The Molecular Control of Blood Cell Development

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The establishment of a cell culture system for the clonal development of blood cells has made it possible to identify the proteins that regulate the growth and differentiation of different blood cell lineages and to discover the molecular basis of normal and abnormal cell development in blood forming tissues. A model system with myeloid blood cells has shown that (i) normal blood cells require different proteins to induce cell multiplication (growth inducers) and cell differentiation (differentiation inducers), (ii) there is a hierarchy of growth inducers as cells become more restricted in their developmental program, and (iii) a cascade of interactions between proteins determines the correct balance between immature and mature cells in normal blood cell development. Gene cloning has shown that there is a family of different genes for these proteins. Normal protein regulators of blood cell development can control the abnormal growth of certain types of leukemic cells and suppress malignancy by inducing differentiation to mature nondividing cells. Chromosome abnormalities that give rise to malignancy in these leukemic cells can be bypassed and their effects nullified by inducing differentiation, which stops cells from multiplying. These blood cell regulatory proteins are active in culture and in the body, and they can be used clinically to correct defects in blood cell development.

THE FORMATION OF DIFFERENT TYPES OF BLOOD CELLS IS essential for normal development. New blood cells belonging to different cell lineages are formed from stem cells during embryogenesis and during the lifetime of the adult. Abnormalities in the normal developmental program for blood cell formation result in various types of hematological diseases. Understanding the molecular control of normal blood cell development makes it possible to answer questions about the origin and possible treatment of these diseases. What are the molecular regulators that control the normal developmental program of cell multiplication and differentiation to different blood cell lineages? How do these regulators interact to give the correct balance between the number of immature and mature cells? What changes cause hematological disease, and can normal blood cell formation be restored in hematologically abnormal individuals by treatment with normal regulators of development? I will mainly describe results obtained with cells of the myeloid blood cell lineages, which have been used as a model system to answer these questions.

To analyze the controls that regulate multiplication and differentiation of normal blood cells to different cell lineages and the changes in these controls in disease, it is desirable and convenient to study the process in cell culture starting from single cells. The analysis, therefore, began with the development of a cell culture system for the cloning and clonal differentiation of different types of normal blood cells. This system made it possible to discover a group of protein hormones that regulates cell multiplication and differentiation of specific blood cell lineages, to analyze the origin of some hematological diseases, and to identify possible ways of treating these diseases with normal regulatory molecules.

Clonal Development of Normal Blood Cells in Culture

In the cell culture system that was developed, normal cells from blood forming tissues from mice were first cultured with feeder layers of other cell types, such as normal embryo fibroblasts. These other cell types were chosen as candidates for the production of the inducers required for clonal growth and differentiation of different blood cell lineages. The first such system, with cells cultured in liquid medium (Fig. 1A), showed that it was possible to obtain clones containing mast cells or granulocytes in various stages of differentiation (*1, 2*). To make it simpler to distinguish and isolate separate clones, this system was then applied to the cloning of different blood cell lineages in semisolid medium containing agar (*3, 4*). An analysis of the first types of clones obtained in agar with these feeder layers showed clones containing macrophages, granulocytes, or both macrophages and granulocytes in various stages of differentiation (Fig. 1, B to D). The macrophage clones in agar contained many metachromatic granules, giving them an apparent morphological resemblance to mast cells (*3, 4*). However, these granules were not present when the cells were cloned in methylcellulose (*5*), and electron microscopy also revealed that these cells, which appeared to be mast cells in agar, were really macrophages that had phagocytosed agar (*6*). The experiments also showed that these clones could originate from single cells (*3, 4, 7*). This assay in agar (*3, 4*) or methylcellulose (*5*) was then applied to cloning and clonal differentiation of normal human macrophages and granulocytes (*8, 9*) and to cloning of all other blood cell lineages, including erythrocytes (*10*), B lymphocytes (*11*), and T lymphocytes (*12*).

