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The Role of Bax/Bcl-2: From T Cell Development to the Cell Death-cell Cycle Interface

Abstract

Transgenic mice were generated overexpressing *baxα* gene in T cells. Thymocytes from the *baxα* transgenics show accelerated apoptosis in response to multiple stimuli. However, while *baxα* transgenic thymocytes in a p53-/- background still show accelerated apoptosis in response to glucocorticoid treatment they do not exhibit a restored response to DNA damage induced apoptosis. We show that Baxα overexpression can lead to a defect in T cell maturation which reflects a perturbation of T cell development and that the Baxα protein is involved in distinct apoptotic pathways. We further demonstrate that Baxα overexpression can facilitate the entry of T cells into S phase whereas Bcl-2 delays this entry via modulation of the level of $p27^{Kip1}$ protein.

Introduction

Apoptosis, or programmed cell death, is a widespread process used to eliminate unwanted or damaged cells from multicellular organisms. For instance, apoptosis serves to ensure the selection of appropriate lymphoid populations during thymic T cell development (Surh and Sprent, 1994). Apoptosis of immature T cells and T cell hybridomas can be readily induced by DNA damaging agents such as ionising radiation and etoposide (Clarke *et al.*, 1993) as well as glucocorticoids (Wyllie, 1980) and various agents that induce activation (Green and Scott, 1994). Much effort is now being expended to elucidate the genetic and biochemical mechanisms of apoptosis. Prominent among the genes already discovered to be involved is the *bcl-2* oncogene.

Bcl-2 was first isolated by virtue of its translocation into the immunoglobulin gene locus in some follicular B cell lymphomas (Cleary *et al.*, 1986; Tsujimoto and Croce,1986). Bcl-2 overexpression was subsequently shown to block cell death in an IL-3 dependent cell line upon withdrawal of the cytokine (Vaux *et al.*, 1988). Bcl-2 and its relative Bcl-X_L (Boise *et al.*, 1993) are potent inhibitors of apoptosis induced by various stimuli such as irradiation, glucocorticoid treatment, calcium ionophores or cytotoxic drugs (Sentman *et al.*, 1991; Strasser *et al.*, 1991; Chao *et al.*, 1995).

Another member of the *bcl-2* gene family is *bax*. The Bax protein was isolated by virtue of its interaction with Bcl-2 (Oltvai *et al.*, 1993). Alternate splicing within the

bax gene produces three classes of transcript of which the α mRNA encodes the 21 KDa protein that can heterodimerise with Bcl-2 (Oltvai *et al.*, 1993). In this same study, the overexpression of Baxα in an IL-3 dependent cell line is seen to accelerate apoptosis upon removal of the cytokine. Furthermore, the ratio of Baxα to Bcl-2 appears to be critical since a predominance of Baxα accelerates apoptosis in response to factor withdrawal whereas overexpressed Bcl-2 greatly diminishes apoptosis.

As mentioned above, DNA damaging agents can exert an apoptosis response in many cell types, particularly lymphocytes. A critical regulator of the cellular response to DNA damage is the transcription factor encoded by the p53 tumour suppressor gene (Cox and Lane, 1995). When non-irradiated cells are forced to express high levels of p53 they undergo either growth arrest (Michalovitz *et al.*, 1990) or apoptosis (Yonish-Rouach *et al.*, 1991). Analysis of mice in which both p53 alleles have been inactivated (p53-/-) by gene targetting have demonstrated that p53 is required for the induction of apoptosis in the thymocytes by γ-radiation and by some DNA damaging drugs (Clarke *et al.*, 1993; Lotem and Sachs, 1993; Lowe *et al.*, 1993). The way in which p53 induces apoptosis is not clear. However, evidence has recently been obtained that implicates Bax/Bcl-2. Restoration of p53 in a murine leukaemia cell leads to increases in *bax* mRNA and protein levels accompanied by lower steady state levels of *bcl-2* mRNA and protein (Miyashita *et al.*, 1994). Moreover, p53 has been shown to directly activate transcription of the *bax* gene (Miyashita and Reed, 1995).

