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REVIEW

The Haemopoietic Stem Cell: Between Apoptosis and Self Renewal

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Self renewal and apoptosis of haemopoietic stem cells (HSC[†]) represent major factors that determine the size of the haemopoietic cell mass. Changes in self renewal above or below the steady state value of 0.5 will result in either bone marrow expansion or aplasia, respectively. Despite the growing body of research that describes the potential role of HSC, there is still very little information on the mechanisms that govern HSC self renewal and apoptosis. Considerable insight into the role of HSC in many diseases has been gained in recent years. In light of their crucial importance, this article reviews recent developments in the understanding of the molecular, biological, and physiological characteristics of haemopoietic stem cells.

STEM CELL: BACKGROUND AND PROPERTIES

The most primitive haemopoietic cells are the haemopoietic stem cells (HSC). They are defined as cells with a high potential for self renewal and possess the capacity for dividing into identical copies of themselves without forming any newly differentiated features. Because most mature blood cells have a very short life span, the importance of HSC in sustaining the life of the mammal — for example, through its ability to self renew — is very critical. Stem cells in both embryonic and adult tissues are defined by their ability to undergo self renewal and differentiation in a balanced state with-

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[†]Abbreviations: HSC, haemopoietic stem cells; HEV, high endothelial venules; H-CAM, homing-associated cell adhesion molecule; SP, side population activity; CFU-GEMM, colony-forming units granulocyte, erythroid, monocyte, and megakaryocyte; CFU-G, colony-forming unit granulocyte; CFU-M, colony-forming unit megakaryocyte; pSR, probability of self renewal; pDiff, probability of differentiation; BM, bone marrow; ROS, reactive oxygen species; ESC, embryonic stem cells; Epo, erythropoietin; FasL, Fas ligand; NBM, normal bone marrow; RMGT, retroviral-mediated gene transfer; CML, chronic myelogenous leukemia.

out depleting the stem cell pool. If a progenitor can divide (and in certain circumstances can generate a secondary colony), it does not mean that it is capable of self renewal. All myeloid progenitor cells, except HSC, will terminally differentiate within two months or sooner. In contrast, HSC are capable of maintaining haemopoiesis during the life of the animal or longer if transplanted. So self renewal implies immortality at least within a reasonably long period of time, even as far as lifespan. All other progenitors that eventually extinguish do not self renew; all their daughters are of a progressively decreased quality (in terms of proliferative potential) and, therefore, cannot be considered as copies of the original cell. This topic is currently under huge debate.

HSC are capable of differentiating into at least eight cell lines. The balance between self renewal and differentiation is considered to be critical to the maintenance of stem cell numbers [1-3]. More importantly, stem cells in general are proposed to have a major role in curing many degenerative diseases and cancers [4-7]. Significant efforts have been made in recent years in understanding the mechanism governing HSC generation, self renewal, proliferation, and commitment. Yet understanding the overall process is still far from complete and largely hypothetical. A growing body of research suggests that pathways regulating the self renewal of normal stem cells are dysregulated in cancer stem cells, resulting in continuous expansion of self renewal and tumor development and, therefore, offering hope that new cancer therapies may emerge via this approach [4-10].

Most stem cells are in G_0 phase of the cell cycle, and, therefore, only a small number of stem cells are responsible for stem cell maintenance and for producing mature cells at any specific time [3,11]. Experimental work has indicated that a single primitive progenitor may survive in a quiescent state for more than two weeks in culture before it divides [1-2,12-14].