The Proteins That Control Development of Different Cell Lineages

When cells were cloned in a semisolid substrate such as agar, there was a more solid layer of agar between the feeder layer cells and the

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cells seeded for cloning. This showed that the inducers required for the formation of macrophage and granulocyte clones were secreted by the feeder layer cells and diffused through the agar (*3*). In the next step, it was then shown that these inducers are present in the conditioned medium produced by the feeder cells (*5, 7*). When cells were washed at various times after initiating the induction of clones, there was no further development of either macrophage or granulocyte clones unless the inducer was added again (*13*). Thus, the development of clones with differentiated cells requires both an initial and continued supply of inducer. These inducers were found in the conditioned medium from different types of normal and malignant cells (*14, 15*). These media were used to purify the inducers, which were glycoproteins or proteins without detectable sugars (*16–20*) [sugars do not seem to be necessary for their biological activity (*19*)]. A similar approach was later used to identify the protein inducers for cloning of T lymphocytes (*21*) and B lymphocytes (*22*).

In cells of the myeloid lineages, four different proteins that induce cell multiplication and, thus, the formation of clones (growth-inducing proteins) have been identified (*23–28*). The same proteins have been given different names. After they were first detected in cell culture supernatant fluids (*5, 7*), the first inducer identified was called "mashran gm" from the Hebrew word meaning to send forth with the initials for granulocytes and macrophages (*29*). This and other growth-inducing proteins were then renamed and are now called colony-stimulating factors (CSF) (*30*) or macrophage and granulocyte inducers (MGI) (*16*) -type 1, (MGI-1); and one protein is called interleukin-3 (IL-3) (*20*) (Table 1). Of these four growth inducers, one protein (M) induces the development of clones with macrophages, another (G) clones with granulocytes, the third (GM) clones with granulocytes, macrophages, or both macrophages and granulocytes, and the fourth (IL-3) clones with macrophages, granulocytes, eosinophils, mast cells, erythroid cells, or megakaryocytes (Table 1 and Fig. 2). These proteins induce cell viability and cell multiplication (*31, 32*) and enhance the functional activity of mature cells (*33*).

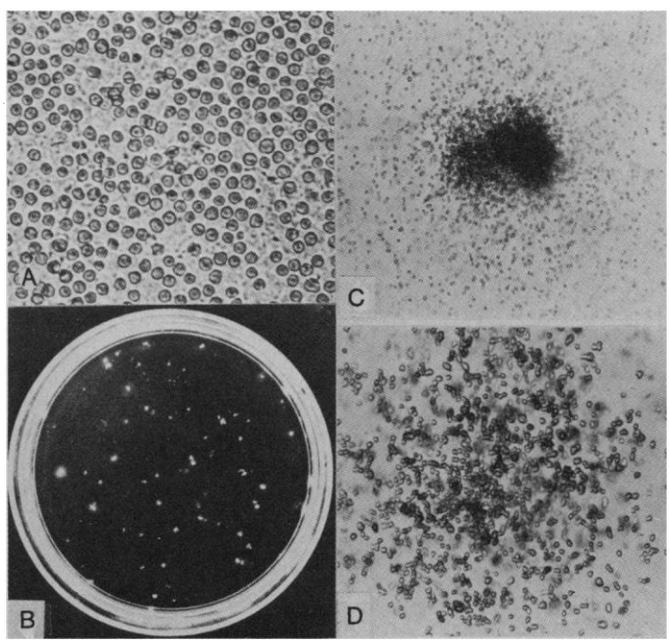

Fig. 1. (**A**) Culture of mouse mast cells that have multiplied and differentiated on a feeder layer of mouse embryo cells (*1*). (**B** to **D**) Clones of macrophages and granulocytes in cultures of normal blood cell precursors incubated with the appropriate myeloid cell regulatory protein. (B) Petri dish with clones (*3*); (C) granulocyte clone (*5*); (D) Macrophage clone (*5*).

