Endogenous retrovirus particles and their repercussion effects on the growth behaviour of continuous hybridoma cultivation processes

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Abstract

Hybridoma cells used for the production of monoclonal antibodies are also known to form growth inhibitory substances. Growth inhibitors already described in the literature belong to the class of peptides and proteins like TGF- β (Transforming Growth Factor- β). The endogenous retrovirus particles – a further potential substance producing this kind of effect – are described here. To examine whether the retrovirus particles participated in growth inhibitory effects hybridoma cells were cultivated in continuous perfusion mode by using a special reactor set-up. A rapid increase of the signal in the supernatant which coincided with a decrease of viability could be observed by monitoring the reverse transcriptase-activity during this type of fermentation process. The examination of concentrated and fractionated supernatant from this period showed growth inhibitory effects in the biological assay (MTT-assay). Investigations of respective fractions demonstrated retrovirus particles with reverse transcriptase-activity. Based on RT-PCR data it was shown that only inhibitory fractions contain retrovirus particles which were of E-MuLV and MCF origin.

Introduction

In the last few years the use of hybridoma cell lines for the production of therapeutic and diagnostic monoclonal antibodies has become very important. By using these cell lines in perfusion fermentation processes, typical cell densities of about 1×10^7 cells ml^{-1} can be reached. During this type of fermentation procedure a decrease of the viability can often be observed. Plausible reasons for this effect are either the consumption of essential medium components or the production of inhibitory substances. In an earlier study (Hawerkamp et al. 1998) we could identify a potential growth inhibitory substance with an approximate molecular weight of > 600 kDa. This complex could be identified as of retrovirus origin and it seems that it participates in the growth inhibitory effect.

Mouse retroviruses occur naturally in mice as well as in certain hybridoma cell lines of mouse origin.

They are released as exogenous, infectious viruses from these cell lines, but are mostly found endogenously, residing in the cellular genome in a silent, but inducible state (Heinemeyer et al. 1997). Since endogenous virus particles may potentially contaminate the products, it is important to characterize and quantitate the expression of endogenous retroviruses in these cell lines used for the production of recombinant-DNA proteins and monoclonal antibodies. The virus testing of monoclonal antibody products is subject to the compliance of the current “Good Manufacturing Practice” regulations (FDA). Their main guidelines describe the necessary virus tests and the stage at which each one should be performed. These documents, the “Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals” for example, describe the testing needed to fully characterize a cell line in detail (Joner and Christiansen 1988). The general concept of GMP is to guarantee

the uniformity, safety, efficiency and high quality of the product. But as far as we know there is no information about possible growth behaviour effects (trigger of apoptosis) of endogenous retrovirus particles on these hybridoma cell lines.

In most cases the murine hybridoma cell lines contain retrovirus-like particles (Deo et al. 1994; Weiss 1982a, 1982b; Bartal et al. 1986) like intracisternal A-type particles (IAP's; Perk and Dahlberg (1974), Wivel and Smith (1971)) and infectious C-type murine leukemia viruses (MuLV). The C-type particles can be either ecotropic (infectious for cell lines of murine origin or closely related species) or xenotropic (infectious for species other than murine (Froud et al. 1997) and they are derived from MuLV-related sequences inserted into the mouse genome. Sequences belonging to the ecotropic and xenotropic group can produce replication-competent viral particles. The examination of these particles is very difficult as the only certain way to identify them is by the process of budding from their plasma membrane (transmission electron micrographs) or by biochemical assays (PCR and reverse transcriptase assay). The outstanding feature of the intracisternal A-type particles is their budding into the cisternae from the membranes of the endoplasmic reticulum (Dalton 1962). They have two concentric shells of approximately equal thickness with an outer diameter of 85–90 nm. Johnson et al. (1997) examined the relationship between cellular metabolism and endogenous retrovirus production. Their results indicate a strong positive relationship between IAP production and cell growth. Mietz et al. (1987) on the other hand describe that IAP elements and their protein products can have important physiological effects. Thus IAP proviral elements can act as endogenous mutagens by transposition to new locations in the genome. Furthermore, Froud et al. (1997) showed that the level of murine C-type retrovirus particles during batch fermentation followed the viable cell density. It appeared that maximum titres of virus particles were found in the exponential growth phase of the cells at optimal nutrient supply. The decrease of nutrient content caused a decrease of virus titres.

