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Apoptosis in the Haemopoietic System

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Apoptosis in the haemopoietic system

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SUMMARY

Our previous studies have shown that haemopoietic stem cells undergo apoptotic death as a consequence of growth factor withdrawal. In this paper we review the new data that has accumulated since this observation and compare it with older data from the 'pre-apoptotic' age. Models of erythropoiesis and granulopoiesis that incorporate apoptosis as a normal physiological process controlling homeostasis are examined. The converse to cell death is cell survival, and we describe experiments which suggest that haemopoietic growth factors can not only act as mitogenic or differentiation stimuli but also act as survival signals. We, and others, have proposed that these growth factor-induced survival signals act through the membrane bound polypeptide receptors and share common features of signal transduction with proliferative responses. Enforced expression of *bcl-2* in haemopoietic stem cells is able to overcome apoptosis following the withdrawal of growth factor, and the cells commit into different lineage differentiation programmes. Such cells spontaneously differentiate without cell division, suggesting a stochastic model of haemopoiesis in which the major role of haemopoietic growth factors is to suppress apoptosis and act as mitogens. We review the evidence that the underlying causes of some haematological diseases may be associated with change in the balance between cell survival and death.

1. INTRODUCTION

Blood cells arise from a small number of pluripotent stem cells that are found in the bone marrow. The process is controlled by a range of cytokines which individually bind to their own or shared receptors and thereby modify the proliferation, differentiation and maturation of these cells. These cytokines include interleukin-1 (IL-1) to interleukin-11 (IL-11), erythropoietin (EPO) and the haemopoietic cell colony-stimulating factors (CSFs), stem cell factor (SCF) (also known as *kit* ligand or mast cell growth factor), transforming growth factor β (TGF-β), macrophage inflammatory protein 1α (MIP-1α) and various others, such as interferons and insulin-like growth factors (IGF) (Heyworth *et al.* 1990; Broxmeyer 1992; Metcalf 1993). For many years the emphasis of haemopoietic growth factor research has been on the coupled proliferative and differentiation functions of these proteins, and most molecular studies are aimed at establishing the precise mechanisms by which individual receptors transduce these different signals. However, another underlying, and more subtle, role of growth factors in haemopoietic cell regulation is as survival factors (see table 1).

Since the general acceptance of apoptosis as a mechanism of cell death and the observation that haemopoietic cells undergo apoptotic death (Williams *et al.* 1990) as a consequence of haemopoietic growth factor redrawal, models of haemopoietic cell regulation have been developed that incorporate this feature (Koury 1992). Moreover, we have previously suggested that cell death is one of the normal physiological processes that controls homeostasis within the haemopoietic system (Dexter *et al.* 1986).

2. ERYTHROPOIESIS

Erythropoietin (EPO) is a 30–34 kDa glycoprotein produced by the kidney, with smaller amounts made in the foetal and neonatal liver and by resident macrophages in adult tissues. It acts as the primary regulator of mammalian erythropoiesis, stimulating both the proliferation and differentiation of immature blast-forming units-erythroid (BFU-E) and their more-mature progeny, the colony-forming units-erythroid (CFU-E). A third role for EPO is that of a survival factor; it acts on all EPO-responsive cell compartments and withdrawal of growth factor results in apoptotic death (see figure 1). Unlike most of the other haemopoietic growth factors, EPO circulates in the blood, its production being controlled by a feedback mechanism through kidney hypoxia. In circumstances where there is a deficiency in erythrocytes (anaemia) EPO production in the kidney is enhanced, whereas in polycythaemic conditions EPO production is reduced.

Early observations showed that the EPO responsiveness of mice maintained in a polycythaemic condition for more than 40 days by red blood cell transfusions was similar to that seen in normal mice. Furthermore the cycling rate of the EPO responsive

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Table 1. *The identified survival and apoptotic characteristics of various haemopoietic growth factors*

(Abbreviations are defined in the text. (?) signifies unknown.)