Cloning of genes from mice or humans for the IL-3 (*34*), GM (*35*), M (*36*), and G (*37*) growth inducers has shown that these genes are unrelated in their nucleotide sequence. This family of genes represents a hierarchy of growth-inducing proteins for blood cell development as cells become more restricted in their developmental program (Fig. 2). There is presumably such a hierarchy of growth inducers for other types of cells. To determine whether this hierarchy is found in normal fetal development, the production of messenger RNA and biologically active protein for the four different myeloid blood cell growth-inducing proteins has been analyzed at various stages of normal mouse fetal development (*38*). The macrophage growth inducer (named MGI-1M, M-CSF, or CSF-1) was the only myeloid cell growth inducer detected as both mRNA and biologically active protein during fetal development. This inducer is produced predominantly in extraembryonic tissues, and the production of growth inducer mRNA in embryogenesis is regulated by transcriptional and post-transcriptional controls (*38*). The early production of MGI-1M is presumably responsible for the early appearance of macrophage precursors in the fetus. Mature macrophages produce MGI-1GM (GM-CSF) (*39*), so that once these cells have differentiated, they can produce a growth inducer for both macrophages and granulocytes. The production of MGI-1M in extraembryonic membranes continues even when the fetal liver is the major hematopoietic organ (*38*). This growth inducer is apparently produced in a separate compartment from the target cells at these development stages. Mature macrophages selectively clear the growth inducer MGI-1M (CSF-1) from the circulation and degrade the protein intracellularly (*40*). This provides a feedback control mechanism by which the rate of macrophage production is determined by the number of mature macrophages (*40*).

How do normal myeloid precursor cells, induced to multiply by growth inducers, develop into clones containing mature cells that stop multiplying when they terminally differentiate? It appears unlikely that a growth factor that induces cell multiplication would also induce differentiation, which stops cell multiplication in mature cells. Indeed, proteins that induce myeloid cell differentiation but not growth have been identified and are called macrophage and granulocyte inducers–type 2 (MGI-2) or differentiation factors (*19, 23–25, 41–44*). Each of the myeloid growth-inducing and differenti-

Table 1. Normal myeloid blood cell growth and differentiation-inducing proteins.

	Nomenclature	Differentiated cell type	Induction of differentiation	
			Direct	Indirect*
Growth inducers	MGI-1M = M-CSF = CSF-1	Macrophages	−	+
	MGI-1G = G-CSF	Granulocytes	−	+
	MGI-1GM = GM-CSF	Macrophages and granulocytes	−	+
	IL-3	Macrophages, granulocytes, and others	−	+
Differentiation inducer	MGI-2	Macrophages and granulocytes	+	−

*Growth-inducing protein activates production of differentiation-inducing protein. References in text.

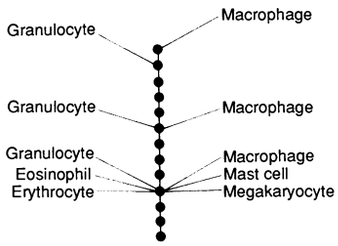

Fig. 2. Hierarchy of myeloid cell growth-inducing proteins. Myeloid precursor blood cells can be induced to multiply by four different growth-inducing proteins. One (IL-3) induces growth in precursors that have the potential to develop into six cell types, the second (GM) induces growth in precursors that develop into one cell type.

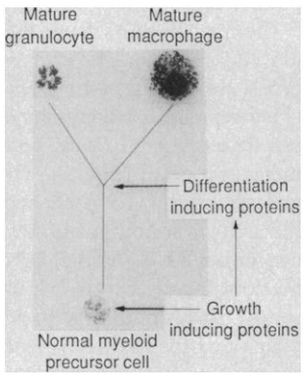

Fig. 3. Growth of normal myeloid blood cell precursors and their differentiation to macrophages or granulocytes are induced by different proteins, growth-inducing proteins and differentiation-inducing proteins. The growth-inducing proteins induce cell viability and multiplication of normal precursors and production in these cells of differentiation-inducing proteins. The induction of differentiation inducer by growth inducer provides a mechanism to couple the multiplication of normal precursor cells and their differentiation.

ation-inducing proteins has a different cell surface receptor (*45, 46*); the receptor for MGI-1M (CSF-1) is related to the proto-oncogene c-*fms* (*47*). Normal differentiation inducers for myeloid cells, but not growth inducers, are proteins that bind to double-stranded DNA (*48*). They can also cause single strand DNA breaks (nicks) (*49*). It will be interesting to determine whether this applies to normal differentiation-inducing proteins for other cell types. The myeloid growth and differentiation-inducing proteins are produced by various cell types and induce growth and differentiation when supplied externally to target cells (*15, 23, 25, 42–44, 50*).