Stem cells are characterized by the expression of CD34 and Thy1 and absence of CD38, CD33, and HLA-DR [15-17]. These cells also lack expression of a great number of markers that are expressed on mature blood cells (lineage negative), and that lineage negativity is as important as other criteria for identifying and isolating these cells. Human HSC are CD34+, while murine HSC are CD34-. CD34 is a transmembrane glycoprotein, expressed in immature haemopoietic cells, fibroblasts, vascular endothelium, and high endothelial venules (HEV) [18-19]. CD34 is a stem and progenitor cell marker in humans. It contains two sites for serine/threonine phosphorylation by protein kinase C (PKC) and a tyrosine phosphorylation site, implying a possible role in signaling [20]. In addition, endothelial CD34 binds to the lectin-like adhesion molecule L-selectin [21]. Surprisingly, however, experimental work on CD34 deficient mice has revealed no major abnormalities either in haemopoiesis or in interactions of haemopoietic progenitor cells with stromal cells [22]. It is well established that even more primitive progenitor cells are present within the CD34 negative fraction [23]. Additionally, haemopoietic progenitor cells express adhesion molecules such as L-selectin [24], integrins [25], and homing-associated cell adhesion molecule (H-CAM) [26]. Alternative methods to isolate HSC include side population activity (SP) or rhodamine efflux, both of which were recently superseded by new markers such as Slamf1 (CD150), or endoglin.

COMMITTED PROGENITOR CELLS: BACKGROUND AND PROPERTIES

This compartment includes stem cell progeny that have been identified by their ability to form colonies of morphologically recognizable cells in semi-solid cultures. In addition, progenitor cells are characterized phenotypically by expression of CD38, CD33, and HLA-DR, and a greater proportion of them are in an active cell cycle compared with stem cells. It is generally assumed that progenitor cells can not self renew. However, considerable self renewal recently has been demonstrated experimentally *in vitro* by replating CFU-GM into secondary cultures [27]. This now has provided an informative clonogenic assay for demonstrating self re-

newal capacity [3]. The size of the progenitor's cell compartment is governed by the balance between the cell gain (self renewal) and the cell loss (apoptosis). Therefore, an imbalance in either of these parameters will result in an elevation or a fall in the progenitor cell mass. The colony-forming units granulocyte, erythroid, monocyte, and megakaryocyte (CFU-GEMM) are intermediate between stem cells and single lineage precursors. They are capable of producing granulocytes, erythrocytes, monocytes, and megakaryocytes. The more committed progenitor cells have only single lineage potential. These are colony-forming unit granulocyte (CFU-G), colony-forming unit macrophage (CFU-M), burst-forming unit erythroid (BFU-E), and colony-forming unit megakaryocyte (CFU-Meg) that produce granulocytes, macrophages, erythrocytes, or megakaryocytes, respectively [28-30].

MODELS OF HSC SELF RENEWAL

Stochastic theory

Stochastic is a term applied to processes that appear random but have associated underlying probability distribution. In the context of haemopoiesis, the fate of individual cells cannot be predicted. An important feature of stochastic processes is that they are associated with an overall probability of an event. Therefore, in haemopoiesis, there is a probability of self renewal (pSR) and a probability of differentiation (pDiff). When cells divide, the values of pSR and pDiff will be 0.5 for steady state haemopoiesis [31]. There is often a misconception that stochastic processes are random and, therefore, that stem cells can not respond to environmental factors. It is sometimes assumed that stochastic processes are regulated intrinsically.

In 1961, Till and McCulloch developed an assay that involved inbred strains of mice, in which the donor and recipient are genetically identical [32]. The recipient mice are irradiated to ablate all haemopoietic cells. Bone marrow cells from donor mice are then injected into the recipient mice. After 10 days, the mice are sacrificed, and the number of spleen colonies are counted. The number of colonies forming on the spleen of lethally irradiated mice correlated with the number of bone marrow (BM) cells transplanted, and different lineages were represented in the developing colonies. It is known from other works that not all CFU-S are capable of giving rise to secondary colonies. Other workers found that replating of erythroid colonies and blast cell colonies showed a similar distribution to that seen in CFU-S [33-34]. Abkowitz and colleagues have shown that stochastic effects may occur in haemopoiesis *in vivo* [35].

Deterministic model of haemopoiesis

This model suggests that decisions to self renew or differentiate are entirely controlled and that responses to a particular set of circumstances will be predetermined [12,36]. Morrison and Weissmann suggested a model based on the purification of three multipotent progenitor cells that had different self renewal capacities. The progenitors from the most primitive population showed a greater self renewal capacity than those from less primitive progenitors. They concluded that self renewal is a deterministic event based on the fact that cell separation could predict self renewal potential [37]. In the deterministic model, the haemopoiesis process is controlled by extrinsic factors such as cell-cell interactions within the haempoietic micro-environment or signalling by cytokines, and, therefore, the cell fate is determined. These obstacles have been reviewed by Metcalf and Enver and Heyworth and Dexter [38-39].

