# Bcl2 Inhibits Apoptosis Associated With Terminal Differentiation of HL-60 Myeloid Leukemia Cells

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**The Bcl2 protein inhibits apoptosis (programmed cell death) induced by a variety of noxious stimuli. However, relatively little is known about its effect on apoptosis that occurs after terminal differentiation. Bcl2 protein levels decrease during differentiation of myeloid cells into granulocytes that subsequently undergo apoptosis, but the potential role of Bcl2 in coupling survival and differentiation remains undefined. To ascertain the relationship between decreasing Bcl2 levels and the onset of apoptosis in differentiating myeloid cells, Bcl2 was hyperexpressed in the HL-60 cell line after retroviral gene transfer. After treatment of HL-60/BCL2 cells with all-*trans* retinoic acid or phorbol myristic acid, Bcl2 levels did not decrease as in normal HL-60 cells but, rather, increased because of activation of the viral promoter. Differentiation of the Bcl2-overexpressing cells was similar to that of normal HL-60 cells, but they showed little evidence for apoptosis and had a prolonged survival. These studies show that the survival-enhancing properties of Bcl2 counteract programmed cell death that accompanies terminal differentiation; however, Bcl2 has no significant effect on differentiation itself, suggesting that apoptosis and differentiation are regulated independently in myeloid cells.**



THE *BCL2* ONCOGENE was initially identified at the breakpoint of the t(14;18)(q32;q21), the most common chromosomal translocation in human lymphoid malignancies.[1-3] This translocation juxtaposes the Ig heavy chain gene enhancer with the *BCL2* gene, resulting in constitutive expression of *BCL2* transcripts[4] with subsequent overexpression of the 24- to 26-kD Bcl2 protein.[5] Overexpression of Bcl2 in lymphoid cells in culture[6-8] and in transgenic mice[9-12] prevents apoptosis (programmed cell death) induced by a wide variety of agents, such as steroids, $\gamma$ irradiation, growth factor deprivation, and other injurious agents. Bcl2 also has been implicated in positive selection of thymocytes and in maintenance of B-cell memory through its effects on survival at specific stages of lymphoid development and differentiation.[10,11] However, the precise role of Bcl2 in the coupling of survival and differentiation or in controlling cell death that accompanies terminal differentiation remains undefined.

A cellular lineage amenable for studying this potential linkage is the myeloid lineage, because differentiation-linked regulation of Bcl2 expression occurs in these cells. Expression of Bcl2 protein has been shown by immunohistochemistry in early myeloid cells in the bone marrow, but decreases with cell maturation, becoming almost undetectable in terminally differentiated neutrophils,[13,14] which subsequently undergo programmed cell death.[15] However, the effects of Bcl2 overexpression in the myeloid lineage have not been well characterized, although Bcl2 has been shown to increase the survival of interleukin-3 (IL-3)–dependent FDC-P1 myeloid leukemia cells under conditions of IL-3 deprivation.[6]

The Bcl2 protein has also been detected in myeloid leukemias and myeloid leukemia cell lines, including the human HL-60 cell line.[14] This cell line, which was derived from a patient with clinical features of promyelocytic leukemia, has been extensively characterized with respect to its proliferation and differentiation properties.[16-18] It can be induced to differentiate along the myeloid pathway into granulocytes with dimethylsulfoxide (DMSO) or all-*trans* retinoic acid (ATRA).[19-21] After differentiation with ATRA, the granulocytes die through apoptosis in a process similar to that seen in normal human neutrophils.[22] HL-60 cells can also differentiate into macrophage-like cells when treated with phorbol myristic acid (PMA).[23] Bcl2 levels decrease during differentiation of HL-60 into neutrophils or macrophage-like cells.[14] However, it is unclear whether a decrease in Bcl2 levels may be required for cells to differentiate or undergo apoptosis.

To address the potential role of Bcl2 in blocking terminal differentiation or its attendant programmed cell death, Bcl2 was hyperexpressed in HL-60 cells after retroviral gene transfer. When these cells were treated with differentiating agents, Bcl2 protein levels did not decline but, rather, increased because of the activation of the retroviral promoter. Overexpression of Bcl2 did not prevent differentiation into granulocytes or macrophage-like cells, but did extend the life of differentiated cells by blocking apoptosis. These studies suggest that, although differentiation and apoptosis occur concomitantly, they are regulated independently in differentiating myeloid cells.

## MATERIALS AND METHODS

*Reagents.* ATRA and PMA were obtained from Sigma Chemical Co (St Louis, MO). ATRA was dissolved in ethanol at 1 mmol/L and used at 1 $\mu$mol/L to induce differentiation. PMA was dissolved in DMSO at 16 $\mu$mol/L and used at 16 nmol/L to induce differentiation.

*Cell culture.* HL-60 cells were grown in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum and 100 U/mL penicillin, 290 $\mu$g/mL L-glutamine, and 100 $\mu$g/mL streptomycin. Cells were maintained at 37°C in a 5% $CO_2$/95% air incubator. HL-60 stock cultures infected with the control neomycin or *BCL2* retroviruses were grown as above in the presence of 500 $\mu$g/mL

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