The development and proper function of an effective immune system requires apoptosis (Cohen *et al.*, 1992). Immature T cells undergo random rearrangement of their antigen receptor genes (reviewed in Davis, 1990). Only those cells bearing T cell receptors (TCRs) of appropriate specificity are positively selected for survival and further differentiation. The rest, which is at least 95% of the CD4+CD8+ T cell precursors, undergo apoptosis (Surh and Sprent, 1994). Those apoptosing cells are either deleted due to expression of self-reactive receptors (negative selection) or die because they failed to receive a surviving signal (death by neglect) (von Boehmer, 1994). The cellular mechanisms involved in positive or negative selection are not yet fully outlined nor is the role of apoptosis related molecules, if any, in the process. However, for example, bcl-2 expression has been shown to be upregulated during or directly following positive selection (Linette *et al.*, 1994).

Results

T cells from Baxα Transgenics show Accelerated Apoptosis in Response to Certain Apoptotic Stimuli

Several transgenic lines of mice were produced carrying a construct containing the human CD2 promoter and LCR element as well as the mouse *baxα* cDNA with a haemagglutinin (HA) epitope attached. The HA epitope was added to the N-terminus of murine *baxα* using PCR followed directly by the cDNA coding for mouse *baxα* so that HABaxα protein produced by the transgene can be distinguished from endogenous Baxα. The CD2 HA*baxα* transgene is shown in Figure 1.

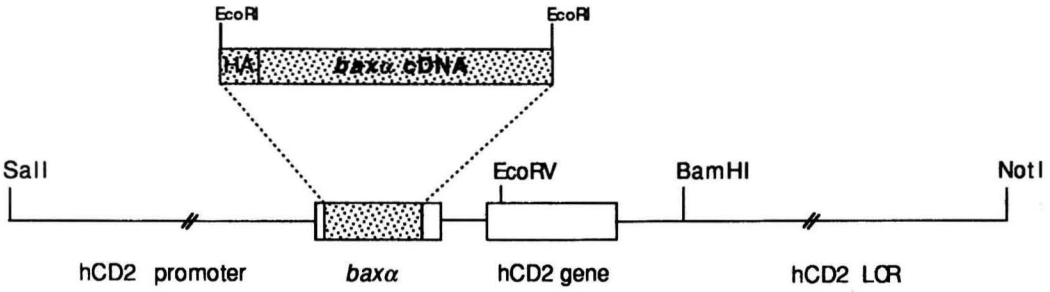

Fig. 1. *Baxα* transgene. *Baxaa* transgene construction. The HA tagged *baxα* insert flanked by EcoRI sites was generated by PCR from the murine *baxα* cDNA. The insert was cloned into the EcoRI site in exon 1 of the human CD2 expression cassette containing the CD2 promoter, shortened CD2 coding region and 3' LCR. The SalI-NotI fragment was isolated for injection into fertilised mouse oocytes.

DNA from the transgenic lines generated was analysed by Southern blot analysis and subsequent studies focussed on the two lines with the highest copy numbers of transgene. These lines of *baxα* transgenic were designated Bax 18 and Bax 25. Western blot analysis was used to examine the level of HABaxα expression in the T cells of the transgenic mice. Baxα expression was detected using a rabbit polyclonal antibody against Baxα and the 12CA5 mouse monoclonal antibody (mAb) against the HA epitope. The addition of the 11 amino acid HA epitope means that the HABaxα protein is larger than the endogenous Baxα. The ratio of HABaxα to endogenous Baxα was determined by phosphorimaging using the actin control to normalise each track. The ratio HABaxα: Baxα was 145% for Bax18 and 155% for Bax25 (Brady *et al.*, 1996a).

To assess the effect of Baxα on survival *in vitro*, thymocytes from *baxα* transgenic mice and control littermates were cultured in medium. Samples were harvested at various time points and the percentage of apoptotic cells determined using a flow cytometry-based technique which measures propidium iodide staining of DNA after cell lysis in a hypotonic buffer (Nicoletti *et al.*, 1991). Over a 3 day period substantially more apoptosis is seen in the baxα transgenic thymocytes than in control thymocytes (Figure 2A). By 72 h 40-50% of control thymocytes had still diploid DNA content whereas virtually all the *baxα* thymocytes were apoptosed. Similarly, splenic T cells from baxα transgenic mice showed accelerated apoptosis compared with splenic T cells from control littermates (data not shown).

