REVIEW ARTICLE

Differentiation and Proliferation of Hematopoietic Stem Cells

By Makio Ogawa

DURING THE last decade there has been an explosion of new knowledge and techniques in the field of hematopoiesis. A number of hematopoietic growth factors (cytokines) have been identified and their genes cloned. Progress in molecular biology techniques has facilitated large-scale protein production, thereby allowing clinical trials of these cytokines. Indications for bone marrow transplantation (BMT), particularly autologous transplantation, have greatly expanded to include many oncologic conditions. Now transplantation of partially enriched marrow cells and use of peripheral blood progenitors are being tested in attempts to ameliorate the complications of BMT. Most recently, manipulation of human hematopoietic stem cells and progenitors, such as in vitro expansion and gene therapy, promises to herald a new era for a number of genetic and oncologic disorders. In 1983 we prepared an overview of the physiology of hematopoietic stem cells in *Blood*.[1] The current report is intended to be another brief review of stem cell physiology based largely on the studies performed in our laboratory. Therefore, it is not intended to be a comprehensive review of the vast literature on the subject of hematopoietic regulation. I hope that the model of stem cell regulation described in this review is simple and clear enough to be testable and may serve as the framework for further research and debate on this subject.

DEFINITION AND KINETICS OF HEMATOPOIETIC STEM CELLS

The turnover of cells of the hematopoietic system in a man weighing 70 kg may be estimated to be close to 1 trillion cells per day, including 200 billion erythrocytes[2] and 70 billion neutrophilic leukocytes.[3] This remarkable cell renewal process is supported by a small population of bone marrow cells termed hematopoietic stem cells. Definitions of the stem cells vary depending on the model of hematopoiesis. In general, "stem cells" are used to refer to cells that are capable of long-term reconstitution of the hematopoietic system of recipient animals. On the other hand, some of the cells that provide relatively short-term reconstitution may also be stem cells according to the stochastic (random) model to be described later. The differences in the duration of reconstitution may be the consequences of chance occurrences. Obviously stem cells must be capable of self-renewal to maintain a long-term supply of progenies and capable of differentiation into multiple hematopoietic lineages, including lymphocytes. While this concept of stem cells was established early, direct studies of the hematopoietic stem cells were hampered by the lack of specific markers for detecting them. Therefore, studies of the mechanisms regulating stem cells depended on functional and indirect assays such as spleen colony assay,[4] clonal[5,6] and suspension culture[7] assays, and reconstitution of lethally irradiated or genetically deficient mice.

It is generally held that, in the steady state, the majority of stem cells are dormant in the cell cycle and only a few cells supply all of the hematopoietic cells at a given time. Lajtha[8] originally proposed the concept of a true resting state and coined the term G_0. He then proposed that hematopoietic stem cells are normally in G_0 and begin active cell cycling randomly.[9] It was reasoned that the G_0 state confers to stem cells time to repair DNA damage, thus allowing maintenance of the genetic integrity of the stem cell populations. There have been a number of studies supporting the concept of cell cycle dormancy of stem cells. Earlier it was demonstrated that brief exposure in vitro of bone marrow cells to thymidine with high specific radioactivity does not reduce the number of multipotential progenitors.[10,11] Treatment of the donor mice with high dose 5-fluorouracil (5-FU) does not inhibit development of late-appearing spleen colonies[12] or blast cell colonies.[13] Evidence for quiescence of primitive human hematopoietic progenitors was also obtained in culture of enriched human progenitors.[14,15] These studies documented that individual progenitors remain as single cells for as long as 2 weeks in culture and begin proliferation upon stimulation by combinations of cytokines. Several in vivo studies of animal hematopoiesis documented significant serial fluctuation of stem cell clones.[16-22] Particularly, studies using retroviral labeling of individual stem cell clones[17-19] provided experimental evidence for "clonal succession" model of Kay[23] and indirectly supported the concept of stem cell dormancy. However, long-term observations of these mice appear to emphasize domination by single or few clones of stem cells in primary and secondary recipients.[21,22] Perhaps stochastic mechanisms of stem cell renewal and differentiation to be discussed next may unify the apparent controversies generated by these observations.

