Journal of Cellular Biochemistry 58:410–415 (1995)

Hematopoietic Stem Cell Cytokine Response

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Abstract The process of hematopoiesis can be modelled on the concept of a pluripotential stem cell able to differentiate and proliferate in multiple lineages. This process proceeds under the permissive or directive influence of "early" and "late" acting hematopoietic cytokines probably acting in synergistic combinations within the context of the *marrow stroma*l microenvironment. Further characterization of the biochemical events that transduce cytokine signalling into cellular events and the ultimate description of the earliest progenitor cell populations and the cytokines which influence them will provide key insights into embryogenesis and tissue maintenance as well as suggest new therapeutic approaches for hematologic and malignant diseases.

Key words: stem cells, growth factors, hematopoiesis

INTRODUCTION

Hematopoiesis is a useful model of "ongoing ontogeny" and has lent itself well to the study of basic events during tissue development. The description of cytokines that direct the survival, proliferation, differentiation, and function of progenitor and mature cells has not only provided the basis for understanding hematopoiesis but has also spawned exciting new therapeutic approaches. Final elucidation of the intracellular signaling pathways activated by cytokine-surface receptor interaction and the subsequent nuclear events which transduce that activation into cellular responses promises to be the vanguard for understanding the basic genetic mechanisms which direct and coordinate overall cell functioning.

HISTORICAL PERSPECTIVE

The existence of the hematopoietic cytokines was suspected as early as the turn of the century with the search for a humoral substance which regulated red blood cell production. Shortly thereafter, far sighted models of hematopoiesis based on a pluripotential stem cell had already emerged as relatively standard concepts which continue to form the basis for the further modelling and investigation of that process.

Received November 10, 1993; accepted November 16, 1993.
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Three major advances unleashed the rapid translation of this early modeling into the much more sophisticated current understanding of hematopoiesis. The development of in vitro culture systems allowed the investigation of hematopoietic proliferation and differentiation to be dissected out independently of the complex environment of the intact organism. Semi-solid cultures were the first available for this investigation and quickly laid the ground work for the identification of multilineage progenitors and the understanding of humoral inputs into the proliferation and differentiation process. Subsequently, the development of liquid culture systems allowed the establishment of a marrow-like environment under controlled conditions that provided the first testing grounds for concepts of cell-cell interaction and renewal processes that are felt to be the basis of marrow function.

Early culture techniques relied on the addition of poorly characterized growth stimulating sources such as animal or human sera, condition medium, or complex cellular extracts to support proliferation. Painstaking and laborious isolation techniques soon confirmed the existence of discrete protein growth factors as the prime directors of this process. Recombinant DNA techniques have now generated an explosion in the identification and availability of the hematopoietic cytokines.

Erythropoietin is the sentinel example of this process whose cloning and characterization has lead not only to a very specific understanding of

the humoral events directing red cell development but whose clinical application has had a dramatic impact on the anemia associated with renal failure and a variety of other disease states. The identification and cloning of erythropoietin was followed shortly by a panoply of other cytokines whose actions on the multiple hematopoietic lineages have now been fairly well characterized and are reviewed elsewhere. [Robinson and Quesenberry, 1990] Whereas initial concepts of cytokine action were relatively simplistic and monolithic as represented by a now anachronistic nomenclature emphasizing specific lineage effects, it has become appreciated that diversity of action and interaction are the rule rather than the exception.

The final quantum leap in the understanding and characterization of hematopoiesis is ongoing. The "holy grail" of hematologists for the better part of this century has been the characterization and isolation of the ultimate pluripotential potential hematopoietic stem cell. It is the cell upon which both modeling of normal hematopoiesis and clinical attempts at transplantation and gene therapy are based. An extensive menu of monoclonal antibody defined cell surface determinants marking progression through hematopoietic proliferation and differentiation has enabled the sophisticated characterization and isolation of subsets of marrow cells which represent very small proportions of overall numbers yet retain the majority share of proliferative capacity, multi-lineage differentiation capability, and the ability to rescue from otherwise lethal radiation. However, even these highly defined subsets remain heterogeneous in phenotype and behavior [Li and Johnson, 1992].

