Variable functions of bcl-2 in mediating bioreactor stress-induced apoptosis in hybridoma cells

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Abstract

It has been demonstrated that the cell lines used for production of biopharmaceuticals are highly susceptible to apoptosis, and that over-expression of the bcl-2 oncogene can protect cells from death. Stress associated with the deprivation of nutrients has been shown to be the main cause of apoptosis in culture. We have extended these studies by investigating the mechanism of cell death under conditions of sub-optimal pH, shear stress and hyperosmolarity, and the protective action of bcl-2 over-expression. At pH 6, there was no clear evidence of protection from cell death. However, at pH 8, the viability of the bcl-2 transfected cells was about 20% higher relative to the control cells. Cultivation of control cells in a flat bottomed bioreactor with a magnetic stirrer bar without a pivot ring resulted in exposure of the cells to a high attrition effect. As a result, cell growth was retarded and a high level of cell death by apoptosis was observed. Under the same conditions, the bcl-2 transfected cell line exhibited a nearly five fold increase in viable cell number. This finding indicates that under apoptosis-suppressed conditions, shear stress can stimulate cell growth. Batch cultivation of both control and bcl-2 transfected cells in 350 and 400 mOsm media resulted in suppression of cell growth, although the effect was most marked in the control cell line. Adaptation of control cells to 400 mOsm proved to be impossible to achieve. However, the bcl-2 transfected cells exhibited resistance to the osmotic stress resulting in long term adaptation to a high salt environment. Specific productivity of bcl-2 transfected cells grown in high osmolarity medium was 100% higher than that produced by non-adapted bcl-2 transfected cells grown in normal osmolarity medium. These results demonstrate that bcl-2 has a beneficial effect on hybridoma cultivation under a wide range of culture stresses.

Introduction

Apoptosis accounts for much of the cell death that occurs during the production of biopharmaceuticals in animal cell lines (Al-Rubeai et al., 1990; Franek and Dolnikova, 1991; Mercille and Massie, 1994; Singh et al., 1994; reviewed in: Al-Rubeai and Singh, 1998; Mastrangelo and Betenbaugh, 1998; Singh and Al-Rubeai, 1998). This cellular suicide programme has been extensively studied at the molecular and morphological level (for recent reviews see: Kroemer et al., 1998; King and Cidlowski, 1998; Thompson, 1998a

and b; Kidd, 1998), which has aided its identification and characterisation in the bioreactor environment. The importance of apoptosis stems from its active and genetically defined nature. As a result, transfection of a range of cell types with genes which block the expression of this suicide pathway has led to the generation of cell lines which are able to tolerate some of the stresses associated with large scale production and intensification. This has resulted in an improvement in protein productivity.

The first and best studied of the anti-apoptosis genes is bcl-2, which encodes a 26 KDa membrane protein (Tsujimoto et al., 1985; Vaux et al., 1988; Chen-Levy et al., 1989). Over-expression of bcl-2

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Figure 1. Schematic representation of the three bioreactor configurations used in the study.

in a range of cell types has been demonstrated to protect against numerous apoptosis-inducing insults (Yang and Korsmeyer, 1996). It has, therefore, been the primary target for studies which have attempted to block apoptosis in commercial cell cultures. For example, over-expression of bcl-2 in Burkitts Lymphoma and murine hybridoma cell lines resulted in the extension of batch culture duration by providing protection from amino acid and glucose starvation (Itoh et al., 1995; Singh et al., 1996; Simpson et al., 1997, 1998). Bcl-2 over-expression also aided cell adaptation to suspension culture and allowed rapid adaptation to serum free media. In cell lines which exhibit a negative correlation between growth rate and antibody productivity, cytostatic agents such as thymidine have been used to improve specific productivity (Fazekas, 1983; Boraston et al., 1984; Reuveny et al., 1986; Al-Rubeai et al., 1990, 1992). However, the main drawback with this strategy is the high level of apoptosis that results