SELF-RENEWAL VERSUS DIFFERENTIATION OF STEM CELLS

In 1964, Till et al[24] proposed an important model of stem cell functions in which the decision of a stem cell to self-renew and differentiate is depicted as a stochastic process. They developed a "birth and death" model for self-renewal and differentiation of stem cells and tested the model by performing a computer simulation based on generation of random numbers and analyzing the distributions of colony-forming units in spleen (CFU-S) in individual spleen colonies. Based on a concordance between computer simulation and

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experimental observations, they proposed that the decision of a stem cell to self-renew or to differentiate is a stochastic process. Humphries et al[25] tested this model in vitro by replating individual macroscopic erythroid colonies and analyzing for secondary macroscopic erythroid colonies. The marked variation in the self-renewal was similar to that observed by Till et al.[24] We also tested this model in vitro[26] by replating individual blast cell colonies.[27] We postulated that production of secondary blast cell colonies is a self-renewal process and that the generation of secondary multilineage colonies is differentiation. The distributions of both types of colonies generated by individual blast cell colonies were very heterogeneous[26] and were similar to the distribution of CFU-S reported by Till et al.[24] Therefore, these in vitro studies of multipotential progenitors were consistent with the stochastic mechanisms of stem cell renewal and differentiation. Investigators in a number of laboratories, including ours, using different mathematical models arrived at distributional parameters "p" for self-renewal of adult murine stem cells that were slightly higher than 0.5.[26,28,29] An interesting and important question is whether "p" is fixed or if it can be regulated by external factors. This holds direct relevance to the current interest in the in vitro expansion of stem cells. In our blast cell colony replating, we observed development of secondary and tertiary blast cell colonies.[26] Because progenitors for the blast cell colonies appear to be in G_0,[13] this observation indicated that, in adult hematopoiesis, the self-renewal process is associated with renewed dormancy in the cell cycle while the differentiation process is characterized by continuous cell doubling.

DIFFERENTIATION (COMMITMENT) OF STEM CELLS

In our previous review,[1] we predicted that commitment of multipotential progenitors to individual myeloid lineages would also be a stochastic process. This prediction was based on identification of several types of multilineage colonies showing variable combinations of lineages, such as murine colonies consisting of neutrophils, macrophages, and megakaryocytes[30] and human bilineage colonies consisting of erythrocytes and eosinophils.[31] Earlier, two deterministic models, ie, the "hematopoietic inductive microenvironment (HIM)" model[32] and the "stem cell competition" model,[33] had been proposed for mechanisms of stem cell commitment. The HIM model envisioned lineage-specific anatomical niches that direct the differentiation of uncommitted progenitors. The stem cell competition model featured regulation of stem cell commitment by humoral factors such as erythropoietin (Ep) and colony-stimulating factor(s) (CSF).[33] More recently, a model of stem cell commitment featuring predetermined, sequential loss of lineage potentials was also proposed.[34] This model predicts that multilineage colonies would show only fixed combinations of lineages. As will be discussed later, our studies using isolated progenitors documented multilineage colonies expressing various combinations of lineages.

Determination of the mechanism of stem cell differentiation requires analysis either with a 100% pure population of multipotential progenitors or analysis of individual hematopoietic progenitors. Fortunately, the latter opportunity was provided by identification of murine blast cell colonies with high secondary colony-forming ability.[27] We examined the commitment of isolated progenitors by use of micromanipulation techniques. Cytologic analysis of multilineage colonies of single cell origin showed a variety of lineage combinations, consistent with the concept that stem cell commitment is a stochastic process.[35] Analysis of colonies derived from paired progenitors (two daughter cells derived from a single parent cell) showed dissimilar combinations of lineages in many instances.[36] Not only were the types of lineages different but also the number of lineages expressed in each of the pairs of colonies differed. For example, we observed a pair consisting of a megakaryocyte colony and a multilineage colony expressing all of the myeloid elements.[36] This observation indicated that the stochastic principle applies not only to the type but also to the number of lineages that are expressed during the differentiation process. We also performed serial micromanipulation of paired daughter cells and cytologic analysis of the resulting colonies.[37] The results were interpreted to suggest that the stochastic commitment takes place at each cell division of the multipotential progenitor. Similar studies of isolated human progenitors suggested that commitment of human hematopoietic progenitors is also a stochastic process.[38,39]