growth factor	proliferation	survival	apoptotic activity
IL-1	+	+	−
IL-2	+	?	−
IL-3	+	+	−
IL-5	+	+	−
IL-6	+	+	−
GM-CSF	+	+	−
G-CSF	+	+	−
M-CSF	+	?	−
EPO	+	+	−
SCF	+	+	−
MIP-1α	−	?	−
IFN-γ	−	+	−
FAS antigen	?	?	+
TGF-β	−	−	+

cells, as measured by [^{3}H]-thymidine killing, also remained unchanged. These results indicate that continuous production and amplification of EPO-responsive progenitor cells continues for long periods in the absence of mature erythrocyte demand (Lord 1976; Wangenheim *et al.* 1977) and furthermore that the size of this population is not changing. It was noted, at the time, that marrow from such animals showed a high degree of spontaneous cell death (B. I. Lord, personal communication). An anecdotal report of two untreated polycythaemia vera patients also showed substantial numbers of phenotypically normal primitive erythroid progenitor cells which failed to mature (Eaves & Eaves 1978). Such results led to the speculation that EPO was required for not only the late-stage erythroid progenitor cell development but more importantly their continued survival. This EPO-dependent period extends from at least the CFU-E stage to the stage at which haemoglobin synthesis begins. Koury & Bondurant (1990) demonstrated that the EPO-dependent cells, isolated from the spleens of mice infected with anaemia-inducing strain of Friend leukaemia virus (FVA cells), deprived of EPO, accumulated DNA cleavage fragments characteristic of those found in apoptotic cells by 2–4 h and began dying by 16 h. In the presence of EPO, the progenitor cells survived and developed into reticulocytes. These and other data led Koury (1992) to propose a general model of blood cell production controlled by the suppression of apoptosis by haemopoietic growth factors. Although this 'supply and demand' model (see figure 1) fits most of what we know about EPO-controlled erythropoiesis, it is dependent on a growth factor produced distal to the bone marrow.

Earlier studies of various EPO-responsive progenitor cell populations indicated that, within a mixed population of erythroid progenitor cells, there can be large differences in EPO sensitivities (Eaves & Eaves 1978). These results were supported by EPO binding studies on purified murine CFU-E and their descendants (Landschulz *et al.* 1989). Several observations arose from this study. First, EPO bound on these cells is in rapid flux having a cell surface half-life for [^{125}I]-EPO internalization of around 5 min. Second, repeated occupancy of the EPO-receptor (EPOR) is required for mitogenic response. Third, there is an apparent disappearance of high-affinity sites and a persistence of low-affinity sites as the cells mature. The authors suggested that at least two gene products mediate EPO-binding. When the cloned cDNA for

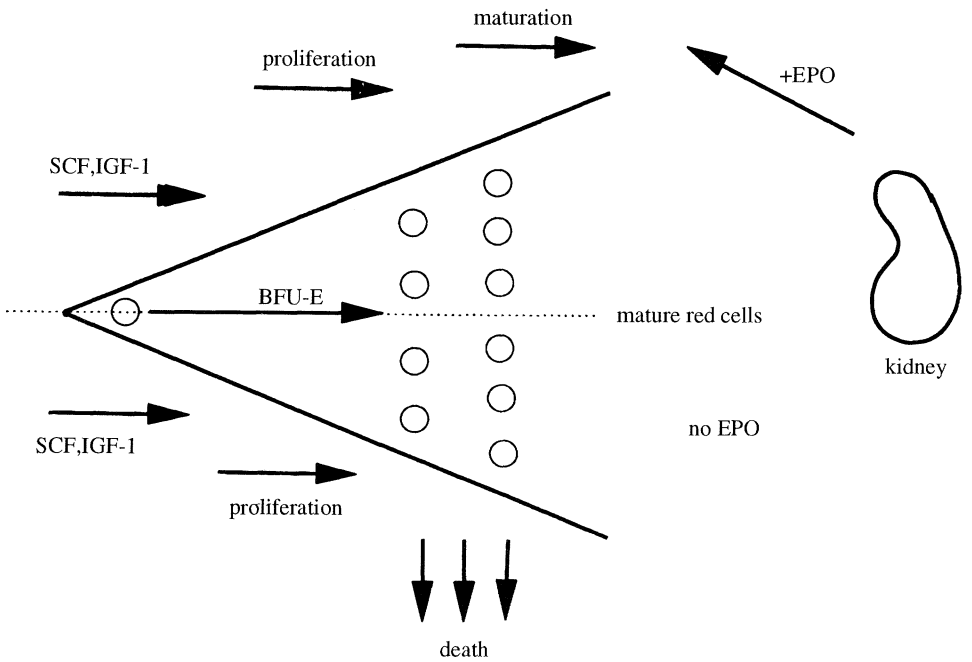

Figure 1. A model of erythropoiesis. Primitive cells (left) can proliferate under the influence of SCF, IGF-1, other conditioning factors and EPO in normal haemopoiesis (top half of diagram), but in absence of EPO (through hypoxic feedback of EPO production or disease), the BFU-Es cannot further proliferate or mature and as a consequence die by apoptosis (bottom half of diagram).