from the growth arrest (Singh et al., 1994). Over-expression of bcl-2 has been demonstrated to offer a high degree of protection under these conditions (Singh et al., 1996; Simpson et al., 1997). Transient virus-based expression systems, such as those employing baculovirus and insect cells, also result in the induction of apoptosis which limits the duration and therefore the productivity of the culture. Expression of bcl-2 results in a reduction in the rate of cell death under these conditions, thus leading to an increase in the duration of the protein productive phase of the cultures (Mastrangelo et al., 1996; Alnemri et al., 1992; Mitchell-Logean and Murhammer, 1997).

Despite the successes outlined above, numerous bioreactor stress factors remain to be assessed, both in terms of the level of apoptosis induced and the influence of bcl-2 expression on survivability. Most notable amongst these is the influence of high and low pH, which can have a significant affect on hybridoma growth and productivity even when the deviation from optimal conditions is very small. Indeed, Wayt et al., (1997) found that a pH difference of 0.1 unit resulted in a 50% reduction in maximum viable cell number but a 50% improvement in antibody titre in a murine hybridoma cell line. The physical culture environment is also a critical factor, and although there is evidence to suggest that hydrodynamic stress can induce an apoptotic response (Al-Rubeai et al., 1995), the influence of bcl-2 expression under extreme levels of hydrodynamic stress has not been investigated.

In the present study, the effect of these two components of the culture environment on the cell death mechanism during hybridoma cultures was investigated, and the influence of bcl-2 over-expression on cell survival was examined. Furthermore, stressful bioreactor conditions, such as cell cultivation in hyperosmolarity medium, can result in substantial improvements in culture productivity (Oyaas et al., 1989; Ozturk and Pollson, 1991; Duncan et al., 1997; Oh et al. 1993). Clearly, however, such strategies are limited by the level of cell death which is triggered by the osmotic stress. The final objective of the present study was, therefore, to establish the effect of bcl-2 over-expression on cell adaptation and productivity under hyperosmolarity conditions.

Figure 2. Influence of sub-optimal pH on hybridoma viability. (Pef: control transfected cell line; Bcl-2: Bcl-2 transfected cell line).

Materials and methods

Cell lines and culture medium

The NS1-derived murine hybridoma used in this study, TB/C3, produces Immunoglobulin G (IgG) monoclonal antibody specific to the hapten C γ 2 domain in the Fc region of human IgG. This cell line was transfected using the bcl-2 expression vector pEF bcl2-MC1neopA and control vector pEF-MC1neopA as previously described (Simpson et al., 1997).

Cells were maintained at 37 °C in RPMI 1640 medium (Gibco, U.K.) supplemented with 5% (v/v) Fetal Calf Serum (FCS) (Sigma, U.K.). Levels of bcl-2 expression were monitored at regular intervals by immunostaining of the bcl-2 protein followed by flow cytometric analysis as previously described (Simpson et al., 1997). Cells were grown in 50 and 250 mL T-flasks, and were removed from the mid-exponential phase of the culture for use in experimental work.

Effect of pH

RPMI 1640 culture medium was adjusted to pH 6 and inoculated with control and bcl-2 cells at a cell number of around $1.5 \times 10^5 \text{ mL}^{-1}$. Samples were taken every 5 hr for evaluation of viability and viable cell number by the trypan blue exclusion method. The level of apoptosis was determined by fluorescence microscopic analysis of nuclear morphology as previously described (Simpson et al., 1997). At regular intervals, the pH was re-adjusted to the desired level by the manual addition of 1 mM HCl or NaOH as required.

