



## Prolongation of murine hybridoma cell survival in stationary batch culture by Bcl-xL expression

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### Abstract

While the ectopic expression of the anti-apoptotic protein Bcl-2 has been shown to significantly increase both cell viability and antibody production in batch culture, some cell lines are refractory to these manipulations. For example, the NS/O and the P3x63Ag8.653 murine myelomas, which express high endogenous levels of the Bcl-2 homologue Bcl-xL, are both resistant to the anti-apoptotic effect of Bcl-2. This indicates that, in these cells, Bcl-2 and Bcl-xL may be functionally redundant. In order to define the role which Bcl-xL plays in hybridoma cultures, we used the Sp2/0-Ag14 cell line. This murine hybridoma expresses low levels of Bcl-xL and is highly sensitive to apoptosis induction by cycloheximide (CHX) and by amino acid depletion. Bcl-xL-transfected Sp2/0-Ag14 cells were more resistant than the wild type and the plasmid-containing cells to apoptosis induced by CHX and by glutamine depletion. Moreover, when compared to the vector-transfected control, Bcl-xL-Sp2/0 cells exhibited a substantial increase in viability in stationary batch culture. Interestingly, Sp2/0-Ag14 cells overexpressing Bcl-xL showed a growth behaviour that was similar to the parent myeloma cell line P3x63Ag8.653. Our results suggest that Bcl-xL expression levels are sufficient to account for the relative robustness of some hybridoma cell lines in stationary batch cultures.

**Abbreviations:** ActD, actinomycin D; CHX, cycloheximide; Gln, L-glutamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; PMSF, phenyl methyl sulfonyl fluoride; SDS, sodium dodecyl sulphate.

### Introduction

Because of the high costs related to the large-scale production of mammalian cell-derived products, intense efforts have been made in the past decades to optimise cell culture conditions (Cotter and Al-Rubeai, 1995; Dickson, 1998). In recent years, a major advance in cell biotechnology was made with the realisation that most stresses prevailing in batch or perfusion culture (e.g. nutrient depletion, anoxia) did not induce passive cell death by necrosis, but triggered an active process of cell suicide called apoptosis (Franek and Dolnikova, 1991; Franek et al., 1992; Mercille and Massie, 1994; Singh et al., 1994). Cells dying

by apoptosis are characterised by a succession of specific morphological changes including loss of cellular volume, chromatin condensation, and cell fragmentation into apoptotic bodies (Nagata, 1996). Moreover, apoptotic cell death involves specific, highly regulated molecular signalling pathways that trigger a variety of biochemical changes (including protein and DNA degradation) that culminate in the execution of the death sentence (Ashkenazi and Dixit, 1998; Green and Reed, 1998).

Several attempts have been made to manipulate the cell's death machinery in order to improve cellular viability in culture and, consequently, increase protein production (Mastrangelo and Betenbaugh, 1998;

Mosser and Massie, 1994; Singh et al., 1996a). In particular, attempts were made to genetically modify mammalian cell lines by transfecting the apoptosis-inhibitory gene Bcl-2. Bcl-2 is the founding member of a family of structurally related proteins that play a crucial role in the modulation of the cell death process (Adams and Cory, 1998; Chao and Korsmeyer, 1998). These proteins are characterised by the presence of at least one of four Bcl-2 homology (BH) domains, and by their capacity to either inhibit or induce apoptosis. In addition to Bcl-2 itself, mammalian anti-apoptotic members include Bcl-xL, Mcl-1, Bcl-w and A1, most of which containing all four BH domains. The pro-apoptotic Bcl-2 family members are more numerous and include Bax, Bok, Bak (Bax subclass), and contain domains BH1, BH2 and BH3. A distinct subclass of pro-apoptotic proteins, bearing only the BH3 domain, includes Bad, Bid, Bik, Bim, Hrk, Bnip3 and Blk. Members of the Bax subclass have been shown to be able to induce apoptosis on their own and to be antagonised by the anti-apoptotic Bcl-2 proteins (Adams and Cory, 1998; Chao and Korsmeyer, 1998). On the other hand, proteins of the BH3 subcategory mainly act by regulating the activity of the anti-apoptotic Bcl-2 members.