Despite the dramatic advances in the identification of building blocks of hematopoiesis and in particular in the characterization and clinical application of the various cytokines involved in that process, the basic model of hematopoiesis has remained relatively unchanged throughout the five decades of this revolutionary research. The ultimate basis of hematopoiesis, the pluripotential stem cell, remains an elusive target whose ultimate characterization, identification, and manipulation awaits further investigation.

THE SPECIFIC ROLE OF CYTOKINES IN DIRECTING THE EVENTS OF HEMATOPOIETIC PROLIFERATION

Although the various hematopoietic cytokines have as a rule multiplicity of action, some general themes are apparent. Cytokine action can be thought of as "early" or "late." Early acting factors tend to impact on more proliferative, multi-lineage potential progenitors while late acting factors act on terminal differentiation of phenotypic expression and ultimate functionality of their target cell populations. Individual factors may show preferential activity in one compartment, such as steel factor acting primarily on early progenitors while G-CSF, CSF-1, and erythropoietin primarily act on more mature cells in the granulocyte, macrophage, and erythrocyte lineages. Nevertheless, cytokines typically manifest a spectrum of effects crossing the boundaries between early and late action and between the specific lineages (see Table I).

In vitro analysis of early hematopoietic progenitors has emphasized a requirement for synergistic interactions between multiple cytokines but, by design, cannot always distinguish hierarchical action by these cytokines in space and time. It is now clear that even within early compartments progenitors may also be subsetted as "early" vs. "late" in replating experiments and that these subdivisions may be further defined by selective growth factor responsiveness at various points during differentiation. Recent data has confirmed this temporal sequencing of growth factor action in a single assay system not requiring replating and has furthermore suggested that critical primary factors at appropriate concentration may "anchor" response to additional factors in combination and thereby allow an augmented responsiveness to markedly reduced concentrations of multiple other factors acting in synergy [Lowry, 1992].

The characterization of cell surface receptors and their serial modulation by previously acting cytokine receptor interactions provides a specific mechanism for the hierarchical and temporal response to growth factor combinations [Jacobsen et al., 1992; Testa et al., 1993]. "Early" acting factors are seen in these models as necessary for survival and initial proliferation and would in turn modulate the number or responsiveness of subsequent growth factor receptors directing the ultimate lineage commitment and maturation of that particular hematopoietic clone.

The specific biochemical reaction cascades that transmit cytokine-receptor signalling to the nucleus are being rapidly clarified. The appreciation that different growth factor receptors may share subunits and work through common sec-

TABLE I. Summary of Hematopoietic Cytokines

Factor	Primary activity
"Early" Factors	
Steel Factor (MGF, SCF, c-kit ligand)	Potent synergistic activity in stimulation of early progenitors. Some terminal stimulation in granulocyte, mast cell, and erythroid lineages.
Interleukin-3 (IL-3)	Also known as "multi-CSF" with action on early and intermediate progenitors. Some synergistic effects on terminal erythroid differentiation.
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Activity on early and intermediate progenitors particularly in granulocyte and macrophage lineages
Interleukin-6 (IL-6)	Activity on early progenitors. Also regulates B cell proliferation and immunoglobulin secretion
Interleukin-11 (IL-11)	Early progenitor and megakaryocyte progenitor stimulation.
"Late" Factors	
Granulocyte colony-stimulating factor (G-CSF)	Granulocyte formation, differentiation, and functional modulation. Also stimulates early progenitors
Colony-stimulating factor-1 (CSF-1)	Also known as Macrophage-CSF (M-CSF). Macrophage differentiation and function.
Erythropoietin (Epo)	Red cell formation.
Interleukin-5 (IL-5)	Eosinophil differentiation. B cell differentiation and immunoglobulin secretion.
Interleukin-9 (IL-9)	Erythroid and possible megakaryocyte differentiation.
Additional interleukins	
Interleukins 1, 2, 4, and 7 involved in B and T cell development and immunoglobulin secretion.	
Other growth factors with hematopoietic effects	
Basic Fibroblast Growth Factor, Platelet Derived Growth Factor, Hepatocyte Growth Factor, Insulin-like Growth Factor II, Leukemia Inhibitory Factor, Membrane Bound Burst-Promoting Activity	
Inhibitory Cytokines	
Transforming Growth Factor-beta, Interferons, Tumor Necrosis Factors, Prostaglandins E_1 and E_2, Inhibin, Lactoferrin, Macrophage Inflammatory Protein-1α and -2α, Platelet Factor 4, Interleukin 8	