Effect of hydrodynamic stress

Control and bcl-2 hybridoma cells were used to inoculate the following bioreactor configurations (Figure 1) at a cell number of around $2 \times 10^5 \text{ cells mL}^{-1}$:

System 1. An Infors Sixfor flat-bottomed bioreactor with a 300 mL working volume was used. Agitation at 100 rpm was achieved using a flat magnetic stirrer bar which lacked a pivot ring. As a result, the cells were exposed to high levels of shear due to the grinding action of the magnetic bar on the bottom of the bioreactor. The culture was surface aerated with 5% CO₂/Air and temperature was controlled at 37 °C.

System 2. A 300 mL glass spinner flask was used. Agitation was achieved by means of a magnetic bar with a pivot ring rotating at 100 rpm. Together with the convex-shaped bottom of the flask, these features prevented the grinding action observed in system 1.

System 3. An Infors Sixfor bioreactor with a 500 mL working volume was used. Agitation at 100 rpm was achieved by means of a magnetic stirrer bar with stainless steel support and shaft. The culture was surface aerated with 5% CO₂/air.

Samples were removed daily from each culture in order to determine the viability, viable cell number and the level of apoptosis as described previously (Simpson et al., 1997).

Figure 3. Viable cell numbers during batch cultures of control (a, c, and e) and bcl-2 transfected (b, d, and f) hybridoma cells in the three culture systems using 300, 350 and 400 mOsm culture medium. (Key: Diamonds: 300 mOsm; Squares: 350 mOsm; Triangles: 400 mOsm.)

Figure 4. Level of viability, apoptosis and necrosis (each as a percentage of total cells) during batch cultures in culture system 1. (Key: Diamonds: viability; Squares: apoptosis; Triangles: necrosis).

System 2

Figure 5. Level of viability, apoptosis and necrosis during batch cultures in system 2. (Key as for Figure 4.)

Influence of bcl-2 expression on adaptation to hyperosmolarity culture conditions

Batch cultivation in high osmolarity medium

Cells were inoculated at a cell number of around $2 \times 10^5 \text{ mL}^{-1}$ in reactor systems 1, 2 and 3 (described above) in medium at 300, 350 and 400 mOsm. Medium was prepared by the addition of a 0.1 g mL^{-1} sodium chloride solution to RPMI medium supplemented with 5% FCS. The cultures were sampled daily for the determination of viable cell number, viability and the level of apoptosis and necrosis.

Experiments on the effect of hydrodynamic forces and high osmolarity were repeated 3 times at different times using different inocula. The results of one typical experiment are reported.

Adaptation to high osmolarity medium

Cells were inoculated in reactor system 3 in 400 mOsm medium at a cell number of $2 \times 10^5 \text{ mL}^{-1}$. The cells were subcultured every 50 hr by replacing 50% of the cell suspension with the same volume of fresh medium.

Spin filter cultivation

An LSL Biolafitte (LSL Group, Luton, U.K.) cell culture vessel (1.5 L working capacity), equipped with a four blade Rushton turbine (diameter: 4.4 cm), was used with a $25 \mu\text{m}$ spin filter mounted on the agitator shaft. Agitation speed was 110 rpm and dissolved oxygen was maintained at 50% of air saturation by a bubble-free oxygenation system using immersed silicone tubing. The tubing was 4.8 mm in diameter with a wall thickness of 0.6 mm and a total length of 3 m. pH was controlled by using sparged CO_2 and 0.1 M NaOH.

Samples were taken daily and viable and total cell numbers were determined. Monoclonal antibody was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) using human IgG coated plates and sheep anti-mouse IgG peroxidase conjugate as the second antibody. The optical density at 490 nm was recorded by an ELISA reader after treating the plates with the peroxidase substrate, o-phenylenediamine dihydrochloride. All immunological reagents were obtained from Sigma (Poole, U.K.).