Two cellular mechanisms may be considered for the stochastic commitment. One model features random restriction in lineage potentials of the progenitors. A schematic presentation of this model is shown in Fig 1A. A major feature of this model is the assumption of the existence of oligopotential progenitors. In an alternative model of stochastic differentiation which is shown diagrammatically in Fig 1B, a pluripotent stem cell may reproduce itself (self-renewal) or may be randomly committed to the expression of only one lineage.[40] This model may be envisioned as a random activation of a group of differentiation genes involved in single-lineage expression. In contrast to the model presented in Fig 1A, progressive restriction in lineage potentials would not take place and therefore there would be no oligopotential progenitors. Detection of two or three lineages within a colony would be caused by the presence of different monopotent progenitors. Either model of differentiation can account for the various types of multilineage colonies documented by the studies using micromanipulation techniques discussed earlier.[35-39] In one of the studies,[37] we performed serial micromanipulation of paired progenitors and developed presumptive genealogic trees of the progenitors based on the cytologic analysis of the colonies. In one experiment, we saw four pairs of colonies each consisting of a macrophage colony and a neutrophil/macrophage colony. This observation appeared to suggest the existence of bipotential neutrophil/macrophage progenitors. However, observation of one such genealogy does not confirm the presence of oligopotential progenitors.

While the stochastic process of commitment depicted in Fig 1 and the mechanisms of cytokine actions to be discussed in later paragraphs appear to provide a reasonable framework for hematopoietic regulation, it should be noted again that it is a model based on studies of isolated progenitors in an artificial culture system. It is possible that the variation in the colony types may be generated by interactions of cytokines

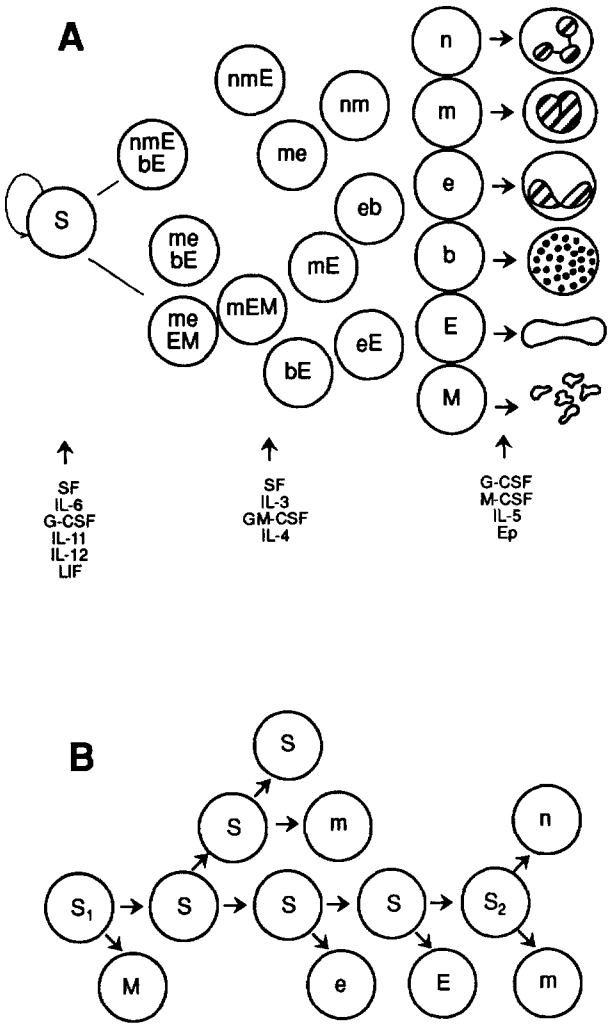

Fig 1. Two cellular mechanisms for stochastic differentiation of hematopoietic stem cells. Circles indicate the progenitors and letters indicate various lineages. The abbreviations are: n, neutrophil; m, macrophage; e, eosinophil; b, basophil; E, erythrocyte; M, megakaryocyte; S, pluripotent stem cell. (A) A model featuring random restriction in lineage potentials of progenitors. This model assumes the existence of oligopotential progenitors. Targets of the growth factors are also illustrated in this figure. (B) An alternative model of stochastic differentiation featuring random commitment of a stem cell to a monopotential state. This model can also account for the various lineage combinations observed in the colonies derived from micromanipulated single progenitors. S_1 would produce a colony expressing neutrophil, macrophage, eosinophil, erythrocyte, and megakaryocyte lineages and possess self-renewal ability. A bilineage neutrophil/macrophage colony would result from S_2.