The finding that Bcl-2 can inhibit apoptosis induced by a wide variety of insults (including ionising and non-ionising radiation, nutrient deprivation, growth factor withdrawal, chemotherapeutic drugs, some cytokines) (Adams and Cory, 1998; Chao and Korsmeyer, 1998) has prompted several groups to test the efficiency of Bcl-2 in prolonging cell viability under batch or perfusion culture conditions. The reported increases in cell viability following Bcl-2 overexpression in murine hybridoma cells were however variable, and increases in antibody production ranged from 0% to 300% (Fassnacht et al., 1998; Itoh et al., 1995; Murray et al., 1996; Singh et al., 1996b; Terada et al., 1997). This indicated that the cellular context pertaining to individual hybridoma cell lines might influence the efficiency of Bcl-2 in prolonging cell viability in culture. Interestingly, the NS/O and the P3x63Ag8.653 myelomas, both of which are refractory to the effect of ectopic Bcl-2 expression (Fujita et al., 1997; Murray et al., 1996), express elevated levels of Bcl-xL (Gauthier et al., 1996; Murray et al., 1996, this report). This raised the possibility that Bcl-xL might be sufficient to protect myeloma/hybridoma cell lines against culture-related stresses and that, in the NS/O and P3x63Ag8.653 cells, Bcl-2 and Bcl-xL would be functionally redundant. However, the ef-

fect of Bcl-xL on cell viability in myeloma/hybridoma batch cultures has not been tested yet.

In order to define the role which Bcl-xL plays in hybridoma cultures, we used the murine Sp2/0-Ag14 cell line. This hybridoma, derived from the fusion of the P3x63Ag8.653 myeloma and murine spleen cells, does not express detectable levels of Bcl-2 mRNA, and low levels of Bcl-xL mRNA are present (Gauthier et al., 1996). This cell line and hybridomas derived thereof are highly susceptible to apoptosis induced by various treatments, including amino-acid deprivation (Mercille and Massie, 1994; Perreault and Lemieux, 1993a) and the inhibition of gene expression by actinomycin D (ActD) or CHX (Perreault and Lemieux, 1993b). Interestingly, apoptotic cell death can be significantly delayed in Sp2/0-Ag14 by the ectopic expression of Bcl-xL or Bcl-2 (Gauthier et al., 1996). In contrast, the murine myeloma P3x63Ag8.653 expresses high levels of Bcl-xL, no Bcl-2, and is more resistant to apoptosis induction than Sp2/0-Ag14 (Gauthier et al., 1996). We therefore generated Sp2/0-Ag14 cells expressing Bcl-xL protein levels similar to the P3x63Ag8.653 cell line. The resulting clones were more resistant than wild type Sp2/0-Ag14 to apoptosis induced by CHX or glutamine deprivation. Most importantly, the viability of the Bcl-xL-Sp2/0 cells in stationary batch culture was significantly extended when compared to the control and was similar to that observed for the P3x63Ag8.653 cell line. Our results suggest that Bcl-xL plays an important role in the regulation of hybridoma cell survival in batch culture.

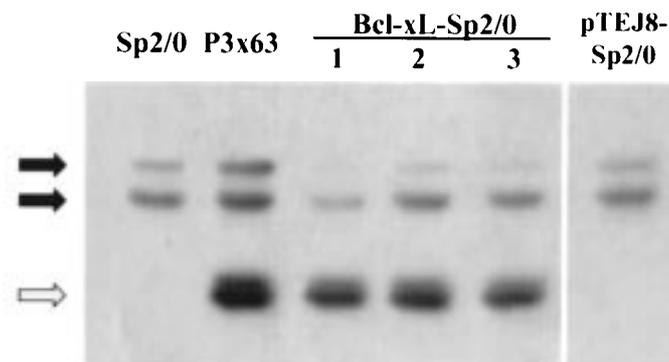
## Materials and methods

### *Reagents*

Unless otherwise stated, all reagents were obtained from Canadian Life Technologies (Burlington, ON, Canada).