Results

Effect of sub-optimal pH

Figure 2 shows the effect of pH 6 and 8 on the viability of the two cell lines. Clearly, acidic pH values had the greatest affect on culture viability, with approximately 90% of cells having died within 40 hr. Generally, during the course of the culture, there was little difference in viability between the control and bcl-2 cell line. At pH 8, over the same time period, the viability had only declined by 20% and 40% in the bcl-2 and control cultures respectively, giving a clear indication of the activity of bcl-2 at this pH. Repeated experiments with different inocula confirmed this trend at both pH values and demonstrated the marginal protective effect of bcl-2 in an alkaline environment.

Effect of hydrodynamic stress and hyperosmolarity medium

When control cells were cultured in reactor System 1 using normal (300 mOsm) medium, there was an initial lag period of around 50 hr after which time the cell number increased from around $2 \times 10^5 \text{ mL}^{-1}$ to $6 \times 10^5 \text{ mL}^{-1}$ at 96 hr (Figure 3a). Fluorescence microscopic analysis of the dead cells indicated that over 10% of cells were apoptotic after only 24 hr, and this figure increased steadily such that after 168 hr about 96% of cells were apoptotic (Figure 4a). Cells over-expressing bcl-2 also exhibited a 50 hr lag phase after which cell number increased steadily, reaching a peak of $22.5 \times 10^5 \text{ mL}^{-1}$ after 120 hr (Figure 3b). The level of apoptosis remained well below 10% for in excess of 100 hr, clearly demonstrating the protective effect of bcl-2 (Figure 4b). When significant levels of cell death did occur, apoptosis was predominant. However, even by the end of the experiment, when no viable cells could be detected by means of trypan blue assay, analysis of nuclear morphology by fluorescence microscopy revealed that viability was still around 50% which, as discussed below, indicates the limitations of trypan blue staining.

The cultures described above were repeated in medium at 350 and 400 mOsm in order to compare batch growth under hyperosmolarity conditions. The control cell lines exhibited suppressed growth due to the extreme hydrodynamic conditions outlined above at all three osmolarity levels (Figure 3a). Fluorescence microscopic analysis revealed apoptosis as the major form of cell death in all cases, with a similar rate of fall at each osmolarity level (Figure 4a, c,

e). However, bcl-2 transfected cells exhibited significantly higher viable cell number and culture duration, although there was clear evidence that high osmolarity had suppressed cell growth relative to normal culture conditions (Figure 3b). Fluorescence microscopy indicated that the level of apoptosis remained low during the growth phase of the culture. However, once peak cell density had been attained, cell death by apoptosis ensued (Figure 4b, d and f). As in other experiments with the bcl-2 transfected cells in system 1, there was a slight disagreement in the viability data produced by fluorescence microscopy and trypan blue analysis.

The hydrodynamic environment of culture system 2 proved to be much less stressful to the control cell line, which reached a maximum cell number in excess of $15 \times 10^5 \text{ cells mL}^{-1}$ in 300 mOsm medium (Figure 3c). A similar maximum cell number was achieved by the bcl-2 transfected cell line. However, as expected, the death phase of the bcl-2 transfected cells was considerably longer with viable cell number falling to around $5 \times 10^5 \text{ mL}^{-1}$ after 200 hr (Figure 3d). By comparison, the control cell line had fallen to $5 \times 10^5 \text{ mL}^{-1}$ after only 100 hr. Substantial levels of apoptosis were seen in the bcl-2 cultures after 144 hr of cultivation (Figure 5b).

Because of the milder hydrodynamic environment in culture system 2, the effect of increased osmolarity on cell growth was clearly evident. Maximum viable cell number was suppressed by 33% and 50% at 350 and 400 mOsm respectively (Figure 3c). Notably, the high degree of growth suppression at 400 mOsm enabled the cells to exhibit a reduced rate of cell death, presumably due to the greater availability of nutrients at this stage of the culture. Maximum cell number for the bcl-2 transfected cell line was 50% and 100% higher than control cells at 350 and 400 mOsm respectively. Whilst a slight increase in culture duration for the bcl-2 cell line was evident in the 350 mOsm medium, no real difference could be detected at 400 mOsm (Figure 3d). In all experiments, analysis of nuclear morphology indicated that the majority of cell death occurred by apoptosis (Figure 5). However, under high osmolarity conditions the rate of cell death in the control cell line cultures appeared to be lower than that seen in the bcl-2 transfected cell line.