during colony formation. Regarding the latter point, Metcalf[41,42] proposed that the commitment of granulocyte/macrophage (GM) progenitors is regulated by early acting factors such as interleukin-3 (IL-3) and GM-CSF. Specifically, he reported that higher concentrations of GM-CSF alone[41] or GM-CSF and IL-3, but not G-CSF, in synergy with steel factor (SF)[42] augment production of granulocyte progenitors.

Ultimately the mechanisms of stem cell commitment should be resolved at the genetic level. The rearrangement of immunoglobulin and T-cell receptor genes may be considered precedent for the stochastic process in the hematopoietic system. Recently, Borzillo et al[43] developed early pre–B-cell lines that show parental Ig gene rearrangements and morphologic and functional characteristics of macrophages. Although this observation is made with cell lines, it may support flexibility in the physiologic mechanisms of stem cell commitment that was suggested by McCulloch[44] earlier.

PROLIFERATIVE POTENTIALS OF COMMITTED PROGENITORS

In general, there is a correlation between the number of lineages expressed and the size of the colonies indicating general symmetry of hematopoiesis. However, when we analyzed murine colonies derived from paired progenitors we observed significant asymmetric proliferation in colonies expressing the same lineages.[36] In addition, cytologic analysis of human hematopoietic colonies derived from single cells showed that the proliferative potentials of committed progenitors may be extremely variable.[38] Differential counting of the total cells in the mixed colonies of single cell origin revealed that the individual lineages are represented by varying numbers of cells. For example, the number of eosinophilic leukocytes in the various types of multilineage colonies ranged from 4 cells to 616 cells per colony. In an extreme case, a bilineage eosinophil/erythrocyte colony consisted of 1,340 erythrocytes and 4 mature eosinophilic leukocytes. Because cell loss during staining was kept to a minimum in this experiment, the observation indicated that the eosinophil progenitor reached terminal maturation only a few cell divisions after commitment to the eosinophil lineage. Earlier, Wu[45] noted that the replating ability of the individual T-lymphocyte colony-forming units was extremely variable. Similarly, the replating incidences of primary mast cell colonies varied over a wide range and the size of the secondary mast cell colonies was very heterogeneous.[46] Taken together, these results indicate that proliferation of committed progenitors is not a rigidly regulated process.

GROWTH FACTOR REGULATION OF SURVIVAL AND PROLIFERATION OF PROGENITORS

While self-renewal and differentiation of stem cells and progenitors appear to be stochastic processes, survival and proliferation of the cells is regulated by cytokines. Recent studies showed that cytokines prevent cells from apoptotic death.[47] A number of direct-acting and indirect-acting cytokines have been identified and their genes cloned. While some factors are primarily stimulatory or inhibitory, most have more complex functions in hematopoietic proliferation. An excellent example is IL-4 which has been shown to exhibit both stimulatory and inhibitory activities on hematopoietic proliferation.[48-51] Many factors also possess activities outside the hematopoietic system. For example, the pleiotropic effects of IL-6 involving immune, hepatic, nephric, and other systems are well known.[52] Even within the hematopoietic system cytokines have been shown to exhibit multiple functions affecting cells at different stages. For example, G-CSF stimulates proliferation and maturation of monopotent neutrophil progenitors while it can also trigger proliferation of cell cycle

dormant primitive progenitors. In addition to the effects on progenitors, many cytokines are capable of activating or enhancing functions of mature leukocytes and monocytes. In this review, I will focus primarily on the hematopoietic effects of cytokines in culture. First, I will discuss a group of stimulatory cytokines and their interactions and later comment on inhibitory cytokines.

