 4: protein A elution of the ultrafiltration concentrate; 5: superdex 200 gelfiltration fraction 9 of the protein A elution; 6: superdex 200 gelfiltration fraction 21 of the protein A elution; 7: superdex 200 gelfiltration fraction 32 of the protein A elution.

Figure 8. Blocking of the adhesion of HL-60 cells to E-selectin transfected CHO cells (CHO-E) with soluble E-selectin-IgG in concentrations of 4.7 to 150 $\mu\text{g ml}^{-1}$. Data corresponds to mean values from 4 parallels in 3 independent series.

Figure 9. Blocking of the adhesion of HL-60 cells to human umbilical vein endothelial cells (HUVEC) with soluble E-selectin-IgG in concentrations of 4.7 to 150 $\mu\text{g ml}^{-1}$. Data corresponds to mean values from 4 parallels in 3 independent series.

After ultrafiltration many low concentrated proteins were detectable (Figure 7, lane 3). After purification with a protein A-sepharose column predominantly E-selectin-IgG chimera was eluted. However this fraction still contained a portion of BSA (Figure 7, lane 4). A subsequent superdex 200 gelfiltration removed the BSA (Figure 7, lanes 5–7) and pure E-selectin-IgG chimera was gained.

Functional activity of the E-selectin-IgG chimera

The functional activity of the produced and purified E-selectin-IgG protein from the three bioreactor harvests was examined using a cellular adhesion assay. The protein was applied for blocking the binding of leukocytes, especially granulocytes, to endothelial cells which naturally occurs during the process of inflammation. The adhesion is mediated by E-selectin. In this competitive assay E-selectin-IgG-chimeric pro-

tein ($300 \mu\text{g ml}^{-1}$) was diluted with medium in a microtiter plate in 1:2 series in $100 \mu\text{l}$ volumes. HL-60 cells were used instead of primary granulocytes and E-selectin transfected CHO cells (CHO-E) and human umbilical vein endothelial cells (HUVEC), respectively, were used instead of primary microvascular endothelial cells. 2.7×10^5 HL-60 cells ($100 \mu\text{l}$ volumes) were added per well and incubated for 1 h at 37°C and 5% CO_2 . 1.35×10^5 cells ($100 \mu\text{l}$ cell suspensions) were then transferred to the HUVEC and CHO-E cell layers (adhesion assay plate). After centrifugation, unbound HL-60 cells were removed with a microplate washer and the adhered cells were counted.

Without blocking an amount of 4312 (sd 317) HL-60 cells per mm^2 bound to the CHO-E cells (Figure 8). A concentration of $150 \mu\text{g ml}^{-1}$ led to 234 (sd 169) HL-60 cells per mm^2 and therefore almost completely abolished the binding capacity. No significant differences between the blocking capacity of the three bioreactor supernatants were observed at identical concentrations. As the binding of HL-60 cells to CHO-E cells is only mediated through E-selectin, the functional activity of the soluble E-selectin-IgG was demonstrated.

Furthermore the functional activity was tested with HUVEC layers (Figure 9). HUVEC do not express E-selectin constitutively. The expression has to be induced by inducers like IL-1 β or TNF- α . After induction of E-selectin expression with 50 ng ml^{-1} IL-1 β for 4 h HL-60 cells were transferred to HUVEC layers. Without blocking 1379 (sd 358) HL-60 cells per mm^2 bound to the HUVEC layers. The lower binding capacity in comparison to the CHO-E layers is possibly caused by a lower expression level of E-selectin. At a concentration of $75 \mu\text{g ml}^{-1}$ E-selectin-IgG 510 (sd 23) HL-60 cells adhered per mm^2 . The appliance of higher concentrations did not significantly improve the blocking capability (487 cells per mm^2 , sd 23). HL-60 cells adhere to endothelial cells additionally through integrin interactions. So the binding can not be blocked completely by soluble E-selectin-IgG. But the blocking capacity was demonstrated as saturation occurred. On that account functional activity of the produced E-selectin-IgG was proven.

Conclusion

Soluble E-selectin is a desired protein due to its medical potency for inflammation and cancer research. The production of large quantities of soluble E-

selectin was demonstrated using three different bioreactors (1 l Super-Spinner, 2 l perfusion bioreactor, and 100 l bioreactor) and two process strategies (repeated batch and perfusion mode). All methods presented in this study have shown to be suitable for producing and purifying recombinant functional E-selectin-IgG protein. The strategy of choice depends on the existing equipment and on the desired product quantity. For the production of 25 mg E-selectin the Super-Spinner system needs 60 days. The 2 l perfusion bioreactor needs less than half of this time (26 days) and is the best choice if a quantity up to 25 mg is desired. More than 25 mg should be produced in a large vessel like the 100 l bioreactor, because the bigger volume is a greater gain than the high cell concentrations caused through cell retention and process time of the perfusion time is limited until blocking of the spin filter occurs.

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