Gordon and Blackett have proposed the stochastic model with observations that cells with different self renewal capacities could be separated into subpopulations. They proposed that self renewal was a stochastic event, but the probability of this process could be modified by extrinsic factors [11].

KINETIC MODELS OF STEM CELL REGULATION

Two theories have been proposed to explain the ability of stem cells to maintain lifelong haemopoiesis. The first suggests that the

embryo has sufficient stem cells to maintain haemopoiesis throughout life. However, this concept fails to explain the ability of a bone marrow transplant (consisting of a small fraction of the total marrow) to restore haemopoiesis to normal levels after the recipient has been exposed to myeloablative therapy [40]. The second theory is that a small number of stem cells are able to sustain lifelong haemopoiesis because they are capable of self renewal when they divide. For steady state haemopoiesis, the probability of self renewal must be 0.5 and the probability of differentiation and/or loss by apoptosis must also be 0.5 [13-14]. Asymmetric division indicates a stem cell when it divides into one new stem cell and one differentiated cell, while symmetrical division is when one stem cell divides into two stem cells and another divides into two differentiated cells. Both models can account for steady state haemopoiesis. However, only the latter model can account for expansion or recovery of the stem cell pool following damage.

Several investigators have shown that the probability of stem/progenitor cell self renewal is not fixed. Metcalf demonstrated that G-CSF decreases the replating ability of the WEHI-3B colony-forming cells [41]. Additionally, Lewis and colleagues have reported that cytokines modify the self renewal kinetics of primary granulocytic and erythroid progenitor cells [42-44]. The control of stem cell numbers can be regulated by the loss of apoptosis, as well as by differentiation, from the stem cell population. However, there is a paucity of information on this point. Despite the wide acceptance and being publicized repeatedly at scientific meetings, still there is a need to re-examine some of the basic concepts in haemopoiesis. Quesenberry and Lowry suggested previously the important need to bring current models of stem cell self renewal and differentiation into line with experimental observations [45-46]. This necessity is further emphasized by the discovery that haemopoietic stem cells may differentiate into non-haemopoietic lineages such as muscle and neural cells [47-48].

The concept of stem cell compartmentalization is a cornerstone of HSC self renewal [49]. There are various known factors that contribute to stem cell functionality, including second messenger systems, transcription factors, and the type and number of growth factor receptors expressed. The interaction of these factors will determine the potential responses of those cells to both internal and external signals. Probability of self renewal is decreased as HSC progresses toward maturity. Ogawa and colleagues [50] pointed out that the heterogeneity of HSC and the inability to measure the self renewal probability of an individual HSC remain among the most significant limitations. It is impossible to measure precisely the self renewal capacity of an individual HSC at the same time as measuring its differentiation capacity. Therefore, the focus should not be on individual cells, but perhaps rather on populations of stem cells by using techniques that have been developed by Jankovic et al. [51]

While progenitor cells do not have the same self renewal capacity as the parent stem cells, they retain some capacity for further divisions. Therefore, the CFU-GM and BFU-E progenitor assays are very useful tools for studying haemopoiesis, and considerable evidence has been accumulated that this might be the case [52]. Till and colleagues developed a technique to detect HSC, and it is widely believed that it detects not only early but also more mature progenitor cells [32]. It has been demonstrated that replating colonies from murine blast cell colonies and committed progenitors such as CFU-GM and BFU-E results in secondary colony formation [27,33-34]. Additionally, murine B cell progenitors can undergo self renewal in the presence of stromal cells and interlukin-7 (IL-7) [53].