ond messenger pathways suggests a certain economy of action that may make their ultimate understanding and characterization more immediately achievable [Miyajima et al., 1993]. Simultaneously, novel mechanisms of receptor action are increasingly appreciated that reveal a richness and complexity of effects beyond the simple cell surface receptor models initially advanced [Heaney and Golde, 1993]. The additional modulating effects of inhibitors and adhesion molecule interactions are only beginning to be incorporated into the modelling process.

The specific multiple cytokine requirement of early hematopoietic progenitors for optimal proliferation dovetails interestingly with the predilection of the earliest hematopoietic progenitors to tightly adhere to marrow stroma. This adherence provides a mechanism to separate early cells from more the more mature progeny which tend to be nonadherent [Tucker et al., 1993], though direct adherence may not be absolutely required to support proliferation [Verfaille, 1992]. The cell surface expression of certain growth factors and the ability of local extracellular matrix to functionally bind various other cytokines suggests that these firmly adherent early progenitor cells are dependent on the multiple local factors presented to them within the local hematopoietic "niche" (see Fig. 1). Extending this model, the specific growth factors and/or adhesion molecule influences that the stem cell encounters within this niche would be seen to direct the critical early events in proliferation and differentiation or at least provide permissive conditions to allow fruition of a stochastically determined commitment program. Subtle differences in the stoichiometry of these influences may specifically determine or allow expression of a particular lineage differentiation.

Later more terminally differentiated and committed progenitors may be more autonomous. Free floating in a less intimate relationship with