The glucocorticoid dexamethasone has been shown to induce apoptosis in thymocytes (Wyllie, 1980). The result of dexamethasone treatment of thymocytes from the Bax 18 and 25 transgenic as well as non-transgenic littermates is shown in Figure 2B. The cells were harvested after 4-5 hours incubation with 2μM dexamethasone. Greater than 60% of the Bax 18 transgenic thymocytes are in apoptosis whereas only 30% or so of those from non-transgenic thymocytes are apoptotic. Similarly approximately 60% of the Bax 25 transgenic thymocytes are apoptotic as opposed to less than 30% for the non-transgenic thymocytes.

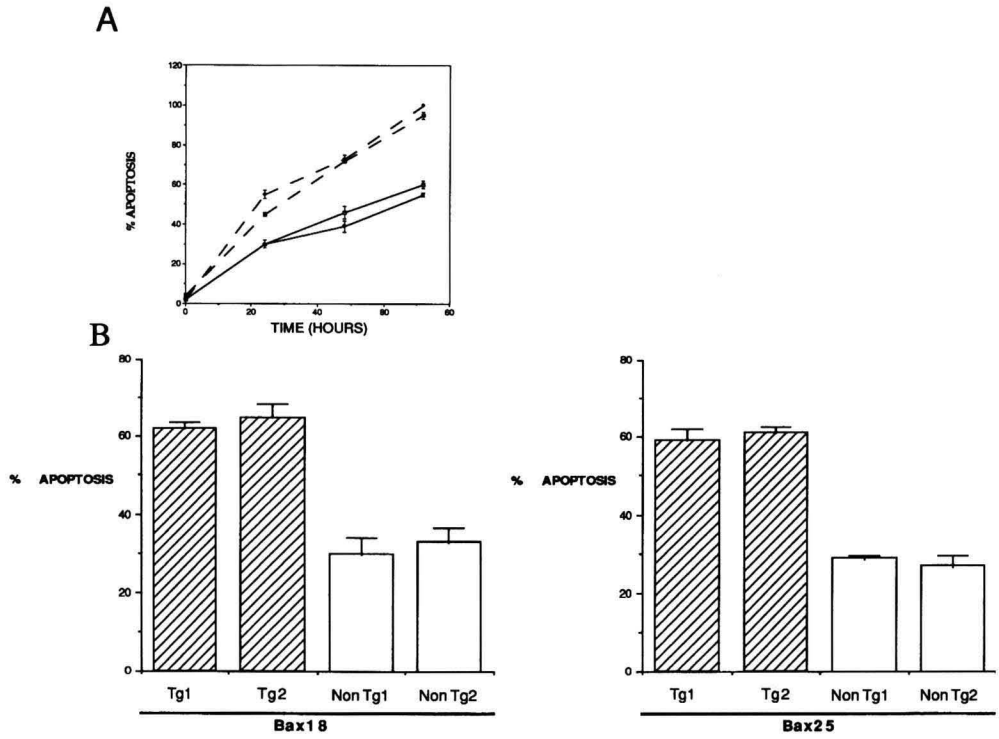

Fig. 2. Baxα accelerates thymocyte apoptosis.
(A) Thymocytes from *baxα* transgenic mice die faster during *in vitro* culture. Thymocytes were isolated from *baxα* transgenic mice and non-transgenic littermates and cultured in medium at 1×10^6 cells/ml. The percentage of apoptosis of *baxα* transgenic thymocytes (open circles and solid squares) is represented by broken lines and control littermate thymocytes (open squares and solid circles) by continous lines. Each data point represents the mean range of duplicate determination of apoptosis in 10,000 cells.
(B) Percentage of apoptosis in *baxα* transgenic thymocytes dexamethasone. Thymocytes were isolated from transgenic (Tg1,2) and non-transgenic littermates (Non Tg 1,2) of the Bax 18 and Bax 25 lines and treated with 2μM dexamethasone. 4.5 hr after treatment the cells were harvested and prepared for FACS to measure the DNA content of the nuclei by propidium iodide staining. The percentage of apoptotic cells shown is normalised to the amount of apoptotic cells found in untreated cultures for the same animal at the same time point.