Here we investigated the implication of retrovirus-like particles to the hybridoma cell growth behaviour in a continuous perfusion fermentation process. Therefore, we used a special reactor set-up (Hawerkamp et al. 1998) with total cell retention which guaranteed a sufficient nutrient supply and the removal of waste products. The existence of retrovirus

particles in growth inhibition supernatant was shown by reverse transcriptase-activity (RTA) determination, RT-PCR and transmission electron microscopy.

Materials and methods

Cell line and medium

A rat-mouse hybridoma (187.1 (HB58), ATCC) cell line was cultured in a serum free medium (Jäger et al. 1988). The medium consisted of a Dulbecco's modified Eagle's medium (DMEM)/ Ham's F12 mix (1:1) supplemented with human transferrin (5 mg l⁻¹) and bovine insulin (5 mg l⁻¹). The amino acids were supplemented to the concentrations necessary.

Cell cultivation

The cells were cultivated in continuous perfusion mode in a 2 litre reactor, Biostat BF2 (B. Braun Biotech International) with total cell retention. The reactor was equipped with bubble free membrane aeration and internal perfusion membrane (Lehmann et al. 1988). The perfusion membrane enabled medium feeding and harvesting of the cell free supernatant. Furthermore, the bioreactor was equipped with separately cooled medium reservoirs for basal medium and glutamine. To avoid high ammonia levels glutamine (100 mmol l⁻¹) was added according to its consumption by the cells and kept on a concentration above 250 µmol l⁻¹ ($K_s = 30\text{--}70\text{ }\mu\text{mol l}^{-1}$; (Heidemann et al. 1998).

The perfusion fermentation process started at a dilution rate of $D=1.75\text{ d}^{-1}$ after inoculation. When a viable cell density of $1\times 10^6\text{ cells ml}^{-1}$ was reached (24–48 h after inoculation) the dilution rate was reduced to $D=0.26\text{ d}^{-1}$ and the pulsed glutamine feed was started to guarantee glutamine was available. Over a 24 h period cell free supernatant was collected and harvested (500 ml per day) for further purification and examinations of retrovirus particles. For reverse transcriptase-activity analysis 10 ml of cell containing sample were taken once a day, centrifuged and the supernatant was used for the activity determination.

Analysis of the amino acids, glucose, lactate and ammonium

Throughout the complete perfusion fermentation the optimal nutrient supply was maintained by daily

monitoring of substrates and the concentration of metabolic products. The intra- and extracellular amino acid concentrations were measured by using an automated reversed phase HPLC system DS 450 (Kontron GmbH) with precolumn derivatization by the OPA method (Büntemeyer et al. 1991) to determine all of the amino acids except for proline, cysteine and the dipeptide cystine. Glucose and lactate concentrations were measured with an automatic glucose and lactate analyzer (Yellow Springs Instruments). To monitor the ammonium concentration during the fermentation process, dissolved ammonium was totally converted into ammonia with 10 M NaOH. The leaking ammonia was then measured with an ammonia-sensitive sensor (WTW).

Monitoring the reverse transcriptase-activity

Fermentation samples

During the fermentation process 10 ml cell containing samples were taken daily. To concentrate only the retrovirus particles a two step centrifugation was done. In the first centrifugation step (3000 rpm, 10 min) whole cells and cell debris were removed. The supernatant was then used for ultracentrifugation (90000 × rpm, 60 min., 4 °C) to concentrate the virus particles.

Gelfiltration samples

Gelfiltration fractions (each with 5 ml) from the Sephacryl S-1000 column were used for ultracentrifugation (19000 × g, 30 min., 4 °C) with Sorvall OTD 50 (rotor: TH 641) to concentrate sample containing retrovirus particles. The resulting pellets were taken for the RTA assay (reverse transcriptase-activity).

Assay principle:

The pellets were incubated with the lysis buffer (lysis-buffer: Tris-HCl, EDTA, Triton X-100, Nonidet P40, DTT; 50 µl per analysing sample) for 30 min at 4 °C. The reverse transcriptase-activity (Mg^{2+} -dependent) was tested in a primary screening by primed synthesis of cDNA on exogenous synthetic templates upon addition of nucleotide substrates by using the ultrasensitive RTA reverse transcriptase assay (Retro-Tech GmbH, Germany). For the standard curve (linear range: 0.1 µU-10 mU RT) and the determination of the retrovirus like particles HIV reverse transcriptase was used. The resulting degree of coloration was measured after time periods of 0, 5, 15, 30 and 45 min. at 405 nm (reference: 492) in a micro-