An important concept that parallels the stochastic model of differentiation is that the role of growth factors is to support survival and proliferation but not to direct the differentiation of progenitors. In this model, the apparent induction of differentiation by a growth factor is interpreted as a consequence of proliferation and maturation of a specific population of progenitors that are supported by that particular factor and concomitant death of the progenitors that are not supported by the same factor. Another important concept regarding cytokine regulation of hematopoiesis is that there is significant functional redundancy among cytokines, particularly early acting cytokines. According to the model of stochastic differentiation presented in Fig 1A, the growth factors may be divided into three categories: (1) late-acting lineage-specific factors, (2) intermediate-acting lineage nonspecific factors, and (3) factors affecting kinetics of cell cycle dormant primitive progenitors. The stages of the progenitors that these cytokines regulate are also indicated in Fig 1A.

LATE-ACTING LINEAGE-SPECIFIC FACTORS

Most of the late-acting factors are lineage-specific and support proliferation and maturation of committed progenitors. For example, Ep is a physiologic regulator of erythropoiesis. M-CSF and IL-5 are considered to be specific for macrophage/monocyte and eosinophil[53] lineages, respectively. While G-CSF regulates proliferation and maturation of neutrophil progenitors, it also serves as a synergistic factor for primitive dormant progenitors as will be discussed in detail later. A number of factors have been shown to stimulate megakaryopoiesis in vitro and/or increase platelet production in vivo.[54,55] These factors are all early acting lineage-nonspecific factors and include IL-6, IL-11, IL-3, IL-1, SF, leukemia inhibitory factor (LIF), and GM-CSF. Whether or not there is a separate late-acting lineage-specific "thrombopoietin" is speculative at this point.

INTERMEDIATE-ACTING LINEAGE-NONSPECIFIC FACTORS

Intermediate-acting lineage-nonspecific factors consist of IL-3, GM-CSF, and IL-4. These factors appear to support the proliferation of multipotential progenitors but only after they exit from G_0. The first clue for this mode of action came from studies of the effects of murine IL-3 on blast cell colony formation from post 5-FU spleen cells.[56] We observed that in the presence of murine IL-3, multipotential blast cell colonies developed after varying periods of time. When the addition of IL-3 was delayed to day 7 of incubation, the earlier-appearing blast cell colonies were eliminated, thus decreasing the number of multipotential blast cell colonies to approximately 1/2 the number observed when IL-3 was added on day 0. However, the delayed addition of IL-3 did not hasten or synchronize the development of late-emerging multipotential blast cell colonies. Based on these observations, we proposed that IL-3 does not trigger cell cycling of dormant stem cells, but rather supports proliferation of multipotential progenitors only after they exit from G_0.[56] IL-3 by itself does not appear to support the terminal stages of hematopoiesis. Studies in our laboratory indicated that the responsiveness of murine multipotential progenitors to IL-3 decreases as they differentiate and mature.[57] Subsequently, Lopez et al[58] reported that human hematopoietic progenitors also lose their responsiveness to human IL-3 as they differentiate to the neutrophil lineage. Together, these results support the concept that hematopoietic effects of IL-3 are restricted to progenitors at the intermediate stages of hematopoietic development and that it does not support the lineage-restricted processes with the possible exception of mast cells/basophils.

While GM-CSF was originally identified as a "lineage-specific" cytokine regulating only progenitors in the granulocyte/macrophage lineages, subsequent studies showed a lack of lineage specificity of GM-CSF. For example, Metcalf et al[59] and Koike et al[60] observed that murine GM-CSF supports a few cell divisions by murine multipotential progenitors. In the human system, there are many reports which show that the target populations of human GM-CSF significantly overlap with those of human IL-3 and include uncommitted multipotential progenitors.[61-64] Recent discovery of the identity between the β-subunit of human IL-3 receptor and the β-subunit of human GM-CSF receptor[65] adds support to the functional overlap between human IL-3 and GM-CSF.