The *baxα* transgenic thymocytes were further assessed for their sensitivity to apoptosis induced by DNA damage. Certain cell types, particularly lymphocytes are known to be highly sensitive to DNA damage induced apoptosis caused by γ-radiation or cytotoxic drugs, as used in chemotherapy (Cohen *et al.*, 1992). This sensitivity is known to be greatly reduced by Bcl-2 overexpression (Sentman *et al.*, 1991; Strasser *et al.*, 1991). Low doses of γ-radiation were administered to the Bax 18 and Bax 25 thymocytes. The doses were in the range 50 cGy to 200 cGy. For both lines the transgenic thymocytes are substantially more sensitive to γ-radiation than non-transgenic littermates. After 8 hr greater than 30% apoptosis is found in Bax 18 mice irradiated with only 100 cGy (Brady *et al.*, 1996a).

Baxα Overexpression in p53 Null T Cells Still Accelerates Glucocorticoid Induced Apoptosis but does not rescue DNA Damage Induced Apoptosis

The *baxα* mice were crossed so as to produce mice homozygous for the p53 null mutation while being *baxα* transgenic. Thymocytes from p53 null mice have been shown to be resistant to apoptosis induced by DNA damaging agents such as γ-radiation or etoposide (Clarke *et al.*, 1993; Lowe *et al.*, 1993). Thymocytes were analysed from mice heterozygote and homozygote for the p53 null allele and then plus or minus the *baxα* transgene. The result of dexamethasone treatment is shown in Figure 3A. High levels of apoptosis were found in the mice transgenic for *baxα* regardless of p53 status. Much lower levels of apoptosis are seen in thymocytes from mice heterozygote or homozygote for the p53 null allele without *baxα*.

A different situation pertains in thymocytes treated with the DNA damaging agent etoposide (Figure 3B) or γ-radiation (Figures 3C and D). High levels of etoposide induced apoptosis are seen in p53 heterozygote thymocytes, somewhat higher in those also expressing HABaxα. There are only very low levels seen in p53 null homozygotes regardless of the presence or absence of the *baxα* transgene. The same situation applies to thymocytes irradiated with 100 cGy (Figure 3C) or 500 cGy (Figure 3D) of γ-radiation. Irradiated p53 null thymocytes showed a slightly increased level of apoptosis with *baxα* than without but we do consider this significant outside of inherent experimental variation. Therefore, it is clear that Baxα overexpression accelerates apoptosis due to glucocorticoid treatment in a p53 independent manner. However, while Baxα overexpression can accelerate apoptosis due to DNA damage this process is dependent on p53 status since, it is not effective in the absence of p53.

Baxα acts in Distinct Apoptotic Pathways

Given that Baxα overexpression accelerates apoptosis in thymocytes in response to DNA damage as well as glucocorticoid treatment, we then investigated whether this also holds true for other pathways leading to cell death. Fas/APO-1 is a cell surface protein that induces apoptosis in immature thymocytes treated with an anti-Fas antibody (Ogasawara *et al.*, 1995). We incubated thymocytes from baxα transgenic mice and control littermates with 1μg/ml anti-Fas antibody (Jo2) for 20h. As on thymocytes from normal mice (Ogasawara *et al.*, 1995), virtually all thymocytes from baxα transgenic mice express Fas/APO-1 (data not shown). No accelerated apoptosis was found in baxα transgenic thymocytes in response to anti-Fas antibody, either with or without cycloheximide (Brady *et al.*, 1996b). A similar result was found when baxα and control thymocytes were incubated with 10 ng/ml of TNFα for 20 h. These data show that overexpression of Baxα does not accelerate the cell death pathways initiated in thymocytes by activating Fas with an agonistic antibody or treatment with TNFα.

