

Hematopoiesis

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Cellular turnover of the hematopoietic system is supported by a small population of cells termed hematopoietic stem cells. Stem cells are capable of self-renewal and differentiation into individual lymphomyeloid lineages. Available evidence indicates that the decision of a stem cell to self-renew or differentiate and the decision of a multipotential progenitor to select a lineage pathway during differentiation (commitment) are intrinsic to 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. Whereas proliferation and maturation of committed progenitors are controlled by late-acting factors such as erythropoietin, macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-5, progenitors at earlier stages of development are controlled by a group of several overlapping cytokines. Interleukin-3, granulocyte/macrophage colony-stimulating factor, and interleukin-4 regulate proliferation of multipotential progenitors only after they are triggered to exit from dormancy state. Triggering of cycling of dormant primitive progenitors and proliferation of lymphohematopoietic primitive progenitors appear to require interactions of early acting cytokines including interleukin-6, granulocyte colony-stimulating factor, interleukin-11, interleukin-12, leukemia inhibitory factor, and steel factor. (J ALLERGY CLIN IMMUNOL 1994;94:645-50.)

Key words: Hematopoietic stem cells, hematopoietic progenitors, differentiation of stem cells, cytokines, interleukin, growth factors

The turnover of cells of the hematopoietic system in a man weighing 70 kg is estimated to be close to 1 trillion cells/day, including 200 billion erythrocytes¹ and 70 billion neutrophilic leukocytes.² This remarkable cell renewal process is supported by a small population of cells termed stem cells. The stem cells are thought to be capable of self-renewal to maintain a long-term supply of progenies and to be capable of differentiation into multiple hematopoietic lineages, including lymphocytes. Although 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 indirect functional assays such as spleen colony assay,³ clonal^{4, 5} and suspension culture⁶ assays, and reconstitution of lethally irradiated or genetically deficient mice.

Abbreviations used

SF:	Steel factor
CSF:	Colony stimulating factor
M-CSF:	Macrophage colony-stimulating factor
G-CSF:	Granulocyte colony-stimulating factor
GM-CSF:	Granulocyte/macrophage colony-stimulating factor
CFU-S:	Colony-forming units in spleen
LIF:	Leukemia inhibitory factor
HIM:	Hematopoietic inductive microenvironment
IL1-12:	Interleukins 1-12

It is generally held that in the steady state, most stem cells are dormant in the cell cycle, and only a few cells supply all of the hematopoietic cells at a given time. It was reasoned that the dormancy (G_0) state confers to stem cells time to repair deoxyribonucleic acid damage, thus allowing maintenance of the genetic integrity of the stem cell populations.⁷ This concept of cell cycle dormancy of stem cells was supported by a number of observations. For example, brief exposure in vitro of bone marrow cells to thymidine with high specific radioactivity did not reduce the number of multipotential progenitors.^{8, 9} Treatment of the

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donor mice with high-dose 5-fluorouracil did not inhibit development of late-appearing spleen colonies¹⁰ or blast cell colonies.¹¹ Cell culture studies of human marrow cells also documented that individual progenitors can remain as single cells for as long as 2 weeks in culture and begin proliferation on stimulation by combinations of cytokines.^{12, 13} Finally, *in vivo* studies using retroviral labeling of individual mouse stem cells¹⁴⁻¹⁶ provided experimental evidence for "clonal succession" model of Kay¹⁷ and indirectly supported the concept of stem cell dormancy.

SELF-RENEWAL VERSUS DIFFERENTIATION OF STEM CELLS

Regarding the decision of a stem cell to self-renew or to differentiate, Till et al.¹⁸ proposed an important model that featured a stochastic (random) process of stem cell self-renewal. They developed a "birth and death" model for self-renewal and differentiation of stem cells and tested the model by carrying out 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 experimental observations, they proposed that the decision of a stem cell to self-renew or to differentiate is a stochastic process. We tested this model *in vitro*¹⁹ by replating individual blast cell colonies.²⁰ 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 heterogeneous¹⁹ and were similar to the distribution of CFU-S reported by Till et al.¹⁸ Therefore these *in vitro* studies of multipotential progenitors were consistent with the stochastic mechanisms of stem cell functions.