plate-reader (Modul Biotek). Levels down to 0.2 pg reverse transcriptase corresponding to approximately 10.000 HIV retrovirus particles were detectable per assay. To calculate the rough number of E-MuLV and MCF particles during the fermentation process and the resulting inhibitory fractions from the gelfiltration we used the following formula. Calculation of retrovirus particles per ml:

$$\text{Particle / ml} = \frac{\text{pg(RT per } 10 \mu\text{l}^*) \times 50 \mu\text{l} / 10 \mu\text{l(lysate)} \times 6,023 \times 10^{23} (\text{molecules per mol RT})}{\text{ml(sample volume)} \times 10^{12} (\text{g RT}) \times 125,000 (\text{MW-RT}) \times 108 (\text{molec. RT per HIV-particle}^{**})}$$

* pg RT in the assay was determined from the specific activity of the HIV-reverse transcriptase and by utilization of the calibration curve and its corresponding function $y = a \times + b$, where $y = \text{OD}/405 \text{ nm}$ and $\times = \text{units or pg HIV-RT}$

** calculation based on: Jurriaans et al. (1994)

RT-PCR

Inhibitory fractions and corresponding negative controls (each 5 ml) of the Sephacryl S-1000 column were first ultracentrifuged (19000 × g, 30 min, 4 °C) with Sorvall OTD 50 (rotor: TH 641). The resulting pellets (0.5 ml) were used for RNA-Isolation with QIAamp Viral RNA kit (Qiagen GmbH). For detection of murine leukemia viruses (E-MuLV and MCF) specific primer (Life Technologies) were used (Irving et al. 1993) in the RT-PCR (PCR kit, Perkin Elmer).

Primer sequences (Irving et al. 1993):

1. E-MuLV	Forward	Primer:
CCGGATCCTGCCAGATGGAGAG-TACAACGCTCTCA		
2. E-MuLV	Reverse	Primer:
CCGAATTCGACGGTTAGATTGCTTACTG		
3. MCF	Forward	Primer:
CCGGATCCTGCAGTGGGGACAATGAC-CGATG		
4. MCF	Reverse	Primer:
CCGAATTCGAC-CGACACCCGAGTCCAGTC		

The RT-reaction with 10 µl per analysed sample containing 2 µl of 25 mM $MgCl_2$, 1 µl of 10 × PCR buffer II, 4 µl of dNTP-Mix (10 mM), 0.5 µl Rnase-Inhibitor, 0.5 µl reverse primer (10 µM), 1.5 µl of the isolated RNA and 0.5 µl MuLV reverse transcriptase (RNA PCR kit, GeneAmp, Perkin Elmer) were performed for 15 min at 42 °C, a subsequent heat

inactivation step of 10 min at 95 °C and then storage at 4 °C. The following PCR was carried out with 10 μ l of the RT reaction, 2 μ l of 25 mM MgCl_2 , 4 μ l of the 10 $\times$ PCR buffer II, 33.3 μ l of DEPC (diethylpyrocarbonat) treated water, 0.5 μ l forward primer (10 μ M) and 0.25 μ l *AmpliTaq* DNA polymerase. Amplifications were performed by an initial heating step at 85 °C for 10 min followed by 35 cycles of 95 °C for 30 s, 63 °C for 30 s and 70 °C for 120 s in a Thermocycler Progene (Thermo-Dux).

Afterwards, the amplification products (4 μ l) were separated by gel electrophoresis on 3% agarose (Sigma) and visualized in a TAE buffer containing ethidium bromide. For the determination of the amplification product a LMVIII Marker (Boehringer) was used. In case of successful amplifications a product of around 118 bp (MCF) and 629 bp (E-MuLV) in length indicated the presence of murine C-type retroviral nucleic acids.

Purification of the cell free harvests

The harvests after the decrease of viability were first concentrated (1:5) by ultrafiltration (Sartocon Micro, cut off: 50 kDa, Sartorius) and the resulting concentrate (8 ml) was separated on a Sephacryl S-1000 (separation range: (600 kDa) C 16/100 with a gel volume of 120 ml (Amersham Pharmacia Biotech). The total volume (V_t) of this column ($V_t = 140$ ml) was specified by using 2% acetone (molecular weight: 54 Da). For equilibration and separation a 20 mM phosphate with 150 mM NaCl buffer (pH 7) was used. Fractions of 5 ml were collected starting with the beginning of sample application. The procedure was performed with a BioPilot-System (Amersham Pharmacia Biotech) at 7 °C.