Baxα Transgenics have decreased Numbers of Mature T Cells in vivo

Having established that baxα transgenic T cells show accelerated cell death *in vitro*, the constitution of T cell compartments *in vivo* was also scrutinised. Thymocytes from baxα transgenic mice and control littermates were analysed by two colour flow cytometry using antibodies against CD4 and CD8. As shown in Figure 4, a

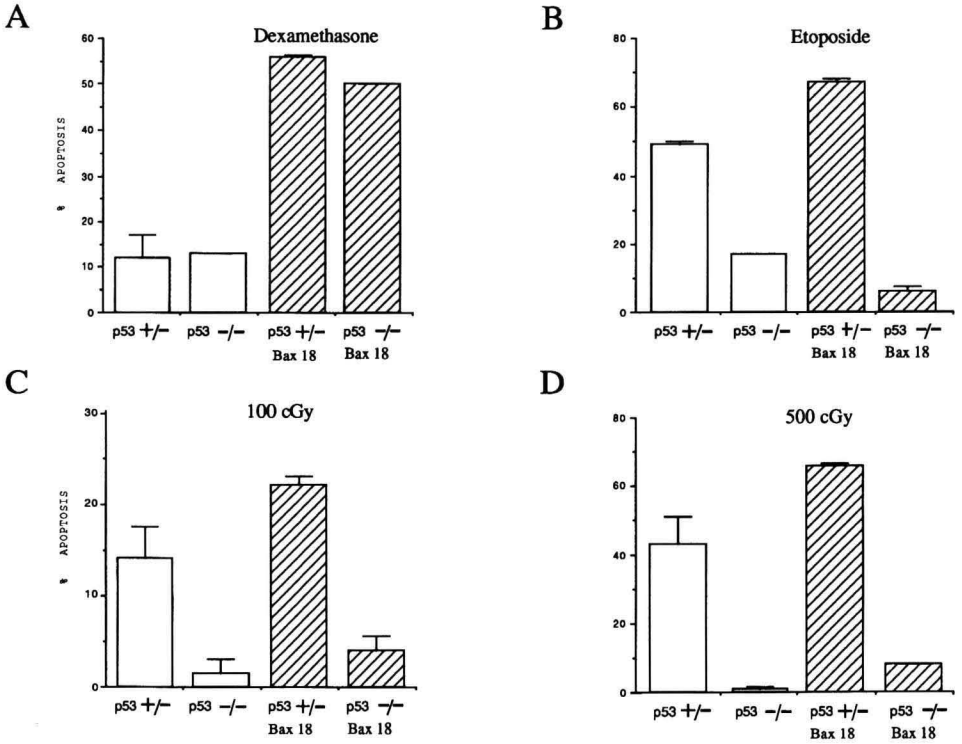

Fig.3. Percentage apoptosis in thymocytes from mice with or without p53 and/or the *baxα* transgene. (A) Thymocytes were isolated from mice heterozygote for a p53 null allele (p53+/-) or homozygote for p53 null alleles (p53-/-) and either containing the *baxα* transgene from the Bax 18 line or not. The cells were treated with 2μM dexamethasone for 4.5 hr, harvested and prepared for DNA content measurement. Thymocytes were also treated with 50 μM etoposide for 7hr (B) or, cultured for 8 hr following 100 cGy of γ-radiation (C) or cultured for 8 hr following 500 cGy of γ-radiation (D), before harvesting and analysis. The percentage of apoptotic cells is shown.

considerable decrease in the number of CD4+ single positive (SP) and CD8+ SP cells was seen in the baxα transgenic mice in comparison to control littermates. The baxα transgenic mice have only 25 to 30% of the control levels of SP cells in the thymus. This was consistent for both transgenic lines studied, Bax18 and Bax25 and could be observed at all the ages studied i.e. 2 to 8 weeks. This change is also reflected in a decrease in the absolute number of SP thymocytes as the total number of thymocytes did not significantly vary between baxα transgenic and control littermates in the age range studied. Concomitant with the decrease in SP T cells in the baxα transgenic thymus is an increase in the number of CD4+CD8+ double positive (DP) immature thymocytes. A similar analysis was carried out on red cell-depleted splenocytes (Brady *et al.*, 1996b). As for thymocytes, a substantial reduction in CD4+ and CD8+ SP T cells was seen in the spleen of baxα transgenic mice. These results show that a consequence of the expression of the baxα transgene in T cells is a major reduction in the numbers of mature T cells *in vivo*.