The hydrodynamic environment of culture system 3 was also less stressful than that of system 1, although the growth profiles were significantly different to those seen for system 2. For the control cell line at 300 mOsm, the lag phase lasted less than 24 hr which was followed by an exponential growth

phase during which cell number reached a maximum of $15.5 \times 10^5 \text{ mL}^{-1}$ after 50 hr (Figure 3e). The culture then went into death phase which lasted 120 hr. The bcl-2 cell line reached a maximum cell number of $12.5 \times 10^5 \text{ cells mL}^{-1}$ after 72 hr (Figure 3f). However, the death phase was considerably longer, with the viable cell number falling to only $2 \times 10^5 \text{ mL}^{-1}$ after around 250 hr. In comparison, the control cell line had completely lost viability by 170 hr. In both cases, cell death was predominantly by apoptosis (data not shown).

At 350 mOsm, viable cell numbers peaked at about $8 \times 10^5 \text{ mL}^{-1}$ for both control and bcl-2 cell lines, although the death phase was much longer in the bcl-2 culture (Figure 3e and f). At 400 mOsm, the control cells exhibited a highly extended lag phase, with only a slight, low rate increase in viable cell number, which peaked at around $5 \times 10^5 \text{ mL}^{-1}$. Viable cell number then declined slowly, falling to just under $2 \times 10^5 \text{ cells mL}^{-1}$ after 170 hr in culture. The bcl-2 transfected cells, by contrast, exhibited a lag phase of only 24 hr, with cell number peaking at around $6 \times 10^5 \text{ mL}^{-1}$ after 50 hr. Following a stationary phase of approximately 75 hr, the cell number rapidly decreased, with complete loss of viability after 150 hr. As in all other experiments, fluorescence microscopic analysis of nuclear morphology revealed that apoptosis was the major form of cell death in each culture (data not shown).

Adaptation to high-osmolarity conditions

The adaptation of control and bcl-2 transfected cells to 400 mOsm medium is shown in Figure 6a and b. Despite initial growth of control cells, the viable cell number gradually declined over subsequent passages, and the culture was lost after the 5th passage. Repeated attempts to adapt the cells to these conditions failed. Bcl-2 expressing cells initially showed high viability, although after subsequent passages there was an increase in the proportion of dead cells. However, in contrast to the control cell line, the culture viability improved after the fourth passage, after which the cells exhibited a growth pattern which was similar to that seen in standard culture medium.

Cell cultivation under high-osmolarity conditions in spin filter perfusion culture

Having adapted the bcl-2 transfected cells to hyperosmolarity conditions, productivity was assessed in

Table 1. Mean and standard deviation (σ) of several time-points of the specific antibody productivities during the cultivation of bcl-2 transfected hybridoma cells at 300 and 400 mOsm in spin filter perfusion cultures

	Antibody specific productivity ($\mu\text{g}, 10^6 \text{ cells hr}^{-1}$)	
	300 mOsm	400 mOsm
Mean	0.305	0.603
σ	0.356	0.227

spin filter perfusion cultures. Comparison with control cells under hyperosmolarity conditions was not possible due to their failure to adapt to a high salt environment. Instead, a comparison is made with bcl-2 cells in normal osmolarity medium. Under such conditions, non-adapted bcl-2 cells reached a maximum of $1.6 \times 10^7 \text{ cells mL}^{-1}$ after around 250 hr in culture (Figure 7a). By contrast, at 400 mOsm, adapted bcl-2 transfected cells reached a maximum of only $8 \times 10^6 \text{ cells mL}^{-1}$, with a consistently higher proportion of dead cells throughout the duration of the culture (Figure 7b). Specific antibody productivity during the course of the two cultures showed that the cells grown at 400 mOsm were approximately twice as productive as cells cultivated under standard conditions (Table 1). More importantly, variability of the specific productivity during the run was significantly lower than that obtained for the control run. The results clearly show a much higher consistence in productivity in the adapted cell line.