### *Cell lines and cell maintenance*

The murine cell lines P3x63Ag8.653 (ATCC # CRL1580) and SP2/0-Ag14 (ATCC # CRL1581) were obtained from the American Type Culture Collection (Rockville, MD). Both cell lines were maintained in Iscove's modified Eagle's media (Media Preparation Lab, Princess Margaret Hospital, Toronto, ON, Canada), supplemented with 5% Fetalclone I (Hyclone, Logan, UT), 100 U ml<sup>-1</sup> penicillin and 100 µg



**Figure 1.** Immunoblot analysis. Bcl-xL expression was analysed in wild type Sp2/0 and P3x63Ag8.653 cells and in transfected Sp2/0 cells. Numbers indicate independently-obtained Bcl-xL-Sp2/0 clones. The open arrow designates the 26 kDa Bcl-xL protein. Black arrows indicate non-specific proteins that cross-reacted with the anti-Bcl-xL antibody.

$\text{ml}^{-1}$  streptomycin. Cell culture was performed at  $37^\circ\text{C}$  under an atmosphere of 5%  $\text{CO}_2/95\%$  air.

#### *Stationary batch culture of hybridoma cells*

Exponentially growing cells were centrifuged and resuspended in fresh culture media at a concentration of  $5 \times 10^4$  cells  $\text{ml}^{-1}$  in  $25 \text{ cm}^2$  flasks and cultured as described above. Cell viability was then determined daily using the trypan blue exclusion assay: an aliquot of cells was diluted in 0.04% trypan blue (Sigma, Oakville, ON) dissolved in PBS (9.1 mM  $\text{Na}_2\text{HPO}_4$ , 1.7 mM  $\text{NaH}_2\text{PO}_4$ , 150 mM NaCl, pH 7.4). The viable (membrane-intact) and dead cells were then counted using a Neubauer hemacytometer. Each result is the average  $\pm$  standard deviation of at least 4 determinations each totalling at least 200 cells.

#### *Glutamine deprivation assay*

Exponentially growing cells were centrifuged and resuspended in Iscove's media lacking L-glutamine (Sigma) at a concentration of  $1 \times 10^5$  cells  $\text{ml}^{-1}$  in  $25 \text{ cm}^2$  flasks. The cells were then cultured as described above, and cell viability was determined daily using the trypan blue exclusion assay. Results are expressed as the average  $\pm$  standard deviation of three independent experiments.

#### *MTT assay*

For the determination of cell viability using the MTT assay (Hansen et al., 1989),  $1 \times 10^5$  cells were incubated in a total volume of  $100 \mu\text{l}$  in 96-well plates in the presence or absence of  $25 \mu\text{g ml}^{-1}$  CHX (Sigma). Cells were then incubated for 2 h before the addition

of  $25 \mu\text{l}$  of MTT dye (Sigma;  $5 \text{ mg ml}^{-1}$  dissolved in PBS). Two hours later,  $100 \mu\text{l}$  of lysis buffer (20% SDS, 50% N-N'-dimethylformamide, pH 4.7) was added to each well and the plates were incubated overnight at  $37^\circ\text{C}$ . Optical density readings were performed at 570 nm using a PowerWaveX microplate reader (Bio-Tek Instruments, Inc). Results are expressed as the average  $\pm$  standard deviation of three independent experiments.

#### *Cell transfection*

The cloning of the mouse BclxL cDNA into pTEJ8 has been described previously (Gauthier et al., 1996). Plasmids ( $10 \mu\text{g}$ ) were linearised with Pvu I and purified by phenol/chloroform extraction and ethanol-precipitated. The linearised plasmid was then added to  $5 \times 10^5$  SP2/0-Ag14 cells resuspended in  $400 \mu\text{l}$  of cold PBS and the mixture was submitted to electroporation (180 V,  $960 \mu\text{F}$ ) using a Gene Pulser Plus unit (Bio-Rad, Mississauga, ON, Canada). After a 30 min incubation on ice,  $10 \text{ ml}$  of culture media was added and the cells were cultured in  $25 \text{ cm}^2$  flasks for 2 days. Geneticin ( $750 \mu\text{g ml}^{-1}$ ) was then added to select for transfected cells. Geneticin-resistant cells were cloned and tested for Bcl-xL expression by Western analysis (described below).

#### *Protein extract preparation and western analysis*

For the preparation of soluble protein extracts,  $2 \times 10^6$  cells were washed in PBS and resuspended in  $500 \mu\text{l}$  of RIPA buffer (1% IGEPAL [Sigma], 1% sodium deoxycholate [Sigma], 0.1% SDS, in PBS) and incubated on ice for 30 min. The sample was then sonicated for 20 sec before adding  $5 \mu\text{l}$  of  $10 \text{ mg ml}^{-1}$

PMSF (Sigma) dissolved in isopropanol, followed by a 30 min incubation on ice. Following centrifugation (12 500 rpm, 4 °C, 20 min), the supernatant was collected and its protein content determined using the DC Protein Assay kit from Bio-Rad. The extract was then stored at -80 °C until needed.