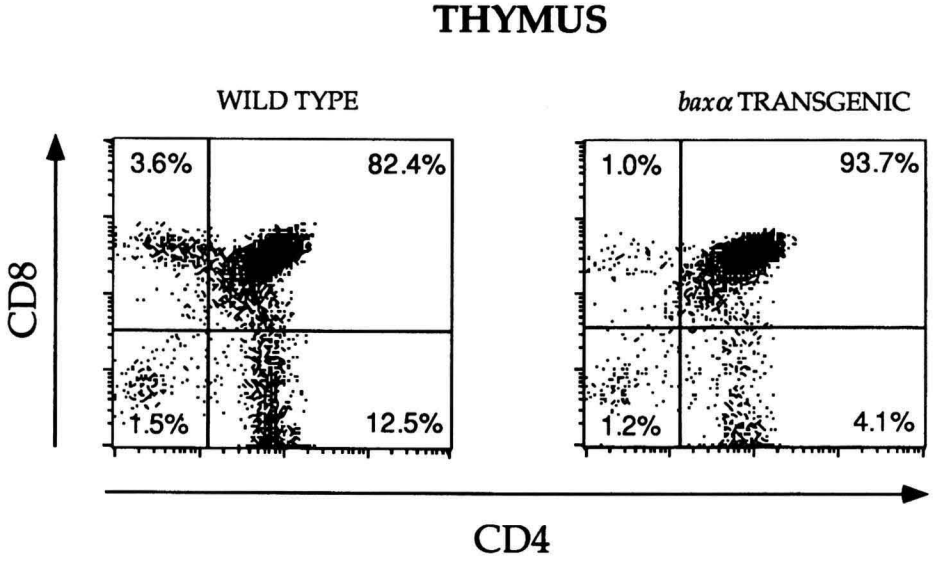

Fig. 4. *Baxα* transgenics have decreased numbers of mature T cells in vivo.
CD4 and CD8 expression on thymocytes and splenocytes from *baxα* transgenics and control littermates. The percentage of cells in each population is indicated.

Baxα Transgenics have an Increased Number of Cycling Cells in the Thymus

In light of the reduced numbers of mature T cells in baxα transgenic mice described above but the unchanged number of thymocytes relative to control littermates, we examined whether this had implications for the number of cycling cells in the thymus. To investigate this *in vivo* we injected the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) intraperitoneally into baxα transgenic mice and control littermates. Four hours later the thymus was removed from each mouse and the BrdU content of the thymocytes measured using a BrdU specific antibody with propidium iodide counterstaining to assess DNA content as an indicator of position in the cell cycle (Begg *et al.*, 1988). We observed that at least twice the number of thymocytes in baxα transgenic compared to control littermates, are in the S phase of the cell cycle (Brady *et al.*, 1996b). *baxα* thymocytes show a concomitant decrease in the number of cells in G0/G1.

Baxα accelerates the Entry into S Phase of Cycling T Cells whereas Bcl-2 delays it

In order to investigate if Baxα can directly affect S phase entry we had to look at a situation in which a synchronised population of primary T cells was allowed to re-enter the cell cycle following an exogenous stimulus. To do this, we incubated splenocytes with ConA for 72 hours. We then starved the resulting T cell blasts of IL-2 for 14 h which resulted in a completely arrested synchronised population, confirmed by propidium iodide staining and flow cytometry (data not shown). Subsequent addition of

IL-2 stimulates the arrested cells to re-enter the cell cycle. Samples were taken at various time points after the addition of IL-2 to allow analysis of cell cycle status as monitored by the kinetics of $p27^{Kip1}$ downregulation as well as BrdU incorporation. T cells induced to leave a quiescent state by IL-2 show a decrease in the level of $p27^{Kip1}$ allowing CDK2 activation and entry into S phase (Nourse *et al.*, 1994; Firpo *et al.*, 1994). As shown in Brady *et al.*, 1996b, the level of $p27^{Kip1}$ decreases with time upon addition of IL-2 to the arrested T cell blasts. However, the overall level of $p27^{Kip1}$ is lower in the baxαtransgenic T cell blasts than in those from control littermates. To confirm that the lower levels of $p27^{Kip1}$ were reflected in an accelerated entry into S phase we also analysed the IL-2 restimulated blasts for BrdU incorporation. The baxα transgenic T cell blasts enter S phase faster than T cell blasts from control littermates as measured by their increased level of BrdU incorporation in the first 18 h after IL-2 stimulation (Brady *et al.*, 1996b).