DIFFERENTIATION OF STEM CELLS

A number of models have been proposed for differentiation (commitment) of stem cells to individual lympho-myeloid lineages. For a long time the prevailing two models were the "hematopoietic inductive microenvironment" model²¹ and the "stem cell competition" model,²² which featured deterministic regulations of stem cell commitment. The "hematopoietic inductive microenvironment" (HIM) model envisioned lineage-specific anatomic 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 and colony-stimulating factor(s) (CSF). In addition, a model of stem cell commitment featuring predetermined, sequential loss of lineage potentials was also proposed.²³

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.²⁰ We examined the commitment of isolated progenitors by use of micromanipulation techniques. Cytologic analysis of multilineage colonies of single-cell origin revealed a variety of lineage combinations, consistent with the concept that stem cell commitment is a stochastic process.²⁴ Analysis of colonies derived from paired progenitors (two daughter cells derived from a single parent cell) revealed dissimilar combinations of lineages in some instances.²⁵ Serial micromanipulation of paired daughter cells and cytologic analysis of the resulting colonies²⁶ further suggested that the stochastic commitment takes place at each cell division of the multipotential progenitor. Studies of isolated human progenitors revealed heterogeneity in lineage expression and indicated that commitment of human hematopoietic progenitors is also a stochastic process.^{27, 28}

PROLIFERATIVE POTENTIALS OF COMMITTED PROGENITORS

In general a correlation exists between the number of lineages expressed and the size of the colonies indicating overall symmetry of hematopoiesis. However, when we analyzed murine colonies derived from paired progenitors, we observed significant asymmetric proliferation in colonies expressing the same lineages.²⁵ In addition cytologic analysis of human multilineage colonies derived from single cells revealed that the individual lineages are represented by varying numbers of cells.²⁷ This observation indicated that proliferation of committed progenitors is not a rigidly regulated process.

GROWTH FACTOR REGULATION OF PROLIFERATION KINETICS OF PROGENITORS

Whereas self-renewal and differentiation of stem cells and progenitors appear to be stochastic processes, survival and proliferation of the cells is

regulated by cytokines. It is thought that cytokines prevent cells from apoptotic death.²⁹ A number of direct-acting and indirect-acting hematopoietic cytokines have been identified and their genes cloned. Although some factors are primarily stimulatory or inhibitory, most have more complex functions in hematopoietic proliferation. An excellent example is interleukin (IL)-4, which has been shown to exhibit both stimulatory and inhibitory activities on hematopoietic proliferation.³⁰⁻³² 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.³³ Even within the hematopoietic system cytokines have been shown to exhibit multiple functions affecting cells at different stages.

An important concept that makes up an integral part of the stochastic model of differentiation is that the role of cytokines in hematopoiesis is to regulate the proliferation kinetics but not differentiation of progenitors. The apparent induction of differentiation by a cytokine is viewed as a consequence of proliferation and maturation of a specific population of progenitors that are supported by that particular cytokine and concomitant death of the progenitors that are not supported by the same cytokine. Another important concept regarding cytokine regulation of hematopoiesis is that there is significant functional redundancy among cytokines. Arbitrarily the cytokines may be divided into three categories: (1) those acting on committed monopotent progenitors, (2) intermediate-acting lineage nonspecific factors, and (3) factors affecting kinetics of cell cycle-dormant primitive progenitors.

CYTOKINES REGULATING COMMITTED MONOPOTENT PROGENITORS

Some cytokines in this group are lineage specific and support proliferation and maturation of only committed progenitors. An example is erythropoietin, which is a physiologic regulator of erythropoiesis. Macrophage CSF and IL-5 are considered specific for macrophage/monocyte and eosinophil³⁴ lineages, respectively. Although granulocyte (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. Several factors have been shown to stimulate megakaryopoiesis *in vitro* and/or increase platelet production *in vivo*.^{35, 36} These factors are all early-acting lineage-nonspecific factors and include IL-6, IL-

11, IL-3, IL-1, steel factor (SF), leukemia inhibitory factor (LIF), and granulocyte/macrophage (GM)-CSF. Whether a separate late-acting lineage-specific "thrombopoietin" exists is speculative at this point.