Biological assay: MTT-assay

To examine the possible growth inhibition effects caused by the presence of retrovirus particles, the MTT-assay was selected. This assay system determines the activity of the dehydrogenases in the mitochondria by colorimetric reaction (Mossmann 1983; Hansen et al. 1989) and therefore, it was an indirect measure of cell growth activity. An important advantage of this assay is that the measured activity is independent from the DNA-synthesis.

For the MTT-procedure the gelfiltration fractions (0.2 μ m filtered) were first diluted 1:1 (45 μ l + 45 μ l) with fresh medium and then inoculated with HB

58 cells (1×10^6 cells ml^{-1}) on microtiter plates (Nunc). 10 μ l MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma) solution (5 mg ml^{-1} in PBS) was added after a proliferation period of two days and the plate was incubated for 3 h at 37 °C in a CO_2 -incubator. Then 100 μ l of the dissolving solution (40 ml N,N-dimethylformamide, 40 ml distilled H_2O and 20 g SDS (Sigma)) were added to each well to dissolve the blue crystals formed by the living cells. The intensity of the degree of coloration was measured at 570 nm in an microplate-reader (Modul Biotek).

Transmission electron microscopy: negative-staining-procedure

To examine whether there are complete virus particles in the inhibitory fractions the negative-staining-procedure was used. Therefore we took 10 μ l of the sample on a formwar coated copper net (400 mesh). The liquid was removed and 10 μ l of a 2% watery uranylacetate solution was added. After a reaction time of 10 s the solution was removed and the net was dried. The transmission electron microscopy examinations were carried out with a Hitachi H 500 with 70 kV.

Results and discussion

Figure 1 shows the time course of a typical continuous perfusion fermentation process with total cell retention and glutamine feed. During the first 5 days the viable cell density reached a maximum of 6×10^6 cells ml^{-1} . From day 5 to day 8 the viable cell density showed a fluctuation between $4\text{--}5 \times 10^6$ cells ml^{-1} . At the beginning of the fermentation the viability was over 90%. Afterwards the viability decreased continuously from day 5 until the end of the fermentation process at day 8. Medium components such as glucose, amino acids, lactate, or ammonia were analysed daily and remained in a concentration range where no limitation or inhibition appeared. Ammonia increased to (6 mmol l^{-1} and the lactate concentration to (30 mmol l^{-1} at the end of the perfusion fermentation.

The time course of the viability described above was contrary to the observed activity of the reverse transcriptase-activity in the harvest. To examine the reverse transcriptase-activity daily taken, cell free samples of a typical fermentation process were used for the RTA-assay. A comparison of the viability and

Figure 1. Time course of a typical perfusion fermentation process in a 2 l Bioreactor with pulsed glutamine feed (100 mmol l^{-1}) from day 2 to day 8 and total cell retention. The dilution rate was $D=1.75 \text{ d}^{-1}$ at the beginning and was reduced to $D=0.26 \text{ d}^{-1}$ when a viable cell density of $1 \times 10^6 \text{ cells ml}^{-1}$ was reached.

the reverse transcriptase-activity during continuous perfusion fermentation processes is shown in Figure 2. The graphical representation shows the typical increase of reverse transcriptase-activity when the viability decreased. To aware that the measured reverse transcriptase-activity does not originate from DNA sequences of cell origin in the cell free but cell debris containing fermentation supernatant, two separate ultracentrifugation steps were performed to remove the respective DNA consecutively. This procedure guarantees that the described reverse transcriptase-activity in Figure 2 is the result of concentrated retrovirus particles in the pellet. Based on these data the retrovirus particles were calculated (Table 1) and thus the number per ml could be determined to be 1.9×10^6 particles per ml at the end of the fermentation process. A comparison of these results regarding the expression level of C-type retrovirus particles (reverse transcriptase-activity, number of particles per ml) during continuous fermentation process including optimal and guaranteed nutrient supply and on the other hand the remove of metabolic substances with growth inhibitory effect with data published in literature was unfortunately not possible, because there were no comparable data available yet.

