Factors Involved in the Regulation of Hematopoiesis

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ABSTRACT

With recombinant deoxyribonucleic acid (DNA) technology and better cell separation techniques, it is possible to demonstrate individual factor activity in the development of uncommitted and committed stem cells and their differentiation into functional mature cells. Much remains to be learned about the individual cell membrane receptors and their interactions with the polypeptide cytokines, as well as with other small molecules such as hemin. Some membrane perturbations must lead to complex and wonderful intracellular machinations to set in motion DNA and ribonucleic acid (RNA) changes leading to cell division and cell differentiation.

Introduction

The application of recombinant DNA technology has aided tremendously in the investigation of lymphokines. These glycosylated polypeptides have diverse and synergistic effects on progenitor cells to produce a variety of functioning terminal cells. Lymphokines are dialysable polypeptides of molecular weight around 15000 to 30000 generated by lymphocyte stimulation in vitro or in vivo. They are nonimmunoglobulin products capable of affecting leukocytes and other cells causing a variety of specific responses. These responses vary with the experimental conditions and the combination of lymphokines used.

Hamblin, in his excellent and concise review,\textsuperscript{10} indicates that nearly 100 factors have been isolated from supernatants of sometimes ill defined cell cultures. The term “interleukin” has been adopted for the most well characterized factors, especially those defined by recombinant DNA technology. Several other cloned proteins still retain their activity based names. Neither approach is completely satisfactory since the interleukins have been found to influence cells other than leukocytes and most factors have multiple activities depending upon experimental conditions and the cell lines being tested.

It appears lymphokines have roles in five areas of immunology: T cell activation, B cell activation, hematopoiesis, toxicity, and inflammation. This paper will deal with hematopoiesis.

Recent Observations

Lymphokines appear to be glycoproteins that are, for the most part, N glyco-
The role of the carbohydrate groups is not entirely clear because recombinant proteins produced in *Escherichia coli* are not glycosylated and yet appear to have similar function in cell cultures as their glycosylated counterparts. Work by Dube et al.\(^{5}\) has suggested that a similar protein, erythropoietin, must be glycosylated to be processed and secreted by cultured BHK cells. These cells, transfected with c DNA constructs that code for erythropoietin lacking carbohydrate groups at specific locations, failed to produce erythropoietin in supernatant culture fluid, although they produced ample intracellular messenger RNA. Although it is early in this work, one could speculate that glycosylation may be necessary for lymphokines to be processed and secreted.

The most prominent hematopoietic growth factors appear to be interleukins 1, 3, and 5, and the colony stimulating factors G, M, and GM-CSF and erythropoietin\(^4,6,12,13,17,21,22,23,25,30,36,39\) (table I). Both IL 4 and 6 are thought to be synergistic with other factors.\(^3,16\) Hemin has been described by three different groups as a prominent cofactor in erythroid colony formation.\(^24,26,33\) The interleukins generally are provided by macrophages/microcytes or lymphocytes while the colony stimulating factors come from other nonleukocyte lines.\(^7,9,10,15,28,31\) With the exception of IL 1, 2, 4, 37 the target of the interleukins is the hematopoietic stem cell,\(^8,11,12,13,19,20,21,22,24,32,34,35\) while the colony stimulating factors (CSF) act on partially developed or committed stem cells producing more specific differentiation.\(^1,6,8,13,14,17,18,19,20,22,29,36\) It is not completely settled as to whether or not the CSFs merely stimulate growth (meaning division) of differentiated cells or whether or not the CSFs provide for the maintenance of differentiated cells that have already divided. It may be a combination of both.\(^10,11,13,14,17,22,23,34,35,36\)

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**TABLE I**

<table>
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<th>Characteristics of Cytokines</th>
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<tr>
<td>Hematopoietic Factor Source Target Action Reference</td>
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<tr>
<td>Monocytes &amp; neutrophils Endothelial cells &amp; fibroblasts Increases production of CSFs while decreasing immune system regeneration 2,4,9,18,23,25,31,37</td>
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<tr>
<td>T Cells (Tg) Uncommitted hematopoietic stem cells Stimulates 6,12,13,20,21,24,28,32,36,37</td>
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<tr>
<td>Uncommitted hematopoietic stem cells B cell growth eosinophils differentiations 10,30</td>
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<tr>
<td>Fibroblasts &amp; endothelial cells Committed hemopoietic stem cells Stimulates 6,12,13,18,30,34,36,37</td>
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<tr>
<td>Renal cortex BFU-E Development 1,7,8,11,14,17,29</td>
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<tr>
<td>Interstitial BFU-E (CFU-GEMM with IL 3) Development 24,26,33</td>
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Interleukin 1 (formerly Hemopoietin 1) is produced by the monocyte/macrophage system after a variety of stimuli including infection, mitogens, hemolysis (tissue destruction), – all of which present new chemical forms (antigens) for processing by the macrophage\(^4,9,18,23,25\) (figure 1). The IL 1 produced has been demonstrated to interact