For Western analysis, equal amounts of proteins were loaded into the wells of a 12% PAGE-SDS polyacrylamide gel and resolved using a Bio-Rad Mini-Protean II electrophoresis unit. The proteins were transferred onto an Hybond-P membrane (Amersham-Pharmacia, Baie d'Urfé, QC) as instructed by the manufacturer. The membrane was then processed for Western analysis using a rabbit polyclonal Bcl-xL antibody (S-18, Santa Cruz Biotechnology Inc., Santa Cruz, CA) and a goat anti-rabbit IgG secondary antibody coupled to horseradish peroxidase (Santa Cruz Biotechnology). Detection was performed using the ECL-Plus Chemiluminescence Kit (Amersham-Pharmacia) following the manufacturer's instructions.

#### DNA laddering assay

DNA fragmentation analysis was performed using a modification of a procedure previously described by Smith et al (1989). Five hundred thousand cells were collected and washed once with PBS. The cell pellet was lysed with 50  $\mu$ l of lysis buffer (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8, 0.5% N-lauroyl sarcosine, 0.25 mg ml<sup>-1</sup> proteinase K and 0.02 mg ml<sup>-1</sup> RNase A). The mixture was incubated for 5 min at 50 °C. Fifty microliters of sample buffer (40% sucrose, 0.08% bromophenol blue) was then added to the sample. The sample was loaded into the wells of a 2% agarose gel and electrophoresis was performed using a Bio-Rad Mini Sub Cell electrophoresis unit in TBE Buffer (0.089 M Tris base, 0.089 M boric acid, 0.002 M EDTA pH 8) at 95 V for 45 min. The DNA was then stained for 5 min with ethidium bromide (0.7  $\mu$ g ml<sup>-1</sup>) before visualisation under UV illumination.

## Results

#### Generation of Bcl-xL-transfected Sp2/0-Ag14 hybridoma cells

To examine the effect of Bcl-xL on the behaviour of the Sp2/0-Ag14 hybridoma in stationary batch culture, we transfected these cells with the pTEJ8 expression vector containing the murine Bcl-xL cDNA (Gauthier