IL-4 also appears to belong to the group of lineage-nonspecific factors that regulate cycling, multipotential progenitors.[48-51] For example, a combination of IL-4 and Ep can support the formation of mouse multilineage colonies from highly purified progenitor populations.[51] Because of the intermediate positions of the target cells of IL-3, GM-CSF, and IL-4, these molecules can effectively interact with late-acting growth factors in the production of more mature cells[66] as well as with the factors that initiate the cycling of dormant progenitors, which are to be discussed next. It is postulated that production of these factors is accelerated only when serious cytopenias call for rapid expansion of the progenitor cell pool.

According to the alternative model of stochastic differentiation presented in Fig 1B, there are no intermediate oligopotential progenitors. If this model is correct, the roles of IL-3, IL-4, and GM-CSF are to function primarily as synergistic factors for lineage-specific factors.

FACTORS INVOLVED WITH CYCLING OF DORMANT PROGENITORS

For many years, the mechanisms regulating proliferation of cell cycle-dormant primitive progenitors remained unknown. Recently, several factors have been identified that appear to be involved in the triggering of cell divisions in the dormant hematopoietic progenitors. Earlier, it was proposed that IL-1 (also called hemopoietin-1) acts synergistically with IL-3 in support of proliferation of murine hematopoeitic stem cells.[67,68] In our laboratory, by using mapping studies of blast cell colony formation, we have found that IL-6,[69] G-CSF,[70] IL-11,[71] SF,[72] and, most recently, IL-12[73] act synergistically with IL-3 in support of colony formation from dormant mu-

rine hematopoietic progenitors. In addition to these factors, LIF was found to augment proliferation of primitive human progenitors.[15,74] Because IL-1 did not enhance IL-3–dependent formation of human blast cell colonies from enriched human marrow cells, we suggested that hemopoietin-1 effect of IL-1 is indirect and mediated in part by factors such as IL-6 and G-CSF.[75] However, others have observed synergism between IL-1 and IL-3 in culture of enriched murine[76] and human[77] marrow cells. These synergistic factors also interact with other intermediate-acting factors, including GM-CSF[78] and IL-4,[51,79,80] with minor exceptions. SF[72] and IL-12[73] do not work synergistically with IL-4.

Originally, we proposed that part of the synergistic effect of these factors is to shorten the duration of G_0 of the primitive progenitors. More recent studies with enriched human marrow cells, including a mapping study of blast cell colony formation from isolated progenitors, indicated that these factors appear to trigger cycling by dormant progenitors.[15] Effects of IL-6[81] and G-CSF[82] on primitive progenitors were shown also in long-term suspension culture using stromal cells that were engineered to produce these factors. Recent studies of the mechanisms of cytokine signal transduction have provided possible biochemical explanations for the similarities in function of IL-6, G-CSF, IL-11, IL-12, and LIF. There is structural homology between IL-6 and G-CSF, indicating that these factors share a common ancestoral gene.[83] Receptors for IL-6, LIF,[84] and IL-11[85] share signal-transducing protein, IL-6 gp130. IL-12 is a heterodimer consisting of 35-Kd and 40-Kd proteins each sharing homology with IL-6 and its receptor, respectively.[86] These structural homologies may provide biochemical basis for the functional duplication of the synergistic factors.

Among the synergistic factors regulating the cycling of dormant progenitors, SF appears to be unique. SF interacts with IL-3 and GM-CSF but not with IL-4.[72,78] In addition, SF can interact with other synergistic factors, including IL-6, G-CSF, IL-11,[72,78] and IL-12[73] to support formation of multipotential blast cell and multilineage colonies while the other synergistic factors do not appear to have this capability. SF has also been shown to interact with late-acting factors, in particular with Ep.[87] This apparent ability of SF to support intermediate stages of hematopoietic development is illustrated also in Fig 1A. Groups of cytokines that interact to support proliferation of dormant primitive progenitors are summarized in Fig 2.

