Role of apoptosis in the pathogenesis of B-cell chronic lymphocytic leukaemia

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Accepted: 26 August 2002

ABSTRACT

B-cell chronic lymphocytic leukaemia (B-CLL) is a clinically heterogeneous disease characterised by the accumulation of a clonal population of B lymphocytes. This accumulation is considered to result from the prolonged survival of B-CLL cells arrested in the G_0 stage of the cell cycle. However, when cultured *in vitro*, B-CLL cells die rapidly by apoptosis. It is now clear that a number of factors can delay or postpone the onset of apoptosis, including a number of cytokines and direct contact with different cell types. Although many drugs are now known to cause clinical improvement in B-CLL by causing apoptosis of B-CLL cells, in only a few cases have biological mechanisms been reported to have similar effects. It is now important to understand the role of these mechanisms in the pathogenesis and progression of B-CLL, and to devise strategies to exploit them for therapeutic use.

KEY WORDS: Apoptosis. Genes, bcl-2. Leukemia, B-cell, chronic.

Introduction

B-cell chronic lymphocytic leukaemia (B-CLL) is characterised by the relentless accumulation of a monoclonal population of mature-looking B lymphocytes in the peripheral blood and bone marrow.[1,2] The course of B-CLL is highly variable. In some patients the disease progresses rapidly, but a large number of patients will survive for many years with little or no treatment, and with a gradually increasing number of B-CLL cells in the circulation. Nevertheless, these patients develop a progressive disorder of the immune system, including hypogammaglobulinaemia and autoimmune haemolytic anaemia.[3]

Morphologically, B-CLL cells appear as small resting lymphocytes, with little or no evidence of proliferation *in vivo*. For this reason, B-CLL is often considered to reflect the accumulation of long-lived malignant B lymphocytes *in vivo*, arrested in the G_0 stage of the cell cycle.[4] However, when B-CLL cells are cultured *in vitro* they die rapidly by apoptosis.[5] A number of factors, including co-incubation with cytokines, can rescue B-CLL cells from cell death, and the role of these mechanisms is important in the understanding of the behaviour of the disease *in vivo*.

Phenotype

B-CLL cells have a characteristic phenotype. They express B-lineage markers shared with normal and malignant B cells, such as CD19, CD20 and CD37, but express low levels of CD22 and surface immunoglobulin M (IgM). They also express MHC Class II and the co-stimulatory molecule CD40.[6] In addition, B-CLL cells also express markers often associated with activation, such as CD5 and CD23.

CD5 is found on normal T cells, a subset of normal B cells and can also be induced on activated normal B cells. There is still no consensus as to whether BCLL corresponds to a malignant proliferation of a CD5-positive subset of B cells or to a malignant population of B cells that has acquired CD5 expression. CD5 is also expressed on mantle cell lymphoma cells, but this is clearly a different disease, both clinically and immunologically.[7]

B-CLL cells also express CD23, a B-cell activation marker, and high levels of soluble CD23 are found in the serum of patients with B-CLL. CD23 expression is lost on B-CLL cells following incubation in culture, and evidence suggests that CD23 expression is regulated by interactions with other cell types, such as activated T cells.[8]

B-CLL cells also express CD25, which further suggests an activated phenotype. Evidence that B-CLL cells have received activation signals prompted the study of external stimuli that may lead to proliferation of the malignant clone.

Cell proliferation

Most B-CLL cells appear to be in the G_o stage of the cell cycle, and therefore not actively proliferating. However, in order for the number of B-CLL cells to increase during the course of the disease, a proportion of the cells must be able to proliferate. These are probably not circulating in the peripheral blood but found in the pseudo-follicles seen in the majority of B-CLL patients.[9] However, it is difficult to stimulate B-CLL cells to proliferate *in vitro*, and B-CLL cells are hyporesponsive to a variety of signals that stimulate proliferation of normal B cells in culture.