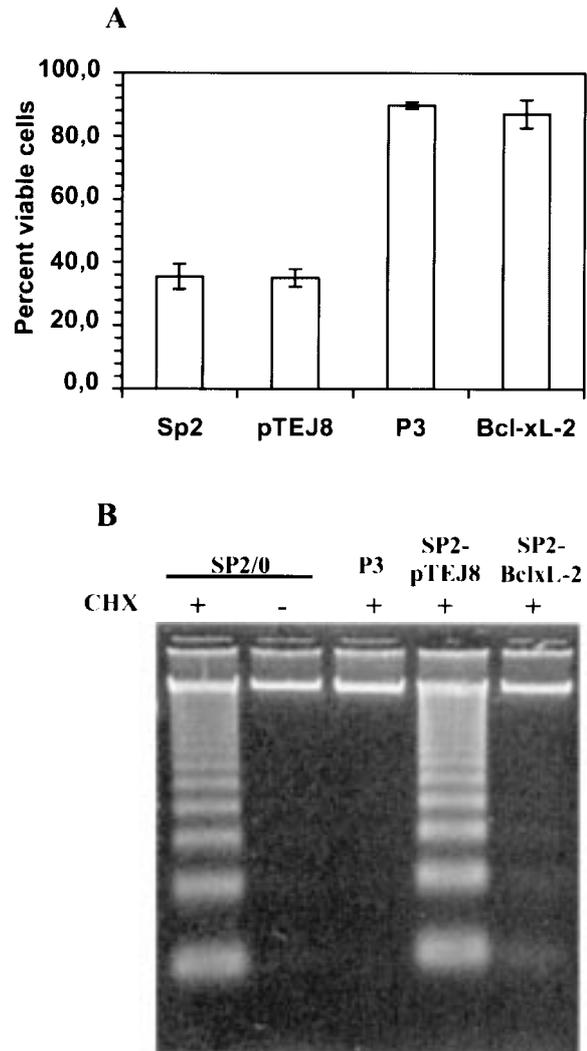
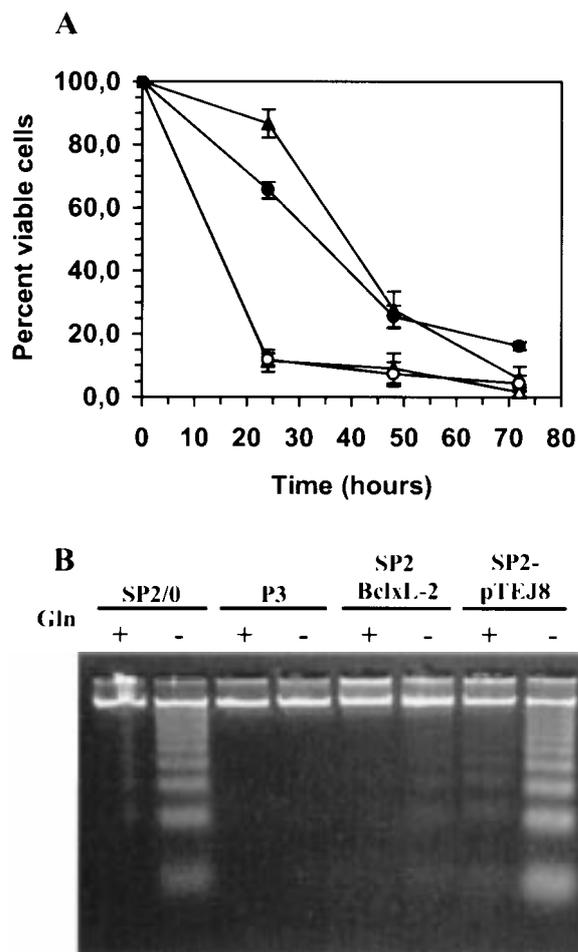


Figure 2. Effect of Bcl-xL expression on CHX-induced apoptosis. Wild type Sp2/0 and P3x63Ag8.653 (P3) cells, pTEJ8-Sp2/0 cells and Bcl-xL-Sp2/0 clone 2 (Bcl-xL-2) were incubated in the presence (+) or absence (-) of CHX for 2 h. MTT cell viability assay (A) and DNA fragmentation analysis (B) were then carried out as described in Materials and Methods. Identical results were obtained for all three Bcl-xL-transfected Sp2/0 clones.

et al., 1996). Several G-418 resistant clones were selected and examined for Bcl-xL expression by Western blot analysis. We compared the Bcl-xL protein levels in the Sp2/0-Ag14 clones to those found in the parent murine myeloma P3x63Ag8.653. Three Sp2/0-Ag14 clones were independently obtained that expressed Bcl-xL at levels similar to the P3x63Ag8.653 cell line (Figure 1). As expected, both the vector-transfected (pTEJ8-Sp2/0) and the wild-type Sp2/0-Ag14 cells



**Figure 3.** Effect of Bcl-xL expression on Sp2/0-Ag14 cell survival in glutamine-deprived media. (A) Viability assay. Cells were incubated in Iscove's media lacking L-glutamine and cell viability was assessed daily using the trypan blue exclusion assay. Open circle: wild type Sp2/0-Ag14; open triangle: pTEJ8-Sp2/0; closed triangle: wild type P3x63Ag8.653; closed circle: Bcl-xL-Sp2/0 clone 2. (B) DNA laddering assay. Wild type Sp2/0 and P3x63Ag8.653 (P3) cells, pTEJ8-Sp2/0 cells and Bcl-xL-Sp2/0 clone 2 were incubated in complete Iscove's media (+) or in culture media lacking L-glutamine (-) for 3 h. DNA fragmentation analysis was then performed as described in Materials and Methods. Identical results were obtained for all three Bcl-xL-transfected Sp2/0-Ag14 clones.

contained no detectable amounts of Bcl-xL protein (Figure 1).