It appears that factors are required for the primitive progenitors to survive in the dormancy state. Recently, Bodine et al[88] and Itoh et al[89] proposed that G-CSF, IL-3, and SF are survival factors for murine hematopoietic stem cells in culture. Using highly enriched bone marrow cells, we have observed that IL-3 and SF, but not G-CSF, independently support survival in G_0 of murine progenitors including lymphohematopoietic progenitors.[90] Regarding human hematopoiesis, our data have indicated that IL-3 and GM-CSF, but not SF, IL-6, IL-11, or G-CSF, maintain the survival in G_0 of the human primitive progenitors.[15]

INHIBITORY CYTOKINES

During the last 3 decades, a number of inhibitory factors have been proposed as physiologic regulators of hematopoiesis. Of these proposed inhibitors, several cytokines deserve attention because they appear to be effective at very low concentrations. These include interferons, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and macrophage inflammatory protein 1α (MIP1α). In general, interferons[91,92] and TNF-α[93,94] appear to be lineage nonspecific inhibitory factors affecting progenitors throughout a wide range of developmental stages. In contrast, the inhibitory effects of TGF-β appear to be directed at the early stages of hematopoiesis.[95-97] It has been shown that TGF-β works antagonistically with a number of early acting cytokines.[97-100] Similarly, MIP1α was reported to inhibit proliferation of primitive hematopoietic progenitors.[101,102] Whether or not TGF-β and MIP1α play a physiologic role in negative regulation of the stem cells in G_0 is not clear at this time. An interesting observation in this regard is the report that antisense TGF-β sequence significantly enhances the frequency of multilineage colony formation in culture.[103]

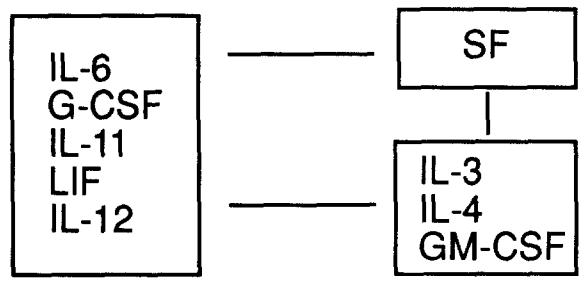

Fig 2. Early acting cytokines may be loosely grouped together based on their functional similarities. Cytokines in each group may interact with those in the other group to stimulate proliferation of primitive progenitors.

LYMPHOHEMATOPOIETIC STEM CELLS

The existence of totipotent lymphohematopoietic stem cells was postulated long ago from clinical observation of hematopoietic reconstitution of patients after BMT and by careful analysis of transplantation in experimental animals. More recently, retroviral labeling of individual hematopoietic progenitors has clearly shown the existence of progenitors common to all hematopoietic lineages, including the lymphocyte lineages.[17,104,105] However, despite all of the in vivo evidence, it has not been possible to detect and quantitate the lymphohematopoietic progenitors by use of culture techniques until recently. Baum et al[106] and Cumano et al[107] reported culture assays for human and murine fetal marrow lymphohematopoietic progenitors that are capable of myeloid and B-cell differentiation using coculture with murine stromal cells. In our laboratory, we have developed a two-step methylcellulose clonal culture system for murine progenitors that have the capacity for differentiation along the myeloid as well as B-cell lineage.[108] Since we isolated individual progenitors from highly enriched marrow cells by micromanipulation, the results provided unequivocal evidence for the single cell origin of lymphohematopoietic progenitors. When enriched marrow cells from day 2 post–5-FU marrow preparation were tested in this assay, about 40% of the progenitors showed B-cell potential. We also found that the combinations

of two factors that included SF plus IL-6, IL-11, G-CSF,[108] or IL-12[73] were all effective in the primary culture in the maintenance of B-lymphoid potentials. Interestingly, IL-3 could neither replace nor act synergistically with SF to support the lymphoid potential of the primary cultures.[108] These results indicated that the reason for our previous failure to recognize lymphohematopoietic progenitors in culture was caused by suboptimal culture conditions and that a significant population of cells that were once thought to be myeloid restricted are actually lymphohematopoietic progenitors. The latter is in agreement with Harrison and Zhong,[109] who documented correlations among T and B lymphocytes and individual myeloid lineages and concurrent development of these lineages in murine transplantation model. These observations further suggest that development of a cell culture assay for lymphohematopoietic stem cells including T lymphocytes may require only identification of permissive culture conditions.