A number of strategies have been used to induce

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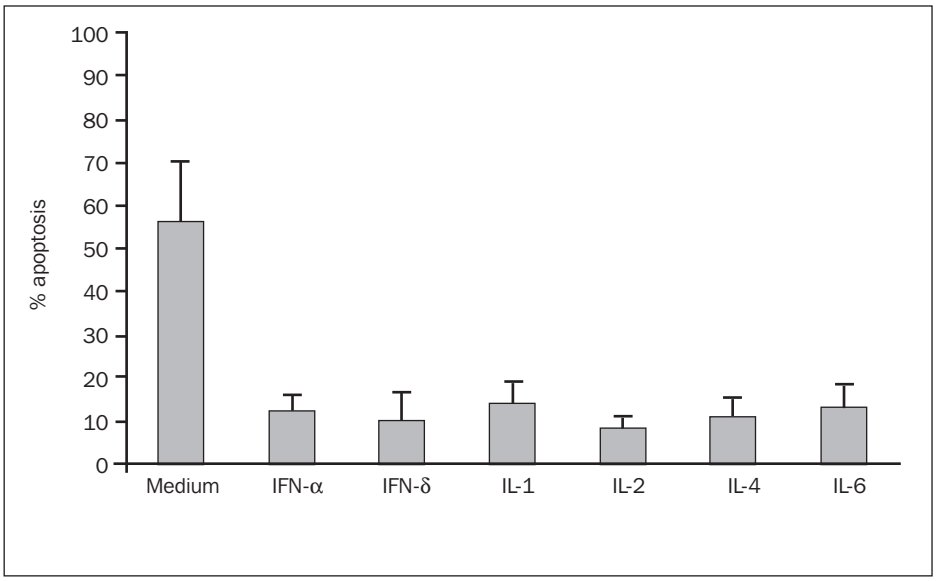

Fig. 1.
Inhibition of apoptosis in B-CLL cells by co-incubation with cytokines, added at the following concentrations: interferon-α, 500 units/mL; interferon-γ 500 units/mL; IL-1, 10 units/mL; IL-2, 500 units/mL; IL-4, 1 ng/mL; IL-6, 10 ng/mL

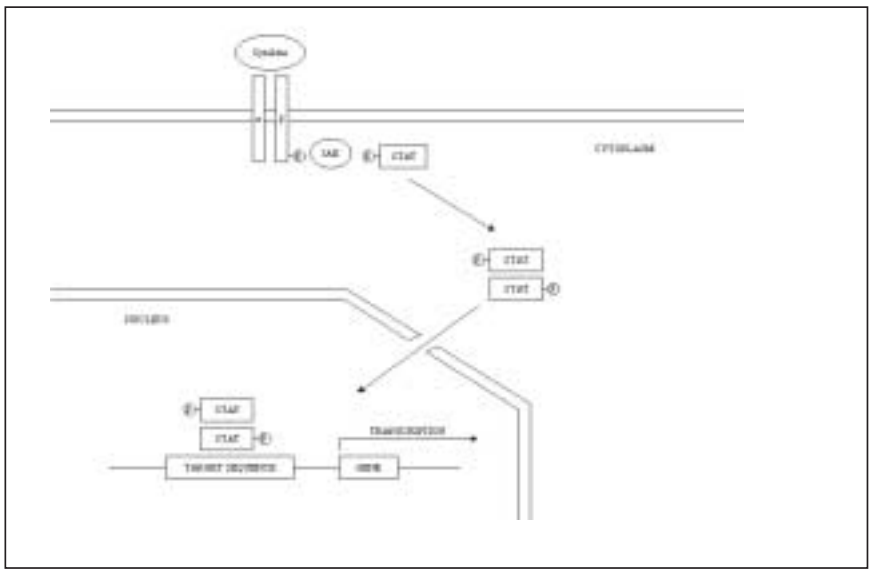

Fig. 2.
Model of signalling through cytokine receptors. Binding of cytokine to the heterodimeric receptor leads to activation of JAK kinases. These phosphorylate STAT signal transduction molecules, which dimerise and migrate to the nucleus. They then bind target sequences and activate transcription. Different cytokine receptors utilise different combinations of JAK and STAT family members.

proliferation in B-CLL cells *in vitro*, including crosslinking of immunoglobulin and stimulation with interleukin-2 (IL-2), co-incubation with tumour necrosis factor (TNF) and direct contact with CD4-positive T cells.[10,11] However, B-CLL cells remain hyporesponsive compared to normal B cells. These results may also be difficult to interpret, as it is difficult to isolate pure populations of B-CLL cells, and residual normal B or T cells in a heterogeneous system may lead to confusion.[12]

Apoptosis

Apoptosis (programmed cell death) is an important physiological process that helps to regulate the normal levels of all haemopoietic cells. Cell division and differentiation are balanced by apoptosis, which shows a number of distinct morphological features, including a decrease in cell volume, chromatin condensation and the formation of membrane-bound apoptotic bodies.