RELATIONSHIP BETWEEN APOPTOSIS AND SELF RENEWAL (I.E., PROLIFERATION)

HSCs are defined by their ability both to self renew and to give rise to any of the haemopoietic cell lineages throughout the lifetime of an animal [54]. To maintain haemopoietic homeostasis in a host, the HSC

numbers need to be precisely regulated. The fate decisions, including life and death and self renewal and differentiation, of HSCs are important processes that regulate the numbers and lifespan of the HSC pool in a host. Defects in these processes can contribute to haemopoietic insufficiencies and the development of haemopoietic malignancies [55]. Failure of HSCs to self renew during aging is believed to depend on several intrinsic (cell-autonomous) and extrinsic (non-cell-autonomous) factors. Products of numerous genes that are involved in either DNA-damage responses or longevity-related signaling contribute to the maintenance of the HSC self renewal capacity. Several intrinsic factors have been identified as potential mechanisms underlying the self renewal of HSC.

Cell cycle checkpoints induced by telomere dysfunction represent one of the major *in vivo* tumor suppressor mechanisms provoking age dependent decline in self renewal and regeneration of tissues and organs. Since stem cells are continuously proliferating throughout their lifetime, most stem cell compartments express telomerase. In stem cells, however, the level of telomerase activity is not sufficient to maintain telomere length during aging. Exhaustion of replicative potential of telomerase appears to be at least partially dependent on the cell cycle regulatory component of the DNA damage response. To overcome telomere activity, stem cells appear to have tighter DNA damage checkpoint control in comparison to somatic cells. These enhanced checkpoint responses may have a detrimental impact on stem cell function by causing increased sensitivity toward senescence or apoptosis induced by telomere shortening [56]. Stresses on stem and progenitor cell pools, in the form of telomere shortening or other genome maintenance failures, have been shown to lower tissue renewal capacity and accelerate the appearance of senescence. Therefore, long-term stem and progenitor cell potential depends on both the genome maintenance mechanisms that counter DNA damage and the cell cycle checkpoint responses to damage [57-58].

In addition to checkpoint factors, proteins that control the normal cell cycle have been shown to regulate stem cell fate. Cdk2 is a major regulator of S phase entry, is activated by mitogenic cytokines, and has been suggested to be involved in antigen-induced apoptosis of T lymphocytes. Analysis of the HSC compartment in mice deficient in Cdk2 revealed normal proportions of stem cells and progenitors. Furthermore, a competitive graft experiment on HSC deficient in Cdk2 showed normal renewal and multilineage differentiation [59], indicating that Cdk2 is not required for proliferation and differentiation of HSC *in vivo*. *In vitro* analyses, however, consider Cdk2 to be a major player in proliferation and apoptosis in HSCs. Therefore, further studies are required to analyze the contribution of this factor and a potential target for therapy.

Reactive oxygen species (ROS) also influence stem cell fate. It has been demonstrated that long-term self-renewing HSCs normally possess low levels of intracellular ROS. If intracellular ROS levels become excessive under pathological conditions, they cause senescence or apoptosis of stem cells and a failure of their self renewal. Correction of the intracellular levels of ROS in HSC by treatment with an antioxidant that scavenges ROS can rescue HSC self renewal [60]. Defining the molecular mechanisms that govern the ROS regulation and strategies that can control the excess levels of ROS could lead to the significant improvement in designing novel therapeutic approaches for haemopoietic diseases, regenerative medicine, and the prevention of leukemia.

There are several transcription factors, such as Foxo3a and Zfx, that play a role in stem cell self renewal. For example, Foxo3a is a forkhead transcription factor that acts downstream of the PTEN/PI3K/Akt pathway. It has been found in mice deficient in Foxo3a that the frequency of HSC is less than normal; the number of colony-forming cells is lowered and the ability of HSC to support long-term reconstitution of haemopoiesis is impaired; HSC showed increased phosphorylation of p38MAPK, an upregulation of ROS; there is defective maintenance of quiescence; and there is increased sensitivity to cell-cycle-specific

myelotoxic injury [61]. Taken together, these results demonstrate that Foxo3a is an important intrinsic factor role for maintaining the HSC pool.

Recent studies have shown that deletion of Zfx, another transcription factor, in murine HSC and embryonic stem cells (ESC) impairs their self renewal, resulting in increased apoptosis and upregulation of stress-inducible genes. By contrast, Zfx directly activated common target genes, including ESC self-renewal regulators Tbx3 and Tcl1, in ESC and HSC [62]. These studies identify Zfx as another factor that is critical for self renewal in embryonic and adult stem cells.