Cascade of Interactions Between Different Proteins in Development

Experiments with normal myeloid precursor cells, which can be separated from other cell types in the bone marrow (*51*), have shown that in these cells any one of the four growth-inducing proteins induces cell viability, cell multiplication, and production of differentiation-inducing protein (*23, 25, 32, 52*). The myeloid differentiation inducers activate differentiation directly, whereas the myeloid growth inducers activate differentiation indirectly by induction of differentiation inducer (Table 1 and Fig. 3). This induction of differentiation inducer by growth inducer serves as an effective mechanism to couple growth and differentiation, a mechanism that may also apply to other cell types. Differences in the time of switch-on of the differentiation inducer would produce differences in the amount of cell multiplication before differentiation. Also, different growth inducers may switch on different differentiation inducers, which may determine the differentiated cell type. A differentiation inducer can in turn switch on production of a growth inducer in myeloid cells (*45*), and this then ensures cell viability and enhances the function of mature cells. Thus, the studies on myeloid cells have shown that there are different proteins, growth inducers, and differentiation inducers participating in development and that there is a cascade of interactions between these proteins in development.

Normal development provides the correct balance between immature and mature cells, and cell multiplication is regulated at two control points. In the first control, growth inducer produces more cells and activates production of differentiation inducer. The second control stops cell multiplication as part of the program of terminal differentiation to mature cells. Growth and differentiation in normal cells are coupled at both these points. Changes in the normal balance between immature and mature cells, by uncoupling growth and differentiation, can lead to nonmalignant or malignant abnormalities, depending on the extent of the changes. Identification of the normal blood cell growth- and differentiation-inducing proteins and of the cascade of interactions between different proteins has made it possible to identify the changes in production or response to these normal regulators that produce hematological disease.

Cell Response to Normal Regulatory Proteins in Hematological Disease

Are abnormal cells in hematological disease still controlled by normal protein regulators of growth and differentiation? Most of the studies on the response of cells to normal regulatory proteins in hematological disease have been carried out with leukemic cells. Normal myeloid growth inducers are produced by various types of cells (*15*) but not by normal myeloid precursor cells (*32*), so that the normal precursors require the production of growth-inducing protein by other cell types for viability and growth. These are leukemic cells from patients with myeloid leukemia that require an external source of myeloid growth-inducing protein (*8, 27, 53*), but other leukemic cells are independent of a growth inducer for viability and multiplication (*15, 23, 25, 41, 54*) because of changes from an induced to constitutive expression of certain genes (*55*). This gives the leukemic cells an advantage over the normal cells when there is a limiting amount of growth inducer in the body. Independence from normal growth inducer can also explain the survival and growth of metastasizing cells in places in the body where growth inducer required for the viability of normal cells is absent.

Can myeloid leukemic cells that no longer require a growth inducer still be induced to differentiate to mature nondividing cells by the normal protein regulators of differentiation? This question has been answered by showing that there are growth inducer–independent clones of myeloid leukemic cells that can be induced to differentiate to mature macrophages or granulocytes through the normal sequence of gene expression by incubation with a normal myeloid differentiation-inducing protein (*15, 23, 25, 50, 54*). These are called D^+ clones (D for differentiation) (*56*). The mature cells, which can be formed from all the cells of a leukemic clone, stop multiplying and are no longer malignant in vivo (*57–59*).

Studies in animals and humans have shown that normal differentiation of D^+ myeloid leukemic cells to mature nondividing cells can be induced not only in culture but also in vivo (*58–62*) (Fig. 4). These leukemias, therefore, grow progressively when there are too many leukemic cells for the normal amount of differentiation inducer in the body. The development of leukemia can be inhibited in mice with these D^+ leukemic cells by increasing the amount of differentiation-inducing protein, either by injecting it or by injecting a compound that increases its production by cells in the body (*58, 59*).