INTERMEDIATE-ACTING LINEAGE-NONSPECIFIC FACTORS

IL-3, GM-CSF, and IL-4 may be considered intermediate-acting lineage-nonspecific factors. These factors appear to support the proliferation of multipotential progenitors but only after they exit from G₀. We studied the effects of murine IL-3 on blast cell colony formation from marrow cells of mice that had been treated with high-dose 5-fluorouracil. In the presence of murine IL-3, multipotential blast cell colonies developed after varying periods of time.³⁷ 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 one half the number observed when IL-3 was added on day 0. The delayed addition of IL-3, however, 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 after they exit from G₀.³⁷ IL-3 alone does not appear to support the terminal stages of hematopoiesis, because subsequent studies in our laboratory revealed that the responsiveness of murine multipotential progenitors to IL-3 declines as they differentiate and mature.³⁸

As illustrated by many cell culture and *in vivo* studies, GM-CSF can support neutrophil, macrophage, and eosinophil proliferation. Metcalf et al.³⁹ and Koike et al.⁴⁰ documented that murine GM-CSF supports a few cell divisions of murine multipotential progenitors. In the human system as well, several studies demonstrated that the target population of human GM-CSF significantly overlaps with that of human IL-3 and that it includes multipotential progenitors. Recent discovery of the identity between the b-subunit of human IL-3 receptor and the b-subunit of human GM-CSF receptor⁴¹ adds support to the functional overlap between human IL-3 and GM-CSF.

IL-4 also appears to belong to the list of lineage-nonspecific factors that regulate cycling, multipotential progenitors.^{30-32, 41} For example, a combination of IL-4 and erythropoietin can support the formation of multilineage colonies from

highly purified murine progenitors.⁴¹ 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 cells⁴² and 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.

FACTORS INVOLVED WITH CYCLING OF DORMANT PROGENITORS

For many years the mechanisms initiating proliferation of cell cycle-dormant primitive progenitors remained unknown. In our laboratory, by using mapping studies of blast cell colony formation, we have found that IL-6,⁴³ G-CSF,⁴⁴ IL-11,⁴⁵ SF (*c-kit* ligand),⁴⁶ and most recently, IL-12,⁴⁷ act synergistically with IL-3 in support of colony formation from dormant murine hematopoietic progenitors. In addition to these factors, LIF was found to augment proliferation of primitive human progenitors.⁴⁸ These synergistic factors also interact with other intermediate-acting factors, including GM-CSF⁴⁹ and IL-4,^{41, 50, 51} with few exceptions. SF⁴⁶ and IL-12⁴⁷ do not work synergistically with IL-4. Originally, we proposed that these factors shorten the duration of G₀ of the primitive progenitors. Subsequent mapping studies of blast cell colony formation from isolated human progenitors indicated that these factors trigger cycling of dormant progenitors.¹³ Recent studies of the mechanisms of cytokine signal transduction may provide possible biochemical explanations for the overlapping functions of IL-6, G-CSF, IL-11, IL-12, and LIF. Structural homology exists between IL-6 and G-CSF, indicating that these factors share a common ancestral gene.⁵² Receptors for IL-6, LIF,⁵³ and IL-11⁵⁴ 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.⁵⁵ 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.^{46, 49} In addition, SF can interact with other synergistic factors, including IL-6, G-CSF, IL-11,^{46, 49} and IL-12,⁴⁷ to support formation of multipotential blast cell and multilineage colonies, whereas 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 erythropoietin.⁵⁶

It appears that some of these factors are required for the primitive progenitors to survive in the dormancy state. Recently Bodine et al.⁵⁷ and Itoh et al.⁵⁸ 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 demonstrated that IL-3 and SF but not G-CSF can support survival in G₀ of murine progenitors including lymphohematopoietic progenitors.⁵⁹ 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 G₀ of the human primitive progenitors.¹³

LYMPHOHEMATOPOIETIC STEM CELLS

The existence of totipotent lymphohematopoietic stem cells was postulated long ago from clinical observation of hematopoietic reconstitution of patients after bone marrow transplantation and by careful analysis of transplantation in experimental animals. More recently, retroviral labeling of individual hematopoietic progenitors has clearly demonstrated the existence of progenitors common to all hematopoietic lineages including the lymphocyte lineages.^{14, 60, 61} 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.⁶² and Cumano et al.⁶³ 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 adult murine progenitors that have the capacity for differentiation along the myeloid as well as B-cell lineage.⁶⁴ When enriched marrow cells from day 2 after 5-fluorouracil marrow were tested in this assay, about 40% of the progenitors revealed B-cell potential. We also found that the combinations of two factors consisting of SF and one of IL-6, IL-11, G-CSF,⁶⁴ and IL-12⁴⁷ were 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 B-lymphoid potential of the primary cultures. Most recently we observed that IL-3 and IL-1a can independently abrogate the B-lymphoid potential of primary colonies

when added to permissive culture conditions.⁶⁵ These results indicated that the reason for our previous failure to recognize lymphohematopoietic progenitors in culture was due to suboptimal culture conditions and that a significant population of cells that were once thought to be myeloid-restricted are actually lymphohematopoietic progenitors. 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.