Evidence has accumulated concerning the participation of certain proliferation promoting factors (such as oncogenes) that are also able to act as potent triggers of apoptosis. Such factors include GM-CSF, Epo, Flt3, and Notch.

Recent experimental evidence also has suggested there may be a positive relationship between apoptosis and proliferation in normal haemopoiesis. For example, Traycoff et al. [63] showed that cord blood and NBM CD34+ cells with a high number of cell divisions in short-term cultures *in vitro* are associated with an increase in the percentage of apoptotic CD34+ cells. It has been demonstrated that both GM-CSF dependent and G-CSF dependent cell lines undergo rapid cell death after removal of the relevant CSF [64]. On the other hand, models proposed by Koury [65] imply that a reduction in apoptosis might result in an increase in proliferation, potentially resulting in an inverse relationship between cell death and cell division. Evidence for a link between apoptosis and proliferation (i.e., self renewal) in haemopoiesis remains unclear; we hypothesize that cell proliferation (i.e., self renewal) and cell apoptosis are linked in normal haemopoietic tissues and that this relationship may be abnormal in chronic myeloid leukemia (CML).

A possible role for apoptosis is illustrated by the relationship between erythropoietin (Epo) levels and erythropoietic activity *in vivo* in mice. When the Epo concentration is elevated due to an increase in demand (such as anaemia), many Epo-dependent progenitor cells that would otherwise rapidly undergo apoptosis will survive. If Epo levels are decreased by hyper-transfusion, Epo-dependent progenitor cells that would normally survive will die rapidly and erythrocyte production is reduced [66]. This hypothesis is supported by results using hyper-transfused mice, which show continual production of erythroid progenitor cells but with no increase in their number following the cessation of erythropoiesis, thus indicating the direct involvement of cell death [66].

Flt3 is a member of the receptor tyrosine kinase family, which plays a critical role in maintenance of haemopoietic homeostasis. Recently, it has been found that the human HSC in both the bone marrow and the cord blood that are capable of long-term reconstitution in xenogeneic hosts uniformly express Flt3. Detailed analysis of Flt3 expression shows that it is expressed not only in early lymphoid progenitors, but also in progenitors continuously along the granulocyte/macrophage pathway, including the common myeloid progenitor and the granulocyte/macrophage progenitor [67]. These studies showed further that an intact Flt3 signaling pathway is required to prevent HSC stem and progenitors from spontaneous apoptotic cell death. This role of Flt3 has been suggested, at least in part, to the up-regulating of Mcl-1, which is an indispensable survival factor for haemopoiesis.

Notch is another receptor that regulates diverse cell fate decisions during development and is reported to promote murine HSC self renewal. Recent *in vitro* studies have shown that constitutive expression of active human Notch 1 intracellular domain in human blood CD34+ cells induced a reduction in the proliferation of the number of CD34+ cell populations, coinciding with inhibited cell cycle kinetics and up-regulation of p21 mRNA expression and induced apoptosis [68]. The results of this study show that activation of the Notch signaling pathway plays an important role in regulation of the proliferation and survival of adult stem cells.

In addition to oncogenes, tumor suppressors like p21 influence stem cell division. We investigated the role of p21 in normal HSC from p21 knockout mice. We found that p21 deletion increases the growth rate of colonies and the multiplication of haemopoietic progenitor cells *in vitro*. We also found an increase in apoptotic percentage on primary haemopoietic cells from mice (unpublished observations). Therefore, in order for a transformed haemopoietic cell to launch a clone and result in leukemia, it is of importance for some defect to provide the cell with an increased probability of self renewal. This is because a self renewal probability greater than 0.5 will not result in clonal expansion; rather, it will attain maintenance of cell number (SR = 0.5) or result in clonal loss (SR < 0.5). These preliminary unpublished results suggest that p21 has the potential to act as a tumor suppressor gene in the myeloid lineage. Additionally, it is now confirmed that p21 is attributed with a role in regulating self renewal (i.e., proliferation) and apoptosis.