The implication of the above is that overexpression of the apoptotic effector molecule Baxα can influence the rate at which cycling cells enter S phase. To test whether this effect has a wider context than Baxα alone we tested whether or not Bcl-2 would exert an opposing effect. We also generated ConA stimulated T cell blasts from the spleens of Eμbcl-2 transgenic mice (McDonnell *et al.*, 1989) and control littermates. The levels of $p27^{Kip1}$ were higher in the bcl-2 transgenic T cell blasts and the rate of decline of these levels was also slower than in controls following IL-2 stimulation (Brady *et al.*, 1996b). The CDK inhibitor $p21^{Cip1}$, is induced upon IL-2 stimulation (Nourse *et al.*, 1994). The kinetics of $p21^{Cip1}$ induction for both the bcl-2 and baxα T cell blasts upon IL-2 addition were measured and remained unchanged in comparison to control blasts (data not shown). To verify that the slower decline in $p27^{Kip1}$ levels in the bcl-2 T cell blasts correlated with a delayed entry into S phase we measured the level of BrdU incorporation after IL-2 addition. As seen in Brady *et al.*, 1996b, the entry of Bcl-2 transgenic T cell blasts into S phase is substantially delayed in comparison to that in control littermates. These data demonstrate that overexpression of a molecule which accelerates apoptosis, such as Baxα, or a molecule that blocks apoptosis, such as Bcl-2, can directly influence the rate at which cells enter the cell cycle: an accelerated rate in the case of Baxα and a delayed rate in the case of Bcl-2.

Discussion

We have demonstrated that the overexpression of Baxα in primary T cells can accelerate apoptosis in these cells in response to different stimuli. Comparing protein expression we find that thymocytes from both Bax 18 and Bax 25 have transgenic HA Baxα levels of approximately 150% that of endogenous Baxα. This level of HABaxα is sufficient to substantially accelerate apoptosis in thymocytes in response to low doses of γ-radiation, dexamethasone and etoposide. This is in direct contrast to thymocytes from *bcl-2* transgenic mice which become more resistant to these apoptotic stimuli (Sentman *et al.*, 1991; Strasser *et al.*, 1991). Our data and the previous work of others point to a situation in T cells where the ratio of Baxα to Bcl-2 is very important for determining response to apoptotic stimuli.

Clearly, p53 has a central role in the regulation of apoptosis in response to DNA damaging agents (Clarke *et al.*, 1993; Lotem and Sachs, 1993; Lowe *et al.*, 1993). p53 also directly activates transcription of the *bax* gene (Miyashita and Reed, 1995). Taken together these findings suggested that Baxα may act downstream of p53 in a p53 dependent apoptosis pathway. The presence of the *baxα* transgene accelerated apoptosis in thymocytes from both p53-/- and p53+/- mice in response to dexamethasone.

In contrast with the response to dexamethasone the presence of the *baxα* transgene accelerated apoptosis in only thymocytes from p53+/- mice after exposure to γ-radiation and etoposide. Thymocytes from p53-/- mice with the *baxα* transgene showed similar resistance to apoptosis by these DNA damaging agents as p53-/- mice without the *baxα* transgene. Therefore, the suggestion that p53 acts to induce apoptosis following DNA damage by altering the Baxα:Bcl-2 ratio in favour of Baxα to accelerate cell death (Miyashita *et al.*, 1994; Cox and Lane, 1995; Miyashita and Reed, 1995) is a part of but clearly not the complete picture. Presumably, p53 is required to induce other necessary factor(s) apart from Bax to hasten cell death or perhaps directly activate such factor(s) by a protein-protein interaction.

There are multiple pathways leading to apoptosis which are subject to distinct regulation (Strasser, 1995). We find that the cell death pathway activated via the Fas receptor is not enhanced by overexpression of Baxα. This suggests that signalling through Fas occurs via an alternative pathway than the one involving Baxα. This correlates with the recent finding that overexpression of Bcl-2 in the lymphoid cells of transgenic mice does not inhibit Fas induced apoptosis (Strasser *et al.*, 1995). Similarly, we find that TNFα induced apoptosis is not accelerated in Baxα transgenic T cells which again tallys with earlier data that Bcl-2 is a poor inhibitor of TNF-induced apoptosis (Vanhaesebroeck *et al.*, 1993).