#### *Bcl-xL-mediated resistance to apoptosis in Sp2/0-Ag14 cells*

The ability of Bcl-xL to protect Sp2/0-Ag14 cells against apoptosis was next examined. Wild type Sp2/0-Ag14 and P3x63Ag8.653 cells, Sp2/0-Ag14 cells transfected with the vector alone and Bcl-xL-

expressing Sp2/0-Ag14 cells (Figure 1) were incubated for 2 h in the presence or absence of CHX. Cell viability was then determined using the MTT assay. The induction of apoptosis was revealed by the analysis of DNA degradation into oligonucleosomal fragments, as described in the Materials and Methods section. In the presence of CHX, there was a significant loss of viability in the wild type Sp2/0-Ag14 and pTEJ8-Sp2/0 cells (Figure 2A). Extensive DNA fragmentation indicative of apoptosis was also detected (Figure 2B). In accordance with previous observations (Gauthier et al., 1996), P3x63Ag8.653 cells were highly resistant to apoptosis induction by CHX (Figure 2). Most notably, the expression of Bcl-xL dramatically increased the resistance of Sp2/0-Ag14 cells towards CHX-induced cell death (Figure 2).

Amino-acid depletion is considered an important limiting factor for hybridoma cell growth in batch culture (Mercille and Massie, 1994; Simpson et al., 1998). We therefore tested the effect of Bcl-xL-expression on the viability of Sp2/0-Ag14 cells in glutamine-deprived media. Exponentially growing cells were transferred into glutamine-deficient Iscove's media, and cell viability was assessed daily using the trypan blue assay. Approximately 85% of wild type and pTEJ8-Sp2/0 cells were dead within 24 h of culture in glutamine-deficient media (Figure 3A). On the other hand, both P3x63Ag8.653 and Bcl-xL-Sp2/0 cells survived longer in the absence of glutamine, with a percentage of viability of 90% and 67%, respectively, after 24 h (Figure 3A). This difference in cell viability was attributable to the anti-apoptotic effect of Bcl-xL. Extensive DNA fragmentation in wild type and pTEJ8-Sp2/0 cells could be observed after 3 h of culture in the absence of glutamine, but not when complete culture media was used (Figure 3B). In contrast, both Bcl-xL-Sp2/0 and P3x63Ag8.653 cells only contained intact, high molecular weight genomic DNA whether they were cultured for 3 h in complete Iscove's or in glutamine-deficient media (Figure 3B).

#### *Bcl-xL overexpression prolongs viability of Sp2/0-Ag14 cells in long-term culture*

The results shown in Figure 3 suggested that Bcl-xL expression could significantly protect Sp2/0-Ag14 cells against apoptosis under conditions known to limit hybridoma cell viability in stationary batch culture. We therefore examined the effect of the expression of Bcl-xL on the growth of Sp2/0-Ag14 cells in culture.