REFERENCES

1. Erslev AJ. Production of erythrocytes. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, eds. *Hematology*. New York: McGraw-Hill, 1983:365-76.
2. Dancy JT, Deubelbeiss KA, Harker LA, Finch CA. Neutrophil kinetics in man. *J Clin Invest* 1976;58:705-15.
3. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961;14:213-22.
4. Pluznik DH, Sachs L. The cloning of normal "mast" cells in tissue culture. *J Cell Physiol* 1965;66:319-24.
5. Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. *Austr J Exp Biol Med* 1966;44:287-99.
6. Allen TD, Dexter TM. Cellular interrelationships during in vitro granulopoiesis. *Differentiation* 1976;6:191-4.
7. Lajtha LG. Stem cell concepts. *Differentiation* 1979;14:23-34.
8. Becker AJ, McCulloch EA, Siminovitch L, Till JE. The effect of differing demands for blood cell production on DNA synthesis by hemopoietic colony forming cells of mice. *Blood* 1965;26:296-308.
9. Hara H, Ogawa M. Murine hemopoietic colonies in culture containing normoblasts, macrophages, and megakaryocytes. *Am J Hematol* 1978;4:23-34.
10. Hodgson GS, Bradley TR. Properties of haematopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell? *Nature* 1979;281:381-2.
11. Suda T, Suda J, Ogawa M. Proliferative kinetics and differentiation of murine blast cell colonies in culture: evidence for variable G₀ periods and constant doubling rates of early pluripotent hemopoietic progenitors. *J Cell Physiol* 1983;117:308-18.
12. Leary AG, Hirai Y, Kishimoto T, Clark SC, Ogawa M. Survival of hemopoietic progenitors in G₀ does not require early hemopoietic regulators. *Proc Natl Acad Sci USA* 1989;86:4535-8.
13. Leary AG, Zeng HQ, Clark SC, Ogawa M. Growth factor requirements for survival in G₀ and entry into the cell cycle of primitive human hemopoietic progenitors. *Proc Natl Acad Sci USA* 1992;89:4013-7.
14. Lemischka IR, Raulet DH, Mulligan RC. Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell* 1986;45:917-27.
15. Snodgrass R, Keller G. Clonal fluctuation within the haematopoietic system of mice reconstituted with retrovirus-infected stem cells. *EMBO J* 1987;6:3955-60.
16. Abkowitz JL, Linenberger ML, Newton MA, Shelton GH, Ott RL, Guttrop P. Evidence for the maintenance of hematopoiesis in a large animal by the sequential activation of stem-cell clones. *Proc Natl Acad Sci USA* 1990;87:9062-6.
17. Kay HEM. How many cell-generations? *Lancet* 1965;2:418-9.
18. Till JE, McCulloch EA, Siminovitch L. A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. *Proc Natl Acad Sci USA* 1964;51:29-36.
19. Nakahata T, Gross AJ, Ogawa M. A stochastic model of self-renewal and commitment to differentiation of the primitive hemopoietic stem cells in culture. *J Cell Physiol* 1982;113:455-8.
20. Nakahata T, Ogawa M. Identification in culture of a class of hemopoietic colony-forming units with extensive capability to self-renew and generate multipotential colonies. *Proc Natl Acad Sci USA* 1982;79:3843-7.
21. Trentin JJ. Influence of hematopoietic organ stroma (hematopoietic inductive microenvironments) on stem cell differentiation. In: Gordon AS, ed. *Regulation of hematopoiesis*. New York: Appleton-Century-Crofts, 1970:161-86.
22. VanZant G, Goldwasser E. Competition between erythropoietin and colony stimulating factor for target cells in mouse marrow. *Blood* 1979;53:946-65.
23. Johnson GR. Is erythropoiesis an obligatory step in the commitment of multipotential hematopoietic stem cells? In: Baum SJ, Ledney GD, Kahn A, eds. *Experimental hematology today*. New York: Springer-Verlag, 1981:13-20.
24. Suda T, Suda J, Ogawa M. Single-cell origin of mouse hemopoietic colonies expressing multiple lineages in variable combinations. *Proc Natl Acad Sci USA* 1983;80:6689-93.
25. Suda T, Suda J, Ogawa M. Disparate differentiation in mouse hemopoietic colonies derived from paired progenitors. *Proc Natl Acad Sci USA* 1984;81:2520-4.
26. Suda J, Suda T, Ogawa M. Analysis of differentiation of mouse hemopoietic stem cells in culture by sequential replating of paired progenitors. *Blood* 1984;64:393-9.
27. Leary AG, Ogawa M, Strauss LC, Civin CI. Single cell origin of multilineage colonies in culture: evidence that differentiation of multipotent progenitors and restriction of proliferative potential of monopotent progenitors are stochastic processes. *J Clin Invest* 1984;74:2193-7.
28. Leary AG, Strauss LC, Civin CI, Ogawa M. Disparate differentiation in hemopoietic colonies derived from human paired progenitors. *Blood* 1985;66:327-32.
29. Koury MJ. Programmed cell death (Apoptosis) in hematopoiesis. *Exp Hematol* 1992;20:391-4.
30. Peschel C, Paul WE, Ohara J, Green I. Effects of B cell stimulatory factor-1/interleukin 4 on hematopoietic progenitor cells. *Blood* 1987;70:254-63.
31. Rennick D, Yang G, Muller-Sieburg C, et al. Interleukin 4 (B-cell stimulatory factor 1) can enhance or antagonize the factor-dependent growth of hemopoietic progenitor cells. *Proc Natl Acad Sci USA* 1987;84:6889-93.
32. Broxmeyer HE, Lu L, Cooper S, et al. Synergistic effects of purified recombinant human and murine B cell growth factor-1/IL-4 on colony formation in vitro by hematopoietic progenitor cells. *J Immunol* 1988;141:3852-62.
33. Kishimoto T. The biology of Interleukin-6. *Blood* 1989;74:1-10.
34. Sanderson CJ. Interleukin-5, eosinophils, and disease. *Blood* 1992;79:3101-9.