Experiments on the properties of D^+ myeloid leukemic clones, which are independent of growth inducer, have shown that after induction of differentiation by a normal differentiation-inducing protein, the normal requirement of growth inducer for cell viability and multiplication is restored (*32, 41*). Growth inducer added to normal myeloid precursor cells induces production of differentiation inducer, and the cells are then induced to differentiate by endoge-

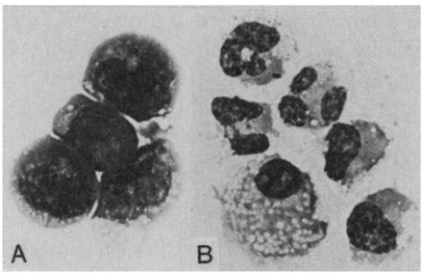

Fig. 4. Differentiation of myeloid leukemic cells in vivo. (**A**) Leukemic blast cells. (**B**) Cells in different stages of differentiation (*60*).

nous differentiation inducer. However, in these D^+ leukemic cells, growth inducer does not induce endogenous production of differentiation inducer, so that there is no induction of differentiation after adding a growth inducer (*32*). The cascade of interaction between growth and differentiation-inducing proteins is genetically blocked in these leukemic cells (*32*).

In addition to the D^+ clones that can be induced to differentiate by a normal myeloid differentiation-inducing protein, there are other D^+ clones that, like normal myeloid cells, can be induced to differentiate by incubating the leukemic cells with a normal myeloid growth-inducing protein (Table 2). Different clones can be induced to differentiate by incubation with MGI-1GM (GM-CSF) (*52, 60*), IL-3 (*52*), or MGI-1G (G-CSF) (*27, 37, 52*). In these clones, the growth inducers presumably induce production of an appropriate differentiation inducer, so that in this type of leukemic cell the cascade does not appear to be blocked. Not all D^+ leukemic clones respond to the same differentiation-inducing protein, MGI-2 (*52*) (Table 2) and, as has been found with myeloid growth-inducing proteins (*28*), there may also be a multigene family of myeloid differentiation-inducing proteins. There is a clonal distribution of response of myeloid leukemic cells to different myeloid regulatory proteins (*52*) and presumably also a clonal distribution in response of normal myeloid precursor cells to these regulators. Most and possibly all of the physiological regulatory proteins of normal myeloid blood cell development can regulate differentiation of D^+ myeloid leukemic cells (*52*).

There is another type of myeloid leukemic clone in which the leukemic cells constitutively produce their own growth inducer and in which the cascade of interaction between growth and differentiation inducer is blocked (*63*). However, in this type of leukemic clone, changes in specific components of the culture medium can unblock the cascade and restore the induction of differentiation inducer by growth inducer (*63*). The study of different clones of myeloid leukemic cells has also shown that, in addition to D^+ clones, there are differentiation-defective clones (*8, 54, 56*), named D^- clones (*56*). Some D^- clones are induced by a normal myeloid regulatory protein to an intermediate stage of differentiation that then slows the growth of the cells; others cannot be induced to differentiate even to this intermediate stage (*15, 23, 25, 50, 54, 64*). Since normal regulatory proteins induce differentiation to mature nondividing cells in the D^+ clones, it has been suggested that D^+ clones are the early stages of leukemia and that the formation of different types of D^- clones may be later stages in the progression of malignancy (*54*). There are D^- clones of myeloid leukemia cells that can no longer be induced to differentiate by proteins that regulate normal myeloid cell development but that can be induced to differentiate by other compounds, including hormones such as certain steroids and insulin (*23, 54, 64, 65*), which induce differentiation by alternative pathways (*23, 25*). Although the cell response to induction of differentiation by normal myeloid regulatory proteins has been altered, these D^- clones have not lost all the genes for differentiation.