Apoptotic factors, such as BCL-2, Fas, and Fas ligand (FasL), also control HSC proliferation. Domen et al. produced Bcl-2 transgenic mice that over-expressed Bcl-2. They found that the HSC from WT mice died after growth factor withdrawal, whereas HSC from Bcl-2 transgenic mice remained viable. More importantly, HSC from Bcl-2 transgenic mice proliferated more rapidly and extensively (in the presence of a cocktail of factors including IL-1, IL-3, IL-6, SCF, Flt3L) than those of WT. Additionally, there was a delay in cell cycle entry. The most dramatic difference between WT and Bcl-2 transgenic mice was revealed when HSCs were cultured in the presence of SCF. Only 20 percent of WT HSC remained viable after one week; whereas, HSC from Bcl-2 transgenic mice showed enhanced survival and more vigorous proliferation. Bcl-2 over-expression and SCF/c-kit signaling were found to be sufficient for HSC proliferation; however, it should be noted that proliferation also resulted in differentiation of myeloid progenitor cells [69-70].

Fas and FasL are also apoptotic factors involved in erythriod differentiation. Peschle's group has demonstrated the possible involvement of Fas and FasL in the regulation of erythropoiesis and immunohistochemistry of normal bone marrow (NBM) samples and determined that several immature erythroblasts undergo apoptosis *in vivo*. These results showed that erythroid blast express Fas; whereas, more mature cells express FasL. These findings suggest the existence of a negative regulatory feedback between mature and immature erythroid cells. Accordingly, the interaction of Fas and FasL might represent an apoptotic control mechanism for erythropoiesis, contributing to the regulation of red blood cell homeostasis [71]. Furthermore, we have shown that Fas, FasL, and caspase activation are likely to play an important role in the regulation of myelopoiesis [72].

Alenzi and colleagues [73] showed greater frequencies of myeloid progenitor cells (CFU-GM) in lpr and gld mice BM (Fas and FasL knockout mice, respectively) compared to wild-type (WT) mice marrow ($p = 0.0008$). The self renewal (i.e., proliferation capacity) was also significantly greater for lpr and gld CFU-GM compared to WT CFU-GM. Retroviral-mediated gene transfer (RMGT) of Fas (apoptotic gene) into lpr marrow reduced CFU-GM self renewal (proliferative capacity) to WT levels. Therefore, Fas is likely to play an important role in regulating myeloid progenitor cell self renewal. This raises the possibility that Fas/FasL are linked to self renewal (i.e., proliferation capacity) in mice. Therefore, our results suggest that the Fas/FasL pathway and caspase activation inhibit progenitor cell proliferation and promote differentiation. They support proposals that caspase activation may have non-apoptotic functions in the regulation of haemopoiesis [74-75]. Thus, it can be inferred that the presence of fully functional genes that regulate both cell proliferation (i.e., self renewal) and apoptosis will maintain the balance between the rate of cell division and apoptosis of any (cell) population *in vivo*. Therefore, malfunction in, or loss of, any of these genes (or inappropriate DNA or RA splicing) may lead to an increase in their self-replication (i.e., self renewal).

We investigated whether changes in the level of apoptosis due to Fas/FasL deficiencies cause changes in the number and kinetics of HSC. For this, mice with mutations in Fas or FasL were used to investigate how this change may affect the self renewal of clonogenic haemopoietic cells. It has been shown previously that haemopoietic activity is increased in lpr mice that have Fas mutations [76], but this was done only at the level of the number of CFU-GM and not in terms of their proliferative activity. The results obtained differ from those of Schneider et al. [77], who did not show a significant increase in the CFU-GM frequency in the bone marrow of lpr mice, although they found no difference in BM cellularity. Instead, they found increased levels of extramedullary haemopoiesis, especially in the spleen, which is a site of haemopoiesis in normal mice. Our results are in line with those of Traver et al. [78], who showed that lpr marrow contains a significantly greater number of myeloid progenitor cells compared to WT mice.