35. Bruno E, Hoffman R. Effect of interleukin 6 on in vitro human megakaryocytopoiesis: its interaction with other cytokines. *Exp Hematol* 1989;17:1038-43.
36. Briddell RA, Brandt JE, Leemhuis TB, Hoffman R. Role of cytokines in sustaining long-term human megakaryocytopoiesis in vitro. *Blood* 1992;79:332-7.
37. Suda T, Suda J, Ogawa M, Ihle JN. Permissive role of interleukin 3 (IL-3) in proliferation and differentiation of multipotential hemopoietic progenitors in culture. *J Cell Physiol* 1985;124:182-90.
38. Koike K, Ihle JN, Ogawa M. Declining sensitivity to interleukin 3 of murine multipotential hemopoietic progenitors during their development: application to a culture system that favors blast cell colony formation. *J Clin Invest* 1986;77:894-9.
39. Metcalf D, Johnson GR, Burgess AW. Direct stimulation by purified GM-CSF of the proliferation of multipotential and erythroid precursors. *Blood* 1980;55:138-47.
40. Koike K, Ogawa M, Ihle JN, et al. Recombinant murine granulocyte-macrophage (GM) colony-stimulating factor supports formation of GM and multipotential blast cell colonies in culture: comparison with the effects of interleukin-3. *J Cell Physiol* 1987;131:458-64.
41. Kishi K, Ihle JN, Urdal DL, Ogawa M. Murine B-cell stimulatory factor-1 (BSF-1)/interleukin-4 (IL-4) is a multi-CSF which acts directly on primitive hemopoietic progenitors. *J Cell Physiol* 1989;139:463-88.
42. Sonoda Y, Yang Y-C, Wong GG, Clark SC, Ogawa M. Analysis in serum-free culture of the targets of recombinant human hemopoietic factors: interleukin-3 and granulocyte/macrophage colony-stimulating factor are specific for early developmental stages. *Proc Natl Acad Sci USA* 1988;85:4360-4.
43. Ikebuchi K, Wong GG, Clark SC, Ihle JN, Hirai Y, Ogawa M. Interleukin-6 enhancement of interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. *Proc Natl Acad Sci USA* 1987;84:9035-9.
44. Ikebuchi K, Clark SC, Ihle JN, Souza LM, Ogawa M. Granulocyte colony-stimulating factor enhances interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. *Proc Natl Acad Sci USA* 1988;85:3445-9.
45. Musashi M, Yang Y-C, Paul SR, Clark SC, Sudo T, Ogawa M. Direct and synergistic effects of interleukin-11 on murine hemopoiesis in culture. *Proc Natl Acad Sci USA* 1991;88:765-9.
46. Tsuji K, Zsebo KM, Ogawa M. Enhancement of murine blast cell colony formation in culture by recombinant rat stem cell factor (rrSCF), ligand for *c-kit*. *Blood* 1991;78:1223-9.
47. Hirayama F, Katayama N, Neben S, et al. Synergistic interaction between interleukin-12 (natural killer cell stimulatory factor, cytotoxic lymphocyte maturation factor) and steel factor in support of proliferation of murine lymphohemopoietic progenitors in culture. *Blood* 1994;83:92-8.
48. Leary AG, Wong GG, Clark SC, Smith AG, Ogawa M. Leukemia inhibitory factor/ differentiation-inhibiting activity/human interleukin for DA cells augments proliferation of human hemopoietic stem cells. *Blood* 1990;75:1960-4.