Myeloid leukemic cells in humans (*66*) and mice (*67*) have chromosome abnormalities. Chromosome changes that alter the balance between genes that allow induction of differentiation and genes that suppress differentiation by normal myeloid regulatory proteins can make D^- leukemic cells become D^+ leukemic cells and vice versa (*67*). The chromosome abnormalities in these leukemic cells are not corrected when the cells are induced to differentiate. In the D^+ leukemic cells, inducing differentiation stops cell multiplication by bypassing the genetic changes that produced the malignant phenotype; that is, loss of requirement for normal growth inducer and inability of growth inducer to switch on differentiation inducer. In the D^- leukemic cells, inducing differentiation by alternative pathways stops cell multiplication by bypassing the genetic changes that inhibit differentiation by normal regulatory proteins. Suppression of malignancy by inducing differentiation does not have to restore all of the normal controls, and genetic abnormalities that give rise to malignancy can be bypassed and their effects nullified by inducing differentiation, stopping the cells from multiplying (*23, 25*).

In Vivo Control of Blood Cell Development

Mature blood cells generally have a short life-span, and large numbers of different cell lineages have to be produced in the body throughout normal life. Many cells from different lineages also have to be produced in emergency situations, such as after infections or loss of blood. Blood cell development in vivo is also regulated by myeloid cell regulatory proteins that were originally identified in vitro. In normal embryogenesis, a myeloid regulatory protein (MGI-1M = M-CSF = CSF-1) is produced during fetal development, predominantly in extraembryonic tissues (*38*). This accounts for the early appearance of macrophage precursors in the fetus. After injection of myeloid leukemic cells into fetuses, the leukemic cells can participate in blood cell differentiation in apparently healthy adult animals (*68*), which also shows that appropriate myeloid cell regulators are present in the fetus.

New blood cells are formed by sequential activation of different stem-cell clones (*69*) found in the bone marrow of adult mammals. Bone marrow contains stroma that consists of endothelial cells, fibroblasts, adipocytes, and macrophages (*70*). Stromal cells produce and bind MGI-1GM (GM-CSF), which can also be bound by glycosaminoglycans of the extracellular matrix (*71*). As has been shown with a cell-associated angiogenesis factor (*72*), a myeloid cell regulatory protein synthesized by stromal or other cells can be stored by binding to certain cells or extracellular matrix and then function directly by cell to cell or cell to matrix interactions or be released when physiologically required (*71, 72*). Changes in cell shape caused by cell attachment, which can alter its response to

Table 2. Differentiation of normal and leukemic myeloid cells after culturing with different normal myeloid regulatory proteins.

Cell type	Differentiation after culturing with				
	MGI-2*	IL-3	MGI-1GM (GM-CSF)	MGI-1G (G-CSF)	MGI-1M (M-CSF, CSF-1)
Normal					
Precursor cells	+	+	+	+	+
Leukemic					
Clone 11	+	−	−	±	−
Clone 7-M12	−	+	+	−	−
WEHI-3B	−	−	−	+	−

*Leukemic clone 7-M12 and WEHI-3B, which are not induced to differentiate by the MGI-2 (differentiation-inducing protein) that differentiates normal myeloid precursors and leukemic clone 11, have few or no receptors for this MGI-2 (*46*). These clones should be useful to identify other types of MGI-2. For further references see (*52*).

hormones and nutrients (*73*), can modulate the development of myeloid precursor cells to differentiate to either macrophages or granulocytes (*74*). Thus, attachment of developing blood cells to stromal cells may also modulate blood cell development by changing their shape. Changes in cell shape after attachment of blood cell precursors to stromal cells may also induce the production of blood cell regulatory proteins in the attached cells.

What happens in emergency situations when a large number of cells are unexpectedly needed? Studies of infection and inflammation have shown that stimulation of the myelopoietic system is associated in vivo with a rapid increase of myeloid regulatory proteins caused by induction of these proteins by various compounds, including bacterial lipopolysaccharide (*75*) and inflammatory agents such as casein or thioglycolate (*76*). Different inducing agents cause different body distributions of these regulators (*76*). A major source of myeloid regulatory proteins appears to be T lymphocytes (*28, 77*), including helper T lymphocytes driven by antigen (*78*). Therefore, an emergency supply of these regulators can be obtained by induction of these proteins in T lymphocytes and other cell types, including macrophages, fibroblasts, and endothelial cells.