It is known from other investigations (Hawerkamp 1999) that only the harvest after the viability decrease contains growth inhibitory substances. To analyse whether there was a potential relationship between the observed decrease of viability caused by increased retrovirus expression the existence of retrovirus like particles in the harvest was examined. To determine the presence and degree of the growth inhibitory effect, cell free harvest after the decrease of viability

Figure 2. Example for development of the reverse transcriptase-activity in the cell free harvest with decreased viability during perfusion fermentation of HB 58 cells.

was first concentrated by ultrafiltration (cut off 50 kDa) then the concentrate of 8 ml was fractionated on Sephacryl S-1000. Afterwards the resulting fractions were examined in the MTT-assay. Figure 3 shows the typical growth inhibitory effects in the range from fraction 18 (90 ml elution volume) to fraction 24 (120 ml elution volume). The greatest extent of negative growth behaviour effects was measured in fraction 22 with a relative activity of 63%. The elution range of 90–120 ml corresponded to the elution volume of blue dextran with a molecular weight of 2000 kDa. Therefore, it was assumed that the inhibitory substance had a molecular weight between 600–2000 kDa. First analysis of these fractions (Hawerkamp et al. 1998) demonstrated the existence of DNA and a protein complex with retrovirus origin. The DNA was detected in most of the gel filtration fractions and was provably not responsible for the growth inhibitory effects. Furthermore, the analysis demonstrated that the protein complex also existed in low concentrations in non-inhibitory fractions.

A further indication for the retrovirus theory can be based on the wavelength spectrum data. The comparison of inhibitory and non inhibitory fractions (data not shown) represented a shifting of the spec-

Table 1. Calculated retrovirus particle number for a representative perfusion fermentation process.

Calculation of particles per ml	
Cultivation Time [d]	Fermentation
2	88,408
3	270,522
4	69,722
5	580,089
6	1,581,300
7	1,804,411
8	1,910,389

Figure 3. Example of a representative MTT-assay with negative growth behaviour effects in the fractions 18 to 24 of the Sephacryl S-1000 column.

trum from 260 nm (RNA, DNA, RNA-containing virus particles) in the inhibitory fractions to 280 nm (proteins) in the non inhibitory fractions (Hull 1985). Therefore it was assumed that the complete retrovirus particle was predominantly responsible for the negative growth behaviour. An indication of negative growth behaviour caused by retrovirus particles was given in earlier studies (Hawerkamp et al. 1998). There it could be shown that the inhibitory effect was removable by ultracentrifugation. Furthermore, to demonstrate that whole retrovirus particles produced these effects, methods like RT-PCR and RTA (reverse transcriptase-activity) were selected for the examination of the inhibitory fractions.

To show that there were whole retrovirus particles in the inhibitory fractions the fractions were concentrated by ultracentrifugation to avoid a DNA amplification and the pellet was used for RT-PCR with MCF and E-MuLV specific primers (Irving et al. 1993). The results in Figure 4a and b document the existence of E-MuLV and MCF relating sequences and therefore indicate with the results shown in Figure 5 the appearance of complete retrovirus like particles in the inhibitory fractions only. A slight signal for the MCF amplification product (118 bp) was obtained in contrast to the strong E-MuLV signal. The course of the E-MuLV signal with 629 bp especially the intensity corresponded with the negative growth behaviour effect of the MTT-assay (Figure 3). The strongest signal could be observe in the fraction 22 which showed the most inhibitory effect. An application of the respective fractions (16–26, see Figure 3) in the reverse transcriptase-assay showed (Figure 5) that complete retrovirus particles were present, because

Figure 4. (a) RT-PCR for E-MuLV (629 bp) detection with the fractions 14 to 28 (M: marker LMVIII; lane 2 to lane 9 fractions 14 to 28) of the Sephacryl S-1000 column. (b) RT-PCR for MCF (118 bp) detection with the fractions 14 to 28 (M: marker LMVIII; lane 2 to lane 9 fractions 14 to 28) of the Sephacryl S-1000 column.

only whole retrovirus particles show reverse transcriptase-activity. The maximal activity of 0,11 mU corresponding to approximately $1,7 \times 10^5$ particles ml^{-1} was reached in the fraction 20.

Figure 5. Course of reverse transcriptase-activity and particle number in the inhibitory fractions of Sephacryl S-1000. The particle number was calculated according to the RT-activity.

A further indication for the existence of complete retrovirus particles was given by transmission electron microscopy (negative staining). Figure 6 represents fraction 20 with an illustration of double shell retrovirus particles with an outer diameter of 90 nm.