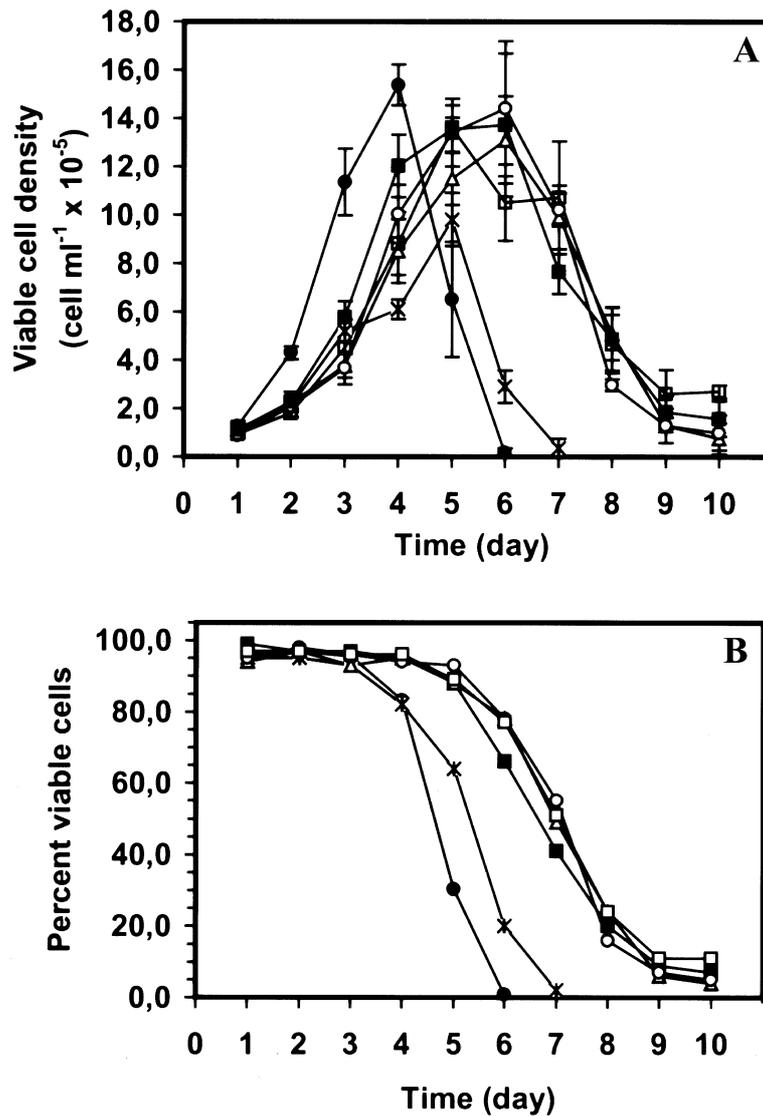


Figure 4. Stationary batch culture. Cells were seeded on day 0 and cell viability was determined daily using the trypan blue assay. (A) Density of viable (membrane intact) cells; (B) Percentage of membrane-intact cells in the culture. Closed circle: wild type Sp2/0; cross: pTEJ8-Sp2/0; closed square: wild type P3x63Ag8.653; open circle: Bcl-xL-Sp2/0 clone 1; open triangle: Bcl-xL-Sp2/0 clone 2; open square: Bcl-xL-Sp2/0 clone 3.

Wild type Sp2/0-Ag14 and P3x63Ag8.653 cells and the transfected Bcl-xL-Sp2/0 and pTEJ8-Sp2/0 cells were seeded at a density of  $5 \times 10^4$  cells ml<sup>-1</sup> and aliquots were taken daily for the measure of cell viability by the trypan blue assay. The results obtained are shown in Figure 4. The wild-type Sp2/0-Ag14 reached a maximal density of viable cells of  $1.5 \times 10^6$  cells ml<sup>-1</sup> on day 4 (Figure 4A); cell viability then decreased sharply and, by day 6, over 95% of the Sp2/0 cells in the culture were dead (Figure 4B). The pTEJ8-Sp2 cells behaved similarly to the wild type, reaching

a maximal density of viable cells around day 5, with a subsequent rapid loss of viability that spanned two days (Figure 4). The slower growth and lower maximal cell density observed for the vector-transfected Sp2/0 cells were obtained with several independent clones (results not shown) and are most likely due to the presence, in the culture media, of the antibiotic used for clone selection.

Remarkably, Bcl-xL expression significantly affected the behaviour of Sp2/0-Ag14 cells in culture. Although the maximal density of viable cells achieved

was essentially the same as the wild type, Bcl-xL-Sp2/0 cells reached their peak of viable density only on day 6 (Figure 4A). Furthermore, the decline in viability was much more gradual for the Bcl-xL-Sp2/0 than for the wild type and the pTEJ8-Sp2/0 control cultures (Figure 4B). Most interestingly, the culture behaviour of all three Bcl-xL-expressing Sp2/0-Ag14 clones analysed in this study was identical to the growth pattern of the P3x63Ag8.653 cell line (Figure 4).

## Discussion

In this report, we show that Bcl-xL plays an important role in modulating the viability of hybridomas in culture. Our conclusions are based on the comparison of two hybridoma cell lines. On the one hand, the P3x63Ag8.653 myeloma expresses high endogenous levels of Bcl-xL and shows resistance to apoptosis induced by ActD, CHX and amino-acid deprivation (Gauthier et al., 1996; Perreault and Lemieux, 1993b). On the other hand, the Sp2/0-Ag14 hybridoma, which is apoptosis-sensitive and exhibits low endogenous Bcl-xL levels (Gauthier et al., 1996), was transfected to express levels of Bcl-xL which were similar to the P3x63Ag8.653 myeloma (Figure 1). Comparison of the sensitivity of these two cell types to the effects of CHX exposure or glutamine deprivation (Figures 2 and 3) clearly indicated that, under these conditions, Bcl-xL expression alone can protect Sp2/0-Ag14 cells against apoptotic cell death to a level that is equivalent to P3x63Ag8.653 cells. This conclusion is further supported by the striking similarity of the growth behaviour observed between the Bcl-xL-Sp2/0 and the P3x63Ag8.653 cells in stationary batch culture (Figure 4).