the EPOR (derived from murine erythroleukaemic cells with only low-affinity receptors) is transfected into monkey kidney cells that have no EPO receptors, both high- and low-affinity receptors are found (D'Andrea *et al.* 1989). This observation and other experiments (Dong & Goldwasser 1993) suggest that an unknown accessory protein or co-factor is required for high-affinity EPORs. These high-affinity sites appear to mediate the growth function of EPO on early progenitor cells and the lingering low-affinity receptors have, as yet, no recognized growth or differentiation function. While it is tempting to speculate that such low-affinity receptors may have a role to play in transducing a survival signal, this has yet to be investigated.

In the EPO-dependent cell line HCD-57, EPO can act as both a mitogen and a survival factor, even in cells that are not terminally differentiating (Spival *et al.* 1991). What of other growth factors which allow the survival of erythroid progenitor cells? Using purified murine CFU-E, IGF-1 (when EPO levels are low) also protected the most primitive cells against apoptosis but lost effectiveness with maturity (Boyer *et al.* 1992). IL-3 and SCF alone have no effect in reducing apoptosis in murine foetal liver cells but, when combined with EPO, enhanced activity was seen (Yu *et al.* 1993). In human erythroid progenitors, apoptosis is decreased by SCF and IGF-1 as well as EPO (Muta & Krantz 1993).

3. GRANULOPOIESIS

The dependence of early progenitor cells and mature cells (such as neutrophils and eosinophils) on exogenous growth factors proved to be a sensitive microassay for colony stimulating factors (Begley *et al.* 1986). Furthermore, the observation that the removal of growth factor, at any stage of the haemopoietic development programme leads to a cessation of growth and to death of the developing clone, led to the suggestion that programmed death has a physiological significance in haemopoiesis (Dexter *et al.* 1986). This was later shown experimentally by demonstrating that the withdrawal of the relevant CSF from haemopoietic precursor cell lines such as FDCP-1 and FDCP-Mix, results in active cell death with morphological features and nucleosomal DNA laddering patterns characteristic of apoptosis (Williams *et al.* 1990). Death was not immediate, but took place over a 20–30 h period and viability could be maintained for this period of time by the addition of cycloheximide. Moreover, sublines of FDCP-Mix that were dependent on either IL-3, GM-CSF or G-CSF, behaved in a similar fashion when deprived of growth factor. These data led to the first proposal that apoptosis is a positive control mechanism that regulates haemopoietic precursor cell survival. Many similar studies followed in which both rodent and human haemopoietic cells as well as leukaemic cell lines were also observed to undergo apoptosis in absence of the various growth factors.

That the ability of growth factors to suppress apoptosis is not restricted to normal or leukaemic cell lines, but also operates on normal early progenitor cell populations, has been shown by several groups. For example, the *c-kit* ligand (stem cell factor (SCF)) has been shown to directly act on highly enriched committed murine progenitor cells in serum-deprived conditions to promote survival, proliferation and development (Heyworth *et al.* 1992); murine mast cells undergo apoptosis on removal of IL-3, an event that is prevented by the addition of SCF, suggesting that these factors act in concert on mast cells (Mekori *et al.* 1993); G-CSF acts as a survival factor in mature neutrophils (Yamamoto *et al.* 1993, Lee *et al.* 1993; Haslett *et al.*, this volume): insulin-like growth factor 1 (IGF-1) has also been shown to delay apoptosis in myeloid precursor cell lines (Rodriguez-Tarduchy *et al.* 1992). These and other data have led us to propose the model outlined in figure 2.