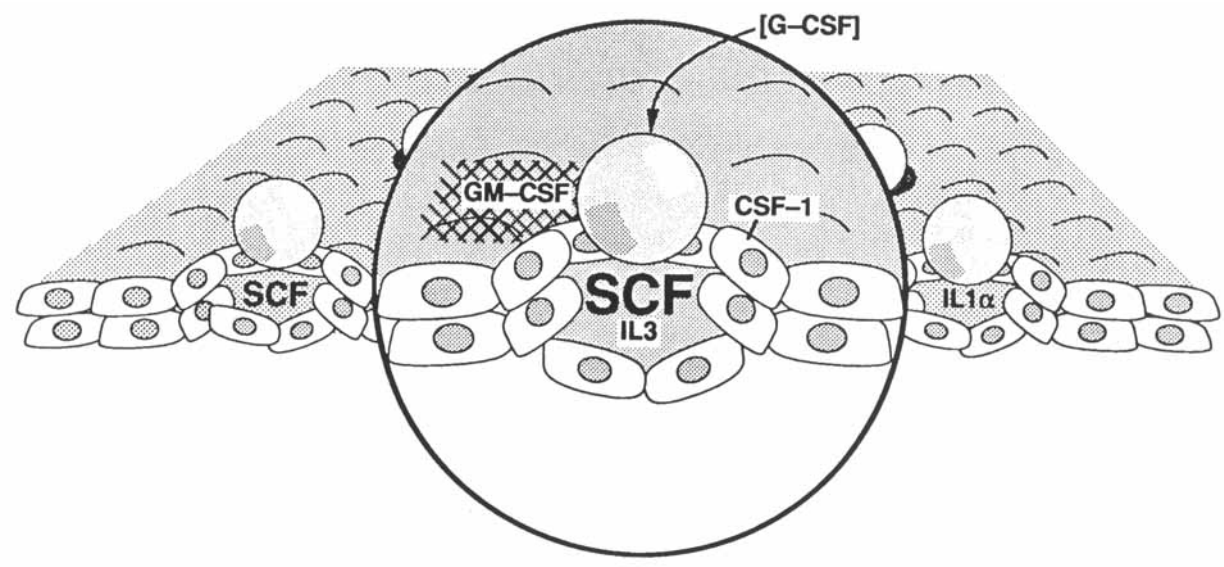

Fig. 1. Model of the hematopoietic microenvironmental "niche" emphasizing the role of growth factors in its definition. Factors such as CSF-1 and SCF can be presented at the stromal cell surface while other factors such as GM-CSF can be locally complexed in active form by extracellular matrix. Other factors may act through diffusion either locally such as IL3 or distantly such as G-CSF. Different niches may induce or permit different differentiation programs through specific factor combinations and/or differing concentration ratios of cytokines. This model neglects the additional influence of adhesion molecules and negative regulators which undoubtedly further modulate hematopoiesis.

marrow stroma, these cells may require only basic hormonal like influences to sustain their ongoing limited proliferation but increasing differentiation program.

STEEL FACTOR AS A PROTOTYPE EARLY CYTOKINE

Steel factor, alternately known as mast cell growth factor (MGF), c-kit ligand or stem-cell factor (SCF) is the prototypical early acting cytokine. Complementary defects in the Sl/Sl and W/W^v mutant mouse strains can now be traced to abnormalities of steel factor and its receptor c-kit. The interaction of this cytokine and its receptor are now recognized as a critical event in hematopoietic proliferation.

Steel factor has only modest independent effects but exerts a potent synergistic influence on early hematopoietic progenitor populations inducing marked proliferation when combined intermediate and late acting factors. The pseudonym "stem-cell factor" highlights the focus on early effects of this factor, though it has also been demonstrated to have late effects in the granulocyte and erythroid lineages. Cells expressing c-kit are among the earliest hematopoietic progenitors identified, and it has been presumed that c-kit expression might be definitional in the appropriate context of the long sought pluripotent stem cell.

More recent findings, however, have cast some doubt on this premise [Ikuta and Weissman, 1992]. Very early events in embryonic hematopoiesis of the Sl/Sl mutant seem to occur normally with ultimate arrest of hematopoietic maturation occurring only after a critical 12–14 day period. Normal embryos show an equivalent steel factor independent early stage of hematopoiesis [Ogawa et al., 1993]. In vitro stromal-based cultures have shown parallel steel factor independent persistence or renewal of progenitors in the very earliest steps of proliferation [Kodama et al., 1992; Wineman et al., 1992]. Thus, though steel factor is certainly an early acting factor, it is probably not the earliest acting factor.

FUTURE PROSPECTS

What, then, are the earliest acting cytokines? Alternatively, this question can be posed: What is the earliest identifiable hematopoietic stem cell and what influences its subsequent survival, proliferation, and differentiation? The answers to these questions remain the central "future prospect" of an ongoing and highly active area of research. The quest for the answers to these

questions concerns not only the specifics of hematopoiesis but is likely to shed light on more general issues of embryonic and tissue development.

One of the more exciting and still controversial reports in the last year has been by Huang and Terstappen [1992] regarding the identification of a single cell capable of giving rise not only to hematopoietic lineages but to a supporting network of stromal cells as well. There is simultaneously growing evidence of the very early action of more general growth factors such as basic fibroblast growth factor and hepatocyte growth factor at the earliest stages of hematopoiesis. These findings return not only to the earliest events of hematopoiesis but also to the original models of almost half a century ago of a unitary stem cell able to give rise to all the resident populations within the bone marrow.

A potential distinction needs to be made between the stem cells present during fetal development, in cord blood at birth, and in the adult organism with the possibility that the nature of the stem cell and its immaturity and multilineage potential may undergo subtle or not so subtle changes at those various stages of life. Ultimately, the pluripotential hematopoietic stem cell may or may not prove to be blurred into a more generalized tissue stem cell. Simultaneously, certain "stem-cell" characteristics such as self-renewal may ultimately prove to be *a population rather than single cell phenomenum.*

Final description of the specific biochemical interactions at the cell surface, within the cytoplasm, and at the nuclear level that underlie the complex mechanisms involved in embryogenesis and ongoing tissue maintenance also holds interest not only for our understanding of these processes in the normal state but also as a key to its disorder in diseases states as a prelude to effective clinical intervention. At least within the hematopoietic lineage, the hematopoietic cytokines seem to be the "conductors" of this process. Though there is remaining debate as to which comes first, the cytokine based determination of nuclear events or stochastic nuclear events which are subsequently expressed in a permissive cytokine environment, it is clear that the hematopoietic cytokines are intimately involved in these signalling and survival mechanisms and ultimately allow hematopoietic proliferation, differentiation, and functionality of terminal hematopoietic cells.