49. Tsuji K, Lyman SD, Sudo T, Clark SC, Ogawa M. Enhancement of murine hemopoiesis by synergistic interactions between steel factor (ligand for *c-kit*), interleukin-11 and other early-acting factors in culture. *Blood* 1992;79:2855-60.
50. Rennick D, Jackson J, Yang G, Wideman J, Lee F, Hudak S. Interleukin-6 interacts with interleukin-4 and other hematopoietic growth factors to selectively enhance the growth of megakaryocytic, erythroid, myeloid, and multipotential progenitor cells. *Blood* 1989;73:1828-35.
51. Musashi M, Clark SC, Sudo T, Urdal DL, Ogawa M. Synergistic interactions between interleukin-11 and interleukin-4 in support of proliferation of primitive hemopoietic progenitors of mice. *Blood* 1991;78:1448-51.
52. Hirano T, Yasukawa K, Harada H, et al. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* 1986;324:73-7.
53. Gearing DP, Comeau MR, Friend DJ, et al. The IL-6 signal transducer, gp130: an oncostatin M receptor and affinity converter for the LIF receptor. *Science* 1992;255:1434-7.
54. Yin T, Taga T, Tsang ML-S, Yasukawa K, Kishimoto T, Yang Y-C. Interleukin (IL)-6 signal transducer, gp130, is involved in IL-11 mediated signal transduction. *Blood* 1992;80(suppl):151a.
55. Merberg DM, Wolf SF, Clark SC. Sequence similarity between NKSF and the IL-6/G-CSF family. *Immunol Today* 1992;13:77-8.
56. Dai CH, Krantz SB, Zsebo KM. Human burst-forming units-erythroid need direct interaction with stem cell factors for further development. *Blood* 1991;78:2493-7.
57. Bodine DM, Crosier PS, Clark SC. Effects of hematopoietic growth factors on the survival of primitive stem cells in liquid suspension culture. *Blood* 1991;78:914-20.
58. Itoh Y, Ikebuchi K, Hirashima K. Interleukin-3 and granulocyte colony-stimulating factor as survival factors in murine hemopoietic stem cells in vitro. *Int J Hematol* 1992;55:139-45.
59. Katayama N, Clark SC, Ogawa M. Growth factor requirement for survival in cell cycle dormancy of primitive murine lymphohemopoietic progenitors. *Blood* 1993;81:610-6.
60. Dick JE, Magli MC, Huszar D, Phillips RA, Bernstein A. Introduction of selectable gene into primitive stem cells capable of long-term reconstruction of the hemopoietic system of W/W^v mice. *Cell* 1985;42:71-9.
61. Keller G, Paige C, Gilboa E, Wagner EF. Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. *Nature* 1985;318:149-54.
62. Baum CM, Weissman IL, Tsukamoto AS, Buckle A-M, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci USA* 1992;89:2804-8.
63. Cumano A, Paige CJ, Iscove NN, Brady G. Bipotential precursors of B cells and macrophages in murine fetal liver. *Nature* 1992;356:612-5.
64. Hirayama F, Shih JP, Awgulewitsch A, Warr GW, Clark SC, Ogawa M. Clonal proliferation of murine lymphohemopoietic progenitors in culture. *Proc Natl Acad Sci USA* 1992;89:5907-11.
65. Hirayama F, Clark SC, Ogawa M. Negative regulation of early B-lymphopoiesis by interleukin-3 and interleukin-1a. *Proc Natl Acad Sci USA* 1994;91:469-73.