Discussion

Influence of bcl-2 on growth at suboptimal pH

Acidification of culture medium due to lactate production can reduce the pH to around 6.6 by the end of the exponential phase of batch culture. This has been demonstrated to be cytotoxic to the cells (Harbour et al., 1989; Macmicheal, 1989), and also adversely affects antibody productivity (Harbour et al., 1989). Addition of sodium hydroxide to control pH can result in regions of high pH, particular in large scale bioreactors due to the increased mixing times (Wayte et al., 1997). Presumably, exposure of cells to large fluctuations in pH will also invoke a stress response and consequently will affect both culture viability and

Figure 6. Viable cell number during adaptation to 400 mOsm medium for (A) control and (B) bcl-2 transfected cell lines.

Figure 7. Spin filter perfusion culture using (a) non-adapted bcl-2 transfected cells at 300 mOsm and (b) adapted bcl-2 transfected cells at 400 mOsm. (Key: Diamonds: total cell number; Squares: viable cell number; Triangles: perfusion rate.)

antibody productivity. However, as confirmed in the present study, cells can tolerate a surprisingly large increase in pH, by as much as 1.1 units above neutral, whereas even slightly acidic conditions are more detrimental to culture viability. The results presented in Figure 2 suggest that cell tolerance to acidic conditions was not significantly influenced by bcl-2 expression. By contrast, there is a clear and consistent indication that bcl-2 expression did offer significant protection at pH 8. Further studies are required to assess the level of protection a) under more extreme culture conditions (i.e. higher than pH 8) and b) under both constant and fluctuating values of pH.

bcl-2 and the hydrodynamic environment

In recent years, two studies have investigated the role of hydrodynamic stress in modulating the apoptotic pathway. Initially, Al-Rubeai et al. (1995) reported that moderate levels of hydrodynamic stress resulted

in the induction of significant levels of apoptotic cell death during murine B-cell hybridoma cultivation. However Dimmler et al. (1996) found that sub-lethal levels of shear stress resulted in suppression of apoptosis in endothelial cells. This different behaviour may indicate that the response to hydrodynamic stress is cell-type dependent and influenced by whether the cells are attached or in suspension when subjected to shear force.

In the present study, in reactor system 1, cells were exposed to a high shear environment generated by the action of the flat stirrer bar on the bottom of the glass culture vessel. In line with previous observations (Al-Rubeai et al., 1995), this resulted in the induction of high levels of apoptosis. As shown in Figure 3a, the expression of bcl-2 in the cells not only provided protection from apoptosis, but also allowed cell growth to levels not observed in standard suspension culture (reproducibility of results was obtained but not pre-

sented). Thus, it would appear that shear stress is extremely effective at stimulating cell growth, which is only evident when apoptosis is suppressed. Hydrodynamic stress has previously been reported to affect hybridoma cellular metabolic activity (Al-Rubeai et al., 1990), and a number of studies have identified genes which are inducible by shear stress in vascular tissue (JIN et al., 1996; Li et al., 1996; Hammond et al., 1997). Given the hydrodynamic environment of the B-cells from which hybridomas are derived, it is quite possible that the biochemical pathways which result in the induction of gene expression in response to physical stress are still active in hybridoma cells. This may explain the shear stress mediated stimulation of cell growth in the bcl-2 transfected cells reported in the present study.