The historic view of cytokines oversimplifies the full extent of interactions required in this process. Our understanding of stromal stem cell interactions, the effect of specific adhesion molecules in passive and active modulation of cytokine response and stem cell localization, and the true complexity of the orchestra of intracellular signals that translate cell surface receptor binding with cytokine into nuclear events remains incomplete.

The final characterization of the pluripotential hematopoietic stem cell or cells and the understanding of the influences of cytokines on the process and product of hematopoiesis will undoubtedly shed light on larger issues of emryogenesis and tissue maintenance. Armed with this knowledge, clinicians will be better able to understand hematologic disease states and design appropriate therapies for them. In particular, the prospect of ex vivo manipulation and expansion of hematopoietic stem cells will open new therapeutic vistas which could revolutionize the treatment of malignant and nonmalignant diseases alike.

REFERENCES

Bodine DM, Seidel NE, Zsebo KM, Orlic D (1993): In vivo administration of stem cell factor to mice increases the absolute number of pluripotent hematopoietic stem cells. Blood 82:445–55.

Brecher G, Redearn WJ, Neben S (1992): Disappearance and reappearance of stem cell clones. Exp Hematol 20:383–385.

Broxmeyer HE (1992): Suppressor cytokines and regulation of myelopoiesis: Biology and possible clinical uses. Am J Pediatr Hematol Oncol 14:22–30.

Broxmeyer HE, Kurtzberg J, Gluckman E, Auerbach AD, Douglas G, Cooper S, Falkenburg JH, Bard J, Boyse EA (1991): Umbilical cord blood hematopoietic stem and repopulating cells in human clinical transplantation. Blood Cells 17:313–329.

Civin CI (1990): Human monomyeloid cell membrane antigens. Exp Hematol 18:461–467.

Dainiak N (1992): Negative regulators of hematopoietic stem cells and progenitors. Experimental Hematology 20: 1154–1155.

Demetri GD (1992): Hematopoietic growth factors: Current knowledge, future prospects. Current Prob Cancer 16:181–259.

Eaves CJ, Sutherland HJ, Udomsakdi C, Lansdorp PM, Szilvassy SJ, Fraser CC, Humphries RK, Barnett MJ, Phillips GL, Eaves AC (1992): The human hematopoietic stem cell in vitro and in vivo. Blood Cells 18:310–307.

Erickson N, Quesenberry PJ (1992): Regulation of erythropoiesis. Med Clin North Am 76:745–757.

Heaney ML, Golde DW (1993): Soluble hormone receptors. Blood 82:1945–1948.

Huang S, Terstappen LW (1992): Formation of haematopoietic microenvironment and haematopoietic stem cell from

single human bone marrow stem cell. Nature 360:745–749.

Ikuta K, Weissman IL (1992): Evidence that hematopoietic stem cells express mouse c-kit but do not depend of steel factor for their generation. Proc Nat Acad Sci USA 89: 1502–1506.

Jacobsen SE, Ruscetti FW, Dubois CM, Wine J, Keller JR (1992): Induction of colony-stimulating factor receptor expression on hematopoietic progenitor cells: Proposed mechanism for growth factor synergism. Blood 80:678–687.

Kmiecik TE, Keller JR, Rosen E, VandeWoude GF (1992): Hepatocyte growth factor is a synergistic factor for the growth of hematopoietic progenitor cells. Blood 80:2454–2457.