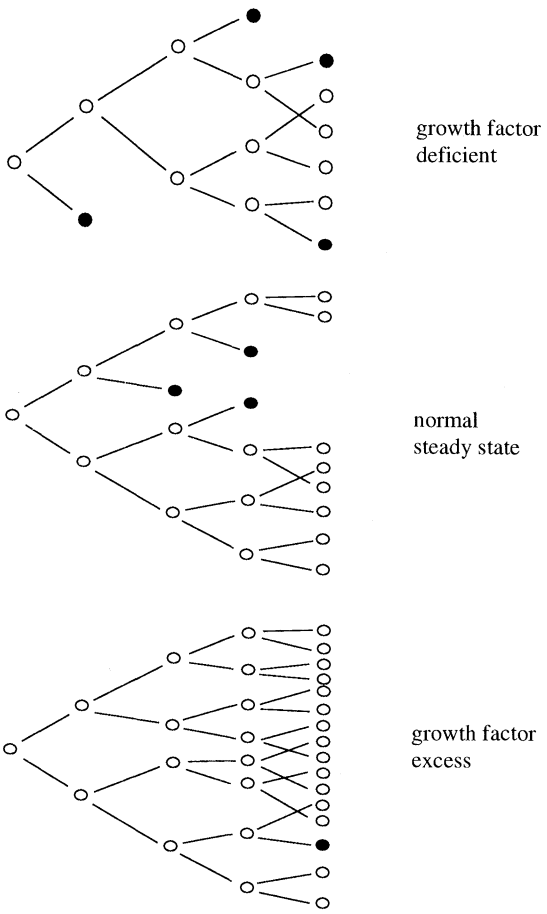

Figure 2. Proposed models of haemopoiesis when growth factor concentration is deficient (top), normal (middle) and in excess (bottom). Open circles are viable cells and closed circles are apoptotic cells. In the primitive compartment of haemopoiesis, stem cells can probably undergo limited self renewal. Withdrawal of the growth factors known to act on the very primitive cell population can lead to apoptosis and therefore such stem cells can die in the absence of growth factor (see top). We speculate that the stroma plays an important role in preventing this happening to any great extent. As stem cells are recruited into the progenitor compartment, they undergo amplification, at each stage (or division) cells can either withdraw from proliferation and differentiate (assuming that the lineage commitment had occurred in the stem cell that they were derived from) or, in the absence of growth factor, die by apoptosis.

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Of the protein factors that are known to be growth inhibitory to haemopoietic stem cells, interferon-gamma (IFN-γ) in the presence of either IL-3 or GM-CSF or M-CSF inhibited highly enriched mouse granulocyte-macrophage colony-forming cells (GM-CFC) forming colonies in soft agar with an overall increase in macrophage differentiation (Kan *et al.* 1991). However, in the absence of growth factors, IFN-γ acted as a survival factor and activated the Na^+/H^+ antiport system suggesting that this factor has a dual effect on GM-CFC, decreasing the rate of death but also limiting the proliferative response to CSFs. Members of the IL-8 family of genes including RANTES, MIP-1β and MIP-1α are known to inhibit the growth of haemopoietic cells by a mechanism which involves removal from the cell cycle (Lord *et al.* 1993). The receptor for MIP-1α has been recently reported and its structure suggests that a heterotrimeric G protein may be involved in its signal transduction (Neote *et al.* 1993). Although acting as a haemopoietic cell growth inhibitory factor, it is unclear whether its mechanism involves the suppression of apoptosis.

Transforming growth factor-β1 (TGF-β1) is an ubiquitous cytokine which inhibits the growth of a wide range of cell types including haemopoietic cells *in vitro* (Hino *et al.* 1988; McNiece *et al.* 1992). Although the actions of TGF-β1 on myeloid cells appear complex, with differing responses depending on the cell population, at least some of the effects of TGF-β can be attributed to its ability to induce apoptosis in normal myeloid precursors (Lotem & Sachs 1990) and myeloid leukaemic cell lines (Lotem & Sachs 1992). It is also intriguing that G-CSF, IL-6 and to a lesser extent IL-1 can inhibit this TGF-β induced apoptosis in at least some cells. In addition, varying patterns of response to TGF-β1 were shown by growth-factor-dependent human AML cell lines as well as primary AML cells. These different responses were shown to be due to differences in the TGF-β1-induced apoptosis of AML-target cells (Taetle *et al.* 1993).