The exhaustion of amino acids, most notably glutamine, has been suggested to be a major trigger for the onset of apoptosis in hybridoma cultures (Mercille and Massie, 1994; Simpson et al., 1998). This process can be inhibited by Bcl-2 (Simpson et al., 1998). In this article, we demonstrated that the effect of glutamine depletion could also be counteracted by Bcl-xL. Although the precise nature of the cell death pathways activated in cultured hybridoma cells remains to be fully elucidated, a direct implication of our observations is that the basic apoptotic mechanisms triggered during batch culture are regulated in a similar fashion in Sp2/0-Ag14 and P3x63Ag8.653 cells. Interestingly, these two cell lines have been shown to express similar

level of the pro-apoptotic protein Bax (Gauthier et al., 1996; results not shown), an important component of the death-inducing machinery (Chao and Korsmeyer, 1998). Although it is possible that other members of this gene family are differentially regulated in these two cell lines, our data clearly suggests that the levels of Bcl-xL expression in Sp2/0-Ag14 cells are critical in the modulation of the sensitivity of this cell line to culture-related stresses and, consequently, in its resulting behaviour in batch culture.

Our results with the hybridoma Sp2/0-Ag14 may also provide an explanation for the lack of effect of Bcl-2 overexpression in some murine myelomas (Fujita et al., 1997; Murray et al., 1996). In light of the data presented here, the endogenous Bcl-xL levels in NS/O and P3x63Ag8.653 cells would 'saturate' the apoptotic machinery, providing sufficient protection against culture-related cell death and making the overexpression of Bcl-2 ineffective. Paradoxically, the adenoviral E1B-19K protein, which is structurally and functionally homologous to Bcl-2 (Chiou et al., 1994), can nevertheless protect NS/O cells against apoptosis (Mercille et al., 1999). Similarly, in CHO and BHK cells, Bcl-xL overexpression afforded a greater protection than Bcl-2 against a number of culture-related stresses (Mastrangelo et al., 2000). This selective, functional redundancy between Bcl-2 family members could be explained by several factors which, alone or in combination, could influence the ability of specific Bcl-2-related proteins to increase the viability of cultured cell lines. First of all, differences in the interaction patterns characteristic of individual Bcl-2 members have been reported to impact on their ability to prevent apoptosis. For example, E1B-19K, Bcl-xL and Bcl-2 each interact with the pro-apoptotic protein Bax, resulting in increased cell survival (Chen et al., 1996; Oltvai et al., 1993). On the other hand, while both Bcl-xL and Bcl-2 have been shown to bind to, and be inhibited by, the BH3-containing protein Bad (Yang et al., 1995), E1B-19K does not interact with Bad (Chen et al., 1996). This difference in Bad-mediated regulation may explain why E1B-19K, but not Bcl-2, can increase the survival of NS/O cells in the presence of Bcl-xL expression (Mercille et al., 1999). Secondly, the relative endogenous expression levels of Bcl-2 family members would impact on the outcome of the ectopic expression of specific anti-apoptotic Bcl-2 proteins. For example, the level of protection afforded by Bcl-2 or Bcl-xL has been shown to be affected by the intracellular levels of the pro-apoptotic protein Bax (Oltvai et al., 1993). Finally, anti-apoptotic Bcl-

2 members are regulated in a specific manner through a complex set of post-translational mechanisms such as phosphorylation, sub-cellular localisation, and proteolytic cleavage (Fadeel et al., 1999). The activity of those regulatory pathways would, in turn, be determined by the cell context and the actual death inducer. Therefore, depending on the nature of the cell death and cell survival machineries operating in a given hybridoma/myeloma cell line, the co-expression of only a specific subset of Bcl-2 family members could be beneficial.

In conclusion, the results reported in this study suggest that Bcl-xL expression levels are sufficient to account for the relative robustness of some hybridoma cell lines in stationary batch cultures. While confirming the importance of adequate expression of anti-apoptotic proteins for hybridoma cell survival, our work also underscores the role which the cellular context may play in determining the success of ectopic gene expression strategies for the improvement of hybridoma cell viability in culture.

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