Virus particles in general, but especially retrovirus particles are also known for being unstable at high temperatures and low pH values. To inactivate retroviruses in harvests the commonly used methods are heat incubation and the short-term reduction of pH. We used these methods to examine whether there is still a growth inhibitory effect visible in the MTT-assay after incubation of growth inhibitory fractions at different temperatures and a reduction of the pH at 3 for 2 h. Figure 7 shows the results after heat incubation of the non inhibitory fraction 30 and the inhibitory fraction 20 with temperatures from 50 °C to 80 °C for each 15 min. A drastic increase of growth activity from 72% to 102% can be reached after an incubation at 80 °C for 15 min. Figure 8 shows the growth activities of HB 58 cells in the fraction 18 and 20 both with growth inhibitory effects, fraction 28 as control (no growth inhibitory effect) and a harvest concentrate after ultrafiltration. All samples were incubated with hydrochloric acid for 2 h on ice (pH 3) followed by a retitration with NaOH to pH 7.2. The growth inhibitory fractions 18 and 20 showed a metabolic activity of 79% (fraction 18), and 61% (fraction 20), respectively before incubation and 111% and 102% after the incubation. The pH change had no effect on the growth behaviour of the HB 58 cells in fraction 28. A strong increase of metabolic

Figure 6. Illustration of a double shell retrovirus particle from a growth inhibitory fraction. The scale bar represent 100 μm (m: membrane of v: virus particle).

Figure 7. Results of the thermostability test. Activities of the inhibitory fraction 20 and the non inhibitory fraction 30 after an incubation with 40, 50, 60, 70 and 80 °C for 15 min. Control: activity of fraction 20 and 30 before incubation.

activity was also observed for the concentrate of the ultrafiltration, from 58% before to 96% after incubation. Therefore, both (Figures 7 and 8) indicate that the growth inhibitory effect could be abolished by short-term heat incubation and reduction of the pH.

In conclusion, these results supported a relationship between retrovirus expression, the occurrence of retrovirus particles in the supernatant and the growth inhibition effects in continuous hybridoma cell cultures. The optimal nutrient supply and the removal of waste products led to the best possible cell growth and retrovirus expression. It seems that the retrovirus particle production affected the cell metabolism which caused a decrease of viability. As a result of the decreased viability the retrovirus titre and therefore the reverse transcriptase-activity in the supernatant increased rapidly. Furthermore, the investigations

Figure 8. Activity [%] of HB 58 cells in the inhibitory fractions 18 and 20 (of the Sephacryl S-1000 column), as well as in the ultrafiltration concentrate before and after incubation at pH 3. Fraction 28 (without inhibitory effect) was used as reference.

Figure 9. Time course of the intracellular amino acid concentrations [mmol/10⁶ cells] during the perfusion fermentation process described in Figure 1 (AA-concentration: Amino Acid concentration).

demonstrated an occurrence of growth inhibitory substances only after a decrease of viability. The purification and fractionation of the supernatant (harvested after decrease of viability) showed retrovirus particles in the inhibitory fractions. MCF, E-MuLV and intracisternal A-type particle were determined. Moreover, the growth inhibitory fractions showed a reverse transcriptase-activity.

Ecotropic murine leukemia retroviruses are known for their ability to infect only murine and rat cells. This specificity is caused by the membrane receptor,

which contains 622 amino acids, divided into 14 membrane domains. Detailed examinations of the receptor could show that the amino acid tyrosine at position 235 in an extracellular loop is crucial for the specificity of this retrovirus for a certain host cell type (Wang et al. 1994, 1992; Yoshimoto et al. 1993). This region is hypervariable and a change of only one amino acid causes an expanded retrovirus specificity. This receptor has also a function as a transport system for basic amino acids (CAT: cationic amino acid transporter). An attachment of virus particles or en-

velope membrane proteins of the virus particles result in a reduced transport of amino acids like arginine, lysine, histidine or ornithine in the cells (Wang et al. 1992; Kavanaugh et al. 1994). Wang et al (1992) and Kavanaugh et al. (1994) therefore assumed that intracellular amino acid limitations by reduced transport activity of the receptor probably caused a decrease of cell viability. During the examination of the intracellular amino acid concentrations in the course of the perfusion fermentation process it could be shown that within the first 5 days a decrease in amino acid concentration, especially of the basic amino acids, could be observed (Figure 9). The concentration of arginine showed a constant decrease during the first 5 days, whereas histidine showed a drastic increase from day 2 to day 3 and a decrease from day 4 to day 5. To get a full insight in the condition of the cells a monitoring of both, extracellular and intracellular amino acid concentrations is advisable. We assume that the observed decrease in the viability could therefore be caused by the retrovirus particles which were responsible for a reduced basic amino acid transport into the cells.

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