Kodama H, Nose M, Yamaguchi Y, Tsunoda J, Suda T, Nishikawa S, Nishikawa S (1992): In vitro proliferation of primitive hematopoietic stem cells supported by stromal cells: Evidence for the presence of mechanism(s) other than that involving c-kit receptor and its ligand. J Exp Med 176:351–361.

Li CL, Johnson GR (1992): Rhodamine 123 reveals heterogeneity within murine Lin^-, $Sca\text{-}1^+$ hemopoietic stem cells. J Exp Med 175:1443–1447.

Lowry PA, Deacon D, Whitefield P, McGrath HE, Quesenberry PJ (1992): Stem cell factor induction on in vitro murine hematopoietic colony formation by "subliminal" cytokine combinations: The role of "anchor" factors. Blood 80:663–669.

Metcalf D (1991): Control of granulocytes and macrophages: Molecular, cellular, and clinical aspects. Science 254:529–533.

Miyajima A, Mui AL-F, Ogoruchi T, Sakamaki K (1993): Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. Blood 82:1960–1974.

Ogawa M (1993): Differentiation and proliferation of hematopoietic stem cells. Blood 81:2844–2853.

Ogawa M, Nishikawa S, Yoshinaga K, Hayashi S, Kunisada T, Nakao J, Kina T, Sudo T, Kodama H, Nishikawa S (1993): Expression and function of c-kit in fetal hematopoietic progenitor cells: Transition from the early c-kit-independent to the late c-kit-dependent wave of hemopoiesis in the murine embryo. Development 117:1089–1098.

Okada S, Nakauchi H, Nagayoshi K, Nishikawa S, Miura Y, Suda T (1992): In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells. Blood 80:3044–3050.

Orlic D, Fischer R, Nishikawa S, Nienhuis AW, Bodine DM (1993): Purification and characterization of heterogeneous pluripotent hematopoietic stem cell populations expressing high levels of c-kit receptor. Blood 82:762–770.

Robinson BE, Quesenberry PJ (1990): Hematopoietic growth factors: Overview and clinical applications. Am J Med Sci 300:163–170, 237–244, 311–321 (Parts I, II, III).

Spangrude GJ, Smith L, Nobuko U, Koichi I, Heimfeld S, Friedman J, Weissman IL (1991): Mouse Hematopoietic stem cells. Blood 78:1395–1402.

Srour EF, Bradt JE, Briddell RA, Grigsby S, Leemhuis T, Hoffman R (1993): Long-term generation and expansion of human primitive hematopoietic progenitor cells in vitro. Blood 81:661–669.

Testa U, Pelosi E, Gabbianelli M, Fossati C, Campisi S, Isacchi G, Peschle C (1993): Cascade transactivation of growth factor receptors in early human hematopoiesis. Blood 81:1442–1456.

Tong J, Gordon MS, Srour EF, Cooper RJ, Orazi A, McNiece I, Hoffman R (1993): In vivo administration of recombinant methionyl human stem cell factor expands the number of human marrow hematopoietic stem cells. Blood 82:784–791.

Tucker D, Bol S, Kannourakis G (1993): Characterization of stroma-adherent colony-forming cells: A clonogenic assay for early hemopoietic cells? Exp Hematol 21:469–474.

Verfaillie CM (1992): Direct contact between human primitive hematopoietic progenitors and bone marrow stroma is not required for long-term in vitro hematopoiesis. Blood 79:2821–2826.

Williams DA (1993): Ex vivo expansion of hematopoietic stem and progenitor cell: Robbing Peter to pay Paul? Blood 81:3169–3172.

Williams N, Bertoncello I, Kaynoudias H, Zsebo K, McNiece I (1992): Recombinant rat stem cell factor stimulates the amplification and differentiation of fractioned mouse stem cell populations. Blood 79:58–64.

Wilson EL, Rifkin DB, Kelly F, Hannocks MJ, Gabrilove JL (1991): Basic fibroblast growth factor stimulates myelopoiesis in long-term human bone marrow cultures. Blood 77:954–960.

Wineman JP, Nishikawa S, Muller-Sieburg CE (1993): Maintenance of high levels of pluripotent hematopoietic stem cells in vitro: Effect of stromal cells and c-kit. Blood 81:365–372.