Monocyte/Macrophage  IL 1 →
Fibroblast/Endothelial Cell  →
GM-CSF (Other CSFs)

GM-CSF →Granulocyte/Macrophage →IL 1

**FIGURE 1.** Proposed amplification route for IL-1.
FACTORS INVOLVED IN REGULATION OF HEMATOPOIESIS

not only with T lymphocytes but also with endothelial cells and fibroblasts in cultures.4,10,25,31,37 These cells, in turn, produce GM-CSF and other CSFs under certain conditions.4,10,31,37 In addition, GM-CSF has been demonstrated to stimulate granulocyte and macrophage development from hematopoietic stem cells.6,13,35,36 These cells, in turn, in certain culture systems have been shown to produce more IL 1.4,18,23 It appears there is the potential for an enhancing cycle to occur to increase the stimulus for granulocytopoiesis.

The most interesting of the interleukins for hematopoiesis is IL 3 (figure 2). Current evidence suggests it is produced almost exclusively by the activated T4 helper cell and, hence, may be directly under the influence of IL 1 from the macrophage.4,12,28 Also, IL 4 (B cell growth factor) interacts synergistically to enhance CFU-GEMM colony growth in the presence of IL 3.3 The pluripotent stem cell is prepared by interaction with IL 3 not only to divide but also to receive further specific differentiation stimuli from CSFs. This preparation may be accomplished by the up regulation of receptor sites as growing cells reach confluence shown by the recent work of Murthy et al.27 Thus, IL 3 may prepare the stem cell surface to receive other polypeptide factors which will cause further differentiation. For example, if there is an increased concentration of erythropoietin in the environment, the cell surface will receive a different stimulus than if there is a high concentration of GM-CSF and will undergo internal activation toward the erythroid differentiation.

Of interest is the effect of another small molecule in this regard. Hemin has been shown by several investigators to induce erythroid cell development in culture in the presence of IL 3 or erythropoietin (figure 3). In addition, in "serum free" culture systems, hemin has been shown to act synergistically with IL 3 to enhance CFU-GEMM growth.24 Monette et al have shown that hemin acts synergistically with IL 3 to promote the growth of CFU-GEMM in serum-free cultures of normal murine bone marrow.24 Morse et al have shown that erythroid colony growth from human peripheral blood stem cells is enhanced when both erythropoietin and hemin are present in the medium.26 Sigounas et al have shown that hemin induces erythropoietin-dependent cell lines in vitro,33 to undergo terminal differentiation and hemoglobin production, but does not by itself sustain viability since the cultures die in the absence of erythropoietin.

Finally, the work of Migliaccio and Adamson22 shows that IL 3 and GM-CSF enhance the growth of erythroid precursors in the presence of erythropoietin in medium containing fetal calf serum; however, fetal calf serum is not a

![Diagram](image_url)

**Figure 2.** Scheme for diversification of uncommitted stem cell CFU-GEMM.

IL 3 or Epo

Stem Cell → BFU-E (CFU-E) → Erythroid Line

**Figure 3.** Position of hemin in development of erythroid line.
requirement for such enhancement. Migliaccio and Adamson conclude that serum-free culture is now possible and that IL 3 and GM-CSF provide for enhancement of differentiation of stem cells from bone marrow or peripheral blood to committed erythropoietin sensitive erythroid burst-forming units. Thus, these three factors, alone in a basic culture medium, are all that are necessary to allow development of end stage erythroid cells from hematopoietic stem cells.

Summary

With recombinant DNA technology and better cell separation techniques, it is possible to demonstrate individual factor activity in the development of uncommitted and committed stem cells and their differentiation into functional mature cells. Much remains to be learned about the individual cell membrane receptors and their interactions with the polypeptide cytokines, as well as with other small molecules such as hemin. Some membrane perturbations must lead to complex and wonderful intracellular machinations to set in motion DNA and RNA changes leading to cell division and cell differentiation.

References


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