B-CLL cells show a high apoptotic rate when cultured *in vitro*, but in the past few years a number of groups have observed that co-incubation of B-CLL cells with a number of different cytokines can inhibit apoptosis (Figure 1). Some of these cytokines (e.g. IL-1, IL-6, IL-8, IL-10 and interferon-γ) are known to be produced by B-CLL cells, and presumably can act in an autocrine fashion.[13] Others, including IL-4 and IL-13, that protect B-CLL cells against apoptosis are not thought to be produced by B-CLL cells, and are considered to be the product of interactions with other cell types such as activated T cells. Interferon-α, which protects B-CLL cells against apoptosis *in vitro*, has not been shown to be produced by B-CLL cells, but is considered to be produced by all nucleated cells in response to viral infection, and may therefore also act in an autocrine fashion.[14]

Both interferon-α and IL-4 have been used therapeutically to treat patients with B-CLL and have led to a reduction in the number of circulating B lymphocytes in a small number

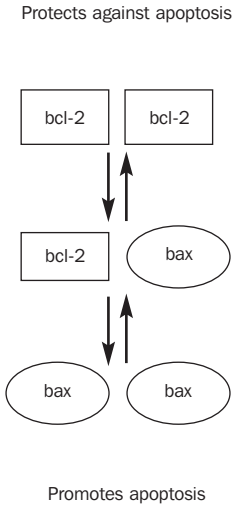

Fig. 3. A simplified model of the relationship between bax, bcl-2 and apoptosis. Dimers of bcl-2 protect against apoptosis but, as bax levels increase, bax dimers increase and apoptosis is promoted.

of patients, presumably through an unrelated mechanism. These cytokines share receptor homology in the cytokine receptor family and utilise a common family of signal transduction molecules (JAK kinases and STAT) (Figure 2). In some cases, this suggests that unrelated receptors may activate common sets of genes within the cells and lead to similar functional consequences. However, there is no evidence that signal transduction pathways are disrupted in B-CLL cells.[15]

Some agents (e.g. corticosteroids and gamma-irradiation) can induce apoptosis in B-CLL cells, both of which have been used to induce remission in patients with B-CLL.[14] Cytokines such as interferon-α and IL-4 can also protect against apoptosis induced by these agents. Furthermore, the new generation of nucleoside analogues, such as fludarabine, also appear to exert a clinical effect by inducing apoptosis in B-CLL cells.[16] Two groups have reported that IL-5 and IL-10 can induce apoptosis of B-CLL cells in culture, but other groups have reported conflicting results using these cytokines.[17]

Role of bcl-2 family proteins

The bcl-2 family of proteins are important regulators of apoptosis in mammalian cells. In B-CLL cells, bcl-2 is expressed at high levels and this favours protection against apoptosis. The level of bcl-2 expression does not correlate with disease progression, but the level of bax (a pro-apoptotic family member) is increased in non-progressive disease. This suggests that bcl-2:bax ratios may correlate with disease progression.[18]

Several groups have correlated the sensitivity of B-CLL cells to drug treatment with bcl-2 expression, but others have shown that bcl-2-independent mechanisms may also be important.[19] Apoptosis of B-CLL cells in culture is associated with decreased bcl-2 expression. Incubation with cytokines that inhibit apoptosis is, in most cases, associated with maintained bcl-2 expression.[14] This suggests that the resistance to apoptosis by the malignant cell clone in B-CLL is not an intrinsic property, but is determined by interactions with other components of the immune system, such as cytokines or cell-mediated mechanisms.

Cell:cell interactions

In addition to being responsive to cytokine-mediated signals, B-CLL cells are also responsive to cell-mediated signals. B-CLL cells express a range of adhesion molecules that determine the tissue distribution of the cells and regulate the ability to recirculate through different immunological compartments.[20]

As cells migrate through tissues, they may interact with other cell types. Interaction with bone marrow stromal cells has been reported to protect B-CLL cells against apoptosis,[21] and direct cellular interaction with CD4-positive T cells to induce proliferation.[11]

In addition, cells may regulate apoptosis through Fas/Fas ligand interactions. Although B-CLL expresses low-levels of Fas, it can be induced to up-regulate Fas by incubation with interferon-α or -β. However, these cells remain resistant to Fas-mediated apoptosis. Furthermore, it has been suggested that B-CLL cells may express and secrete Fas ligand and induce apoptosis in Fas-bearing T cells and hence escape immune surveillance.[22]

Conclusions

It seems likely that the accumulation of the malignant clone in B-CLL is at least partly dependent on the dysregulation of apoptosis, leading to prolonged cell survival. This, however, is not an intrinsic function of B-CLL cells and depends upon continued stimulation by cytokines and cell interactions.

Strategies to disrupt autocrine and paracrine survival pathways may lead to improved clinical management of the disease. Finally, although prolonged cell survival is important in disease progression, it should be remembered that a proliferative response must be fundamental to the disease process and that this may be important in the understanding of this intriguing disease. □

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