Our unpublished results concerning the self-renewal (i.e., proliferation) by NBM progenitors are in line with Marley and colleagues [79], who showed that the self renewal values of CML progenitors are significantly greater than those of normal marrow ($p = 0.0001$). There was a relatively low frequency of apoptotic CD34+ cells in progenitors grown from NBM with a significant difference between the NBM and CML samples. We have accumulated a larger number of CML samples and found a significantly increased level of self renewal (i.e., proliferation capacity) in CML compared to NBM ($n = 100$, $p = 0.01$). There was evidence of a difference in the self renewal (i.e., proliferation capacity) between progenitors grown from NBM or CML samples. The frequency of apoptotic cells in CML progenitors was dramatically increased compared to normal samples that agree that apoptosis is reduced in CML. These data are consistent with those of Thiele et al. [80-86] and others who found a considerable level of apoptosis in CD34+ cells harvested from CML patients. The correlation between apoptosis and proliferation was fairly strongly positive (unpublished observations).

NON-APOPTOTIC ROLE OF FAS

Although several *in vivo* and *in vitro* studies have demonstrated variable sensitivities of haemopoietic precursors to Fas-mediated apoptosis, HSC, at least in murine model, induced to express Fas after treatment with TNF-α showed reduction in their engraftment potential [87]. The induced Fas expression also decreased the self renewal of highly purified progenitors [87]. Furthermore, murine HSC with best haemopoietic reconstituting potential express Fas; whereas, ~50 percent of the colony-forming cells in spleen-derived lineage-negative (lin^{-}) progenitors were resistant to Fas ligation [87]. These data suggest that Fas expression in HSC is not a negative factor in HSC apoptosis. Recent experiments suggest, however, that Fas can improve the engraftment of HSC. With this regard, it was found that haemopoietic progenitors that homed successfully to the BM showed a marked up-regulation in the expression of the Fas and FasL and were more resistant to induction of apoptosis [88-89].

It also was found in a recent study that Fas is capable of transducing growth signals in haemopoietic progenitors, after trimerization of this receptor [90]. Therefore, identification of factors that can up-regulate Fas expression in HSC can be of particular importance to HSC transplantation. Inflammatory cytokines, in particular TNF-α and IFN-γ induced in response to environmental stress factors, are a potent inducer of Fas expression on the HSC and haemopoietic progenitors [91-94]. The acquisition of HSC to apoptotic signals upon Fas up-regulation can help sustain viability of progenitors under stress conditions in case of allogeneic haemopoietic grafts into myeloablative recipients. Ectopic expression of FasL in HSC also showed better survival. For example, it has been reported that FasL-modified lin(-) BM can kill Fas-expressing T cells *in vitro* and that transplanting of allogeneic FasL(+) lin(-) BM into recipient mice treated with nonmyeloablative condi-

tioning regimen resulted in an enhanced short-term engraftment [95].

CLINICAL RELEVANCE

In CML, HSC was found to have defective self renewal and apoptosis. Much attention is now focused on the identification of the features and biological properties of the cells responsible for generating and maintaining this neoplastic clone in patients with CML on their relationship to specific functions of the BCR-ABL gene product and on the development of better experimental models of the human diseases. From such an approach, we can hope that this information, likely to have a greater impact on the treatment of CML, will finally be forthcoming. Improved understanding of apoptosis genes has introduced an entirely new modality for treating leukemias and lymphomas. Although these anti-apoptotic mechanisms are currently obscure, many of these new developments will be implemented rapidly and soon will play an important role in chemotherapeutic strategies in the treatment of cancer. Such strategies are likely to radically change the management of patients with hematological malignancies.

CONCLUSION

Cell population size is determined by a balance between cell loss (apoptosis and differentiation) and cell gain (proliferation and mitosis). In normal haemopoiesis, these factors are controlled so that steady-state kinetics are preserved. In contrast, in myeloid leukemias, myeloid expansion can be explained by an increase in proliferation (self renewal), and reduced apoptosis. There have been huge recent advances in research into stem cells. We are currently in a phase where these developments may be used precisely toward the successful attainment of the use of "intelligent" therapeutics for many diseases, such as CML.

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