Clinical Implications

Identification of the myeloid cell regulatory proteins has suggested novel possibilities for the therapy of nonmalignant and malignant hematological disease (*54*). The concentration of these proteins can be increased in vivo either by injecting the protein or by injecting one of the compounds that induces their production (*58, 59, 75, 76, 79*). Injection of protein stimulates myelopoiesis under normal circumstances and after suppression of myelopoiesis induced by compounds such as cyclophosphamide, with minimal side effects (*59, 79*). In nonmalignant hematological abnormalities, myeloid regulatory proteins should be clinically useful in restoring the normal number and function of the myeloid blood cell population in patients with suppressed myelopoiesis, such as that which occurs after treatment to obtain immune depression for organ transplants or after cytotoxic cancer therapy. These treatments not only suppress myelopoiesis but also destroy cells that produce the regulatory proteins. Addition of these proteins should also increase the success of bone marrow transplant grafts in patients deficient in these proteins and the cells that produce them in the bone marrow. Because of the many functions of mature cells such as macrophages (*80*) and other myeloid cells, the increased function of mature cells induced by these regulatory proteins (*33*) can also be clinically helpful to patients with deficiencies in myeloid cell functions. It has already been shown that injection of the protein erythropoietin, which stimulates the production of erythrocytes, can correct the anemia in patients with end-stage renal disease (*81*), and that MGI-1GM (GM-CSF) is biologically active in leukopenic patients with AIDS (*82*).

In the therapy of malignant disease, suppression of malignancy by inducing differentiation is an alternative approach to the use of cytotoxic doses of compounds that, with the compounds used so far, kill many normal cells as well as tumor cells. Results showing that the development of myeloid leukemia can be inhibited in mice with D^+ leukemic cells by injecting a normal myeloid regulatory protein or injecting a compound that increases the production of this protein in vivo (*58, 59*) indicate a therapeutic potential for these proteins in myeloid leukemia. There are leukemic cells from patients with myeloid leukemia that still require a myeloid growth-inducing protein for cell viability and multiplication (*8, 27, 53*), so that injection of one of these proteins may possibly stimulate the growth of these leukemic cells. However, the D^+ myeloid leukemic cells that no longer require normal growth inducer again require it for cell viability and growth after induction of differentiation (*32*). Despite the requirement of a myeloid growth inducer for growth, the further induction of differentiation can change the balance between multiplying cells and nonmultiplying differentiated cells sufficiently to inhibit the development of leukemia (*58, 59*). Restoration of the requirement for a myeloid growth inducer for viability and growth in these D^+ myeloid leukemic cells also suggests that induction of differentiation to this stage may result in the loss of viability of the induced leukemic cells in vivo if there is not enough growth inducer present.

In addition to hormones such as certain steroids (*64*) and insulin (*65*), there are compounds other than the normal protein myeloid cells regulators that are used at high doses in cancer therapy to kill cells that at lower doses induce differentiation of leukemic cells (*54*). Not all of these compounds are equally active on the same clone. The differences in the ability of clones to be induced to differentiate may help to explain the variations in therapy response in different patients. The induction of differentiation-inducing proteins by some of these compounds (*23, 25*) may also effect the therapeutic results. These findings have led to encouraging clinical results in patients with myeloid leukemia receiving low doses of cytosine arabinoside (*83*), one of the compounds that induces differentiation in myeloid leukemic cells (*54, 64*).

The experiments on induction of differentiation in myeloid leukemic cells have led to the suggestion (*54*) that there can be a form of therapy, based on induction of differentiation, with normal myeloid regulatory proteins and other compounds that will affect mutant malignant cells no longer responding to the normal regulatory proteins alone. Leukemic cells from each patient should be screened to select for the most effective combinations (*84*). Differentiation therapy could also, in some cases, be combined with cytotoxic therapy to reduce the number of malignant cells. With the availability of human recombinant myeloid regulatory proteins (*28*), the study of myeloid blood cell regulatory proteins has now progressed from the original cell culture assays (*1–4*) and discovery of these regulators in cell culture supernatants (*5, 7*) to their application in the clinic.

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85. Supported by the Alfred and Victoria Ebner Foundation for Leukemia Research, the National Foundation for Cancer Research (Bethesda, MD), the Julian Wallerstein Foundation, and the Jerome A. and Estelle R. Newman Assistance Fund.