4. SURVIVAL VERSUS PROLIFERATION SIGNALS

IL-3 can stimulate the activation of an amiloride-sensitive Na^+/H^+ exchange via protein kinase C activation and it was suggested that the resulting increase in intracellular pH acts as a signal for cellular survival and proliferation in myeloid progenitor cells (Whetton *et al.* 1988; Cook *et al.* 1989; Rodriguez-Tarduchy *et al.* 1990). Recent studies, using IL-3 and GM-CSF cell lines and combinations of PKC activators and inhibitors, have further suggested that the sequential activation of PKC and of the Na^+/H^+ antiporter result in the suppression of apoptosis in target cells (Rajotte *et al.* 1992). Little is known of associated cytoplasmic signalling proteins in these processes but is interesting to note that in one report the inhibition of *c-fes*, a member of the cytoplasmic tyrosine kinase family, by antisense oligomers, was shown to increase the rate of apoptosis in HL60 cells following chemical induction to granulocyte differentiation (Manfredini *et al.* 1993). Because of the great diversity of growth factors known or thought to act as suppressors of apoptosis and the differing signal transduction pathways each of these factors use, the 'survival signal' is probably a general cellular change. However, although the activation of the Na^+/H^+ antiport system and the subsequent alkalinization provides an attractive mechanism, such changes are also associated with growth-factor-induced proliferation of cells and it is not known what distinguishes a 'proliferative signal' from a 'survival' one. What is clear, however, is that the survival signal can be uncoupled from proliferation, as low concentrations of growth factors such as M-CSF can promote the survival (but not the proliferation) of target cells, while higher concentrations promote survival and proliferation (Stanley & Guilbert 1981; Tushinski *et al.* 1982). To date, detailed biochemical studies that give a molecular definition of this distinction between survival and proliferation signal pathways have yet to be performed.

(*a*) *Survival effectors*

Little is known of the effector molecules involved in transduction of signals that suppress apoptosis of haemopoietic cells. The inappropriate expression of certain genes, in cells known to undergo apoptosis when deprived of growth factor, has begun to point to their involvement in growth factor-dependent survival. The induction of *c-myc* gene expression is an immediate early response to mitogenic signals and its expression is decreased in response to growth factor deprivation or growth inhibitors. Although the precise biochemical function of *c-myc* still remains unclear, the downregulation of *c-myc* is a required event for cells to withdraw from the cell cycle and either enter differentiation or a quiescent state. Enforced expression of *c-myc* drives cell cycle progression and blocks differentiation whereas reducing *c-myc* expression (by antisense constructs) blocks progression into S phase and promotes differentiation. In both haemopoietic and other cell systems, it has been shown that failure to down regulate *c-myc* expression in response to IL-3 deprivation prevents cells from undergoing G1 arrest and accelerates apoptosis (Evan *et al.* 1992; Askew *et al.* 1993; Selvakumaran *et al.* 1993). On the other-hand, in some cell systems, HL60 (Beere *et al.* 1993) and lymphoid cell-line CCRF-CEM (Yuh & Thompson 1989) *c-myc* expression is not a prerequisite for apoptosis. Although another nuclear protein, p53, when overexpressed as the wild-type form was reported to induce apoptosis in a range of cell types including haemopoietic cells (Yonish-Rouach *et al.* 1991), this effect was not seen in other cell systems expressing high levels of wild-type p53 (see also Lane, this volume).

The *bcl-2* proto-oncogene has been widely associated with the prevention or delay in apoptosis, in response to different apoptotic stimuli, in a variety of haemopoietic cells. A related gene, *bcl-x*, has been isolated and the protein product of the longer mRNA

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form can also prevent apoptosis following growth factor withdrawal in IL-3-dependent haemopoietic cells in a similar fashion to *bcl-2* after growth factor withdrawal whereas expression of the shorter messenger RNA product, *bcl-*x_S, displays an inhibitory effect on the anti-apoptotic activity of both *bcl-*x_L and *bcl-2* (Boise *et al.* 1993). Expression of *bcl-2* prevents apoptosis in response to IL-3, IL-4 and GM-CSF withdrawal (Vaux *et al.* 1988; Nunez *et al.* 1990). Cell death induced by *c-myc* is also inhibited by *bcl-2* (Bissonnette *et al.* 1992; Fanidi *et al.* 1992).