The Baxα mice have only 25-30% the level of mature SP T cells in thymus and spleen as compared to control littermates whereas the number of DP thymocytes is slightly increased. This contrasts with the lymphoid hyperplasia reported in bax (-/-) mice (Knudson *et al.*, 1995). The instance of a slightly increased number of baxα DP thymocytes indicates that until the point of selection there is probably no stimulus to induce apoptosis. When, however, the thymocytes are cultured *in vitro* the baxα DP thymocytes die faster than the control thymocytes.

The depletion of mature T cells *in vivo* suggests that the baxα mice may have a defect in selection. Immature thymocytes that are not positively selected are thought to die via negative selection or neglect. It has been shown that apoptosis is the major process whereby thymocytes die and that the vast majority of apoptotic cells in the thymus seem to be a reflection of failure to undergo positive selection (Surh and Sprent, 1994). Our data indicate that overexpression of an apoptosis accelerating molecule, such as baxα, can directly cause failure of selection.

A further aspect of the thymi of baxα transgenic mice which we observed was the doubling of the number of thymocytes in the S-phase of the cell cycle. This could be attributed to the influence of a homeostatic process within the thymus whereby the depletion of mature T cells, mediated by the baxα transgene, is counteracted by

an increased recruitment of cells into cell cycle within earlier compartments in an attempt to fill up the void. Such a process may exist and result in increased cycling but we were interested whether Baxα overexpression itself could influence T cell entry into cell cycle. To assess this we stimulated splenocytes with ConA to generate T cell blasts, and incubated the cells in medium without IL-2 for 14 h before restimulation by adding IL-2. It has been demonstrated that IL-2 allows CDK activation by causing the elimination of the CDK inhibitor protein p27Kip1 (Nourse *et al.*, 1994; Firpo *et al.*, 1994). p27Kip1 governs CDK activity during the transition from quiescence to S phase in T lymphocytes. We find that Baxα overexpression correlates with overall decreased levels of p27Kip1 after IL-2 stimulation and, furthermore, that this correlates with accelerated entry into S phase as measured by BrdU incorporation and propidium iodide counterstaining. Further confirmation of the finding, that apoptosis regulatory molecules can have an influence on cell cycle, comes from our observation of the opposite effect in bcl-2 transgenic T cells namely, that Bcl-2 delays the downregulation of p27Kip1 after IL-2 stimulation with a concomitant delay in S phase entry. We also observe that the absolute levels of p27Kip1 are higher in the bcl-2 blasts compared to controls.

These data pose the questions how apoptosis regulatory molecules can influence the cell cycle machinery and with what purpose. The two main possibilities for how the interaction can occur are either directly or indirectly. Several analyses (e.g. Makela *et al.*, 1994; Harper *et al.*, 1993) have already taken place for proteins interacting with members of the cell cycle machinery but to date no interaction with Bcl-2 family members has been reported. However, such an interaction cannot be excluded. The indirect association could be via proteases downstream of Baxα/Bcl-2 whose activities might modulate components of the cell cycle. Thus, the activation of these downstream proteases could be a necessary event contributing to the process of downregulation of cell cycle inhibitory proteins like p27Kip1 necessary for cell cycle progression.

Although, our data do not unequivocally position the Baxα/Bcl-2 effect within the cell cycle machinery, we have demonstrated that their overexpression results in modulation of p27Kip1 levels. As a consequence of this Baxα/Bcl-2 levels could influence the timing of the activation of cyclin E/CDK2 and cyclin A/CDK2 complexes. We have shown here the effects of Baxα/Bcl-2 on cell cycle and T cell selection. These may be separate functions of the same proteins or they could be interconnected. One way to explain both effects is based upon the observation that nondividing immature T cells, in particular, are positively selected (Huesmann *et al.*, 1991). Baxα overexpression would lead to more T cells in cycle whereas Bcl-2 would do the reverse.

Acknowledgements

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