Clearly, bcl-2 transfected cells exposed to the grinding action in system 1 will have undergone significant levels of physical damage and consequently will be extremely fragile. When cells in such a state are stained with trypan blue, they gradually become permeable to the stain. Thus, a culture which may initially have appeared to be highly viable, may exhibit a very large proportion of dead cells within a few minutes of staining. This may reflect the inability of stressed cells to tolerate the slight change in medium osmolality which accompanies the addition of trypan blue solution. Such an effect, which has also been observed in previous unreported studies with stressed cells in our laboratory, would explain the difference in viability found between trypan blue analysis and fluorescence microscopic analysis. This clearly demonstrates the limitation of trypan blue staining for the determination of viability of highly stressed cells.

The difference in the growth profiles of the bcl-2 cultures in system 2 and 3 is also very interesting, as it suggests that the level of protection offered by bcl-2 expression varies depending on the exact configuration of the culture vessel. Thus, combining better bioreactor design with the use of apoptosis resistant cell lines may prove to be synergistic, and thereby further improve culture viability. Further studies are required to establish the basis of the differences observed in the present study.

bcl-2 and hyperosmolality

Osmolality of culture media typically ranges between 260 and 320 mOsm, values that were selected to mimic those conditions found *in vivo* (Ozturk and Palsson, 1991). Cell cultivation at osmolality levels

above this range has been reported to result in cellular growth arrest and an increase in specific antibody productivity (Oyaas et al., 1989; Ozturk and Palsson, 1991; Duncan et al., 1997). However, Oh et al. (1993) have found that when cells are initially passaged in 350 mOsm medium, growth arrest was clearly evident, although the specific productivity of the cells was not higher than that seen in control cells. Following a one week adaptation to these conditions, the cells exhibited a gradual increase in growth rate in addition to increased specific antibody productivity. This would indicate that the improved productivity of hybridoma cells under such conditions may not simply be a matter of growth arrest. It has been suggested that the high concentration of Sodium ions may enhance amino acid uptake, thereby stimulating the increased antibody productivity. However, this does not explain the increase in specific productivity seen following treatment with osmolytes such as sucrose and KCl.

The use of hyperosmolality culture medium provides a simple and effective means of improving culture performance at large scale. However, when implementing such an approach, one has to consider the possibility that the resultant stress may lead to high levels of cell death. Clearly, under very high osmolality conditions, the cells will simply undergo extensive damage resulting in passive cell death by necrosis. However, there will be a range of intermediate conditions in which cells will respond to the osmotic stress by undergoing apoptosis. In theory, the suppression of apoptosis by bcl-2 over-expression should allow cell cultivation at significantly higher stress levels than would otherwise be possible, thereby further stimulating antibody production. The results presented in this study support this prediction. As shown in Figure 6a and b, after the initial passage both cell lines exhibited a fall in viability. However, only the bcl-2 cell line recovered, and by the end of the adaptation period culture viability and cell growth were at levels similar to those observed in normal (300 mOsm) medium. Importantly, the cells adapted to 400 mOsm medium exhibited a doubling in specific antibody productivity.

Conclusion

An alkaline pH of 8 proved to be less cytotoxic than an acidic pH of 6. Bcl-2 offers some degree of protection from apoptosis at the higher pH 8, an effect which is not so apparent at pH 6. High shear stress resulted in the induction of a high level of apoptosis

in the control cell line. Expression of bcl-2 provided a high degree of protection under these conditions. Moreover, in the absence of apoptosis, the high shear stress environment stimulated a very high growth rate and resulted in a maximum cell number which was not achieved under standard culture conditions. The over-expression of bcl-2 also enabled adaptation of the cells to 400 mOsm medium, which was not possible in the control cell line. The adapted cell line then exhibited a 100% improvement in specific antibody productivity compared to the non-adapted bcl-2 over-expressing cells cultured in normal (300 mOsm) medium.

Together, these results demonstrate that bcl-2 over-expression results in the suppression of cell death induced by a wide range of stress factors which are not limited to nutrient and oxygen deprivation, growth arrest and viral infection.

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