(*b*) *Differentiate or die*

Enforced expression of *bcl-2* allows cells to survive in a variety of conditions where they would normally die via apoptosis. We have used the enforced expression of the human *bcl-2* gene to overcome the apoptosis that is seen in the murine myeloid progenitor stem cell line, FDCP-mix, after withdrawal of IL-3 and other cytokines (Williams *et al.* 1990). To our surprise, when progenitor stem cells are unable to either proliferate (no IL-3) or die through apoptosis (high expression of *bcl-2*), we found that the cells instead committed into one of the lineage differentiation programmes, i.e. differentiation by 'default' (Fairbairn *et al.* 1993). By monitoring the fate of single cells with time it was established that proliferation was not required for differentiation and that it was not dependent on the constitutive production of several growth factors or serum components. In other words, one conclusion from these experiments is that the major role for haemopoietic growth factors is to suppress apoptosis and act as mitogens and that they are not required for differentiation. Although such data support the stochastic models of haemopoietic development (Nakahata *et al.* 1982; Suda *et al.* 1984), this does not, however, rule out a role for haemopoietic growth factors in modifying the choice of lineage pathway at an early stage of development. Furthermore our ability to uncouple the processes of proliferation, survival and differentiation should allow further analysis of the molecular mechanisms involved in these processes.

5. APOPTOSIS AND HAEMATOLOGICAL DISEASE

Changes in the balance between cell survival and death have clear implications in underlying causes of some haematological diseases including anaemias such as β-thalassaemia (Yuan *et al.* 1993) and chronic myeloid leukaemia. In the latter, for example, the disease is associated with a chromosome translocation (9;22) that results in the expression of a chimeric *bcr-abl* gene product that has elevated tyrosine kinase activity and which appears to be essential for transformation of cells *in vitro*. Although expression of the cellular *c-abl* gene is not critical for development (Tybulewicz *et al.* 1991), the transfection of murine myeloid stem cells with temperature sensitive mutants of the p210 *bcr-abl* gene (Carlesso *et al.* 1994) and p160 *v-abl* gene (Spooncer *et al.* 1994) has shown that expression of *abl* is associated with a reduced rate of apoptosis at low growth factor levels and an exaggerated proliferative response to low levels of growth factor.

Work from this laboratory also shows that expression of the *v-abl* tyrosine kinase (at permissive temperature) in multipotent myeloid progenitor cells leads to a delay in maturation with a concomitant increase in cell production (Spooncer *et al.* 1994). Thus, a combination of delayed apoptosis and enhanced proliferative ability of cell populations in response to reduced growth factor levels may be the mechanism that provides human CML cells expressing *bcr-abl* with a selective advantage over their normal counterparts (figure 3). Although the biochemical mechanisms for this effect are not known, a general reassessment of the role that apoptosis plays in other haematological diseases is clearly warranted.

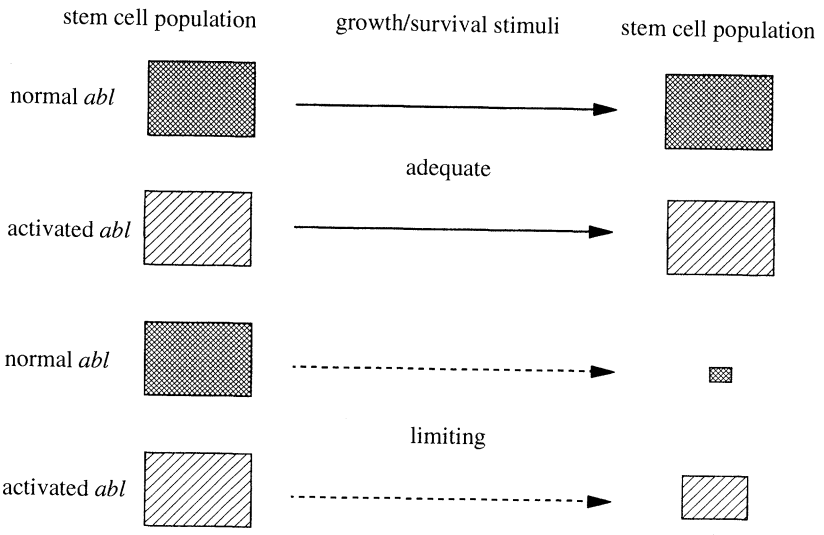

Figure 3. The effect of normal and activated *abl* expression combined with either normal or limited growth factor on the size of the stem cell populations.

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