PHYSIOLOGIC SIGNIFICANCE OF IN VITRO STUDIES

The cellular model presented in this review is based almost exclusively on cell culture studies of murine and human hematopoietic progenitors. Therefore, the physiologic meaning of the model needs to be established in vivo. However, there is remarkable concordance between cell culture observations and the physiologic roles of cytokines, in particular late-acting lineage-specific cytokines. Ep is an erythroid-specific cytokine in culture and is the physiologic regulator of erythropoiesis. IL-5 was identified as an eosinophil-specific cytokine in culture.[53] Coffman et al[110] reported that administration of neutralizing anti–IL-5 antibodies effectively abolishes parasite-induced eosinophilia in mice and established the physiologic importance of IL-5 in eosinophilopoiesis. Administration of G-CSF to patients induces dose-dependent increase in neutrophil counts.[111] Interestingly, prolonged administration of human G-CSF to normal dogs resulted in persistent but reversible neutropenia because of the development of autoantibodies against G-CSF.[112] These observations indicate that G-CSF is an important regulator of neutrophil production.

Regarding earlier acting cytokines, the *W* and *Sl* mutants establish the essential role of SF hematopoiesis. Homozygosity of *W* and *Sl* mutation results in virtual absence of hematopoiesis and mice with genotype W/W^v or Sl/Sl^d show profound anemia and absence of mast cells. Injection of neutralizing anti-c-*kit* antibodies causes profound pancytopenia in adult mice.[113] These observations in vivo agree with the strong synergistic effects of SF observed in culture,[72,78,114-120] in particular the strong synergistic effects of SF in erythropoiesis in culture.[87] While the physiologic role of other early acting cytokines has not been clearly established, in vivo actions of cytokines in general parallel closely to those observed in culture. For example, the multilineage effects of GM-CSF and IL-3 including neutrophil, macrophage, and eosinophil lineages are documented clearly both in vivo[111,121,122] and in culture studies.[59-64] The effects of G-CSF in the cell cycling of primitive multipotential progenitors observed in culture are supported by in vivo effects. Earlier, Mizoguchi et al[123] reported a significant increase in CFU-S number in the nude mice transplanted with a human lung carcinoma known to produce G-CSF. Suicide studies with radioactive thymidine showed that a large fraction of CFU-S are active in the cell cycle in the tumor-bearing animals.[123] G-CSF but not GM-CSF or IL-3 obliterated cycling of neutrophils, platelets, and reticulocytes in the dogs with cyclic neutropenia.[124] G-CSF administration has resulted in recovery of not only neutrophil but also erythroid lineages in some patients with refractory aplastic anemia.[125] Taken together these results support the effects of G-CSF on multipotential progenitors in vivo. Recently, gene "knock-out" experiments have been used for establishing physiologic importance of the gene products. IL-4–deficient mice did not show hematopoietic dysregulation.[126] It is possible that IL-4–based cytokine interactions and activities documented in culture may be less important than other cytokines with overlapping specificity. We have documented a large number of cytokines that appear to be involved in the regulation of cell cycle dormant primitive progenitors. Their order of importance in the physiology of stem cells needs to be established.

SUMMARY

Available evidence indicates that qualitative changes in hematopoietic stem cells and progenitors, such as the decision of stem cells to self-renew or differentiate, or selection of lineage potentials by the multipotential progenitors during differentiation (commitment), are intrinsic properties of the progenitors and are stochastic in nature. In contrast, proliferative kinetics of the progenitors, namely survival and expansion of the progenitors, appear to be controlled by a number of interacting cytokines. While proliferation and maturation of committed progenitors is controlled by late-acting lineage-specific factors such as Ep, M-CSF, G-CSF, and IL-5, progenitors at earlier stages of development are controlled by a group of several overlapping cytokines. IL-3, GM-CSF, and IL-4 regulate proliferation of multipotential progenitors only after they exit from G_0 and begin active cell proliferation. Triggering of cycling by dormant primitive progenitors and maintenance of B-cell potential of the primitive progenitors appears to require interactions of early acting cytokines including IL-6, G-CSF, IL-11, IL-12, LIF, and SF. Currently, this simple model fits our understanding of the interactions of growth factors with hematopoietic progenitors. Naturally the model risks oversimplification of a very complex process. However, because the model is testable, it will hopefully challenge investigators to design new experiments to examine its validity.

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