Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 4742–4749, May 1996
Medical Sciences

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected on April 25, 1995.

The control of hematopoiesis and leukemia: From basic biology to the clinic

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Contributed by Leo Sachs, March 11, 1996

ABSTRACT **Hematopoiesis gives rise to blood cells of different lineages throughout normal life. Abnormalities in this developmental program lead to blood cell diseases including leukemia. The establishment of a cell culture system for the clonal development of hematopoietic cells made it possible to discover proteins that regulate cell viability, multiplication and differentiation of different hematopoietic cell lineages, and the molecular basis of normal and abnormal blood cell development. These regulators include cytokines now called colony-stimulating factors (CSFs) and interleukins (ILs). There is a network of cytokine interactions, which has positive regulators such as CSFs and ILs and negative regulators such as transforming growth factor β and tumor necrosis factor (TNF). This multigene cytokine network provides flexibility depending on which part of the network is activated and allows amplification of response to a particular stimulus. Malignancy can be suppressed in certain types of leukemic cells by inducing differentiation with cytokines that regulate normal hematopoiesis or with other compounds that use alternative differentiation pathways. This created the basis for the clinical use of differentiation therapy. The suppression of malignancy by inducing differentiation can bypass genetic abnormalities that give rise to malignancy. Different CSFs and ILs suppress programmed cell death (apoptosis) and induce cell multiplication and differentiation, and these processes of development are separately regulated. The same cytokines suppress apoptosis in normal and leukemic cells, including apoptosis induced by irradiation and cytotoxic cancer chemotherapeutic compounds. An excess of cytokines can increase leukemic cell resistance to cytotoxic therapy. The tumor suppressor gene wild-type p53 induces apoptosis that can also be suppressed by cytokines. The oncogene mutant p53 suppresses apoptosis. Hematopoietic cytokines such as granulocyte CSF are now used clinically to correct defects in hematopoiesis, including repair of chemotherapy-associated suppression of normal hematopoiesis in cancer patients, stimulation of normal granulocyte development in patients with infantile congenital agranulocytosis, and increase of hematopoietic precursors for blood cell transplantation. Treatments that decrease the level of apoptosis-suppressing cytokines and downregulate expression of mutant p53 and other apoptosis suppressing genes in cancer cells could improve cytotoxic cancer therapy. The basic studies on hematopoiesis and leukemia have thus provided new approaches to therapy.**

"The described cultures thus seem to offer a useful system for a quantitative kinetic approach to hematopoietic cell formation and experimental studies on the mechanism and regulation of hematopoietic cell differentiation." (1)

The formation of different types of blood cells, hematopoiesis, is essential for the development and survival of a normal individual. New blood cells belonging to different cell lineages are formed from stem cells during embryogenesis and throughout the lifetime of the adult to replace cells that have completed their life span. Abnormalities in the normal developmental program for blood cell formation result in hematological diseases including leukemia. Understanding the cellular and molecular controls of normal blood cell development makes it possible to answer questions about the origin and treatment of these diseases. To analyze the controls that regulate viability, multiplication, and differentiation of normal hematopoietic cells, it is desirable and convenient to study the entire process in cell cultures starting from single cells. Therefore, my analysis began with the establishment of a cell culture system for the cloning and clonal development of different types of hematopoietic cells. This cell culture system made it possible to discover a family of cytokines that regulate cell viability, multiplication, and differentiation of different hematopoietic cell lineages; to analyze the changes in normal controls in some hematological diseases including leukemia; and to identify new ways of treating these diseases.

The Cell Culture System for Clonal Development of Normal Hematopoietic Cells

In the cell culture system that was developed to study hematopoiesis, feeder layers of other cell types such as normal embryo fibroblasts were used as possible candidates for cells that produce regulatory molecules required for the viability, cloning, and differentiation of normal hematopoietic cells. In the absence of these feeder layers, the normal hematopoietic cells did not survive. The first system (1), using cells from mice cultured with feeder layers in liquid medium (Table 1), showed that this procedure made it possible to obtain colonies containing mast cells or granulocytes in various stages of differentiation. The cultures also showed differentiation to macrophages. I wrote as the concluding sentence of this paper, "The described cultures thus seem to offer a useful system for a quantitative kinetic approach to hematopoietic cell formation and for experimental studies on the mechanism and regulation of hematopoietic cell differentiation" (1). The system using feeder layers was applied to the development of hematopoietic cell colonies in semisolid medium containing agar (2) or methylcellulose (3). This made it simpler to distinguish and

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Abbreviations: CSF, colony-stimulating factor; M-CSF, macrophage-CSF; G-CSF, granulocyte-CSF; GM-CSF, granulocyte/macrophage-CSF; IL, interleukin; SCF, stem cell factor; TGF, transforming growth factor; TNF, tumor necrosis factor; D^+, differentiation competent; D^-, differentiation defective.

Table 1. Establishment of the cell culture system for cloning and clonal differentiation of normal hematopoietic cells and the use of these cultures to discover colony-stimulating factors (CSFs)

System	Ref.
Cloning and differentiation in liquid medium	1
Cloning and differentiation in agar	2–4
Cloning and differentiation in methylcellulose	3
Inducers for cloning and differentiation secreted by cells	2
Inducers for cloning and differentiation in cell culture supernatants	3, 6

isolate separate colonies and the procedure with agar (2) was then also used by others (4). The colonies obtained in agar or methylcellulose with these feeder layers contained macrophages, granulocytes (Fig. 1), or both macrophages and granulocytes in various stages of differentiation to mature cells. It was also shown that the hematopoietic cell colonies in culture (1–5) can originate from single cells (1, 6, 7) and are therefore clones that develop from progenitor cells that survived, multiplied, and differentiated to mature cells in culture. This culture system was applied to the clonal development of normal human macrophages and granulocytes (8, 9) and other blood cell lineages (10–12).

Discovery of CSFs

The use of feeder layers seeded underneath the agar showed that there was no need for direct contact between the feeder layers and the hematopoietic cells to induce hematopoietic clonal development, because the active factors could diffuse through agar (2). This led to the finding that the feeder layers could be replaced by the active factors found in supernatants secreted by these cells (3, 6) (Table 1). The active factors were found in supernatants from different types of normal and malignant cells (reviewed in ref. 13–15), and the supernatants were then used to purify these factors (16–20). A similar approach was later used to identify the factors required for cloning of T lymphocytes (21) and B lymphocytes (reviewed in ref. 22). It was also shown that the development of clones with differentiated cells requires both an initial and continued supply of colony-inducing factor (23).

In cells belonging to the myeloid cell lineages, four colony-inducing proteins were isolated (reviewed in refs. 24–30). The first colony-inducing factor identified was called *mashran gm* from the Hebrew word meaning to send forth, with the initials for granulocytes and macrophages (31). This and other colony inducing proteins were then renamed with different names including macrophage/granulocyte inducers (16) and macrophage/granulocyte inducer type 1. They are now collectively called CSFs (reviewed in refs. 26 and 30), including one protein called interleukin 3 (IL-3) (20). Of these four CSFs, one (M-CSF) induces the development of clones with macrophages; another (G-CSF) clones with granulocytes; the third (GM-CSF) 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. The CSFs induce cell viability and cell multiplication (reviewed in refs. 26–29, 32, and 33) and enhance the functional activity of mature cells (reviewed in ref. 30). Cloning of genes from mice and humans for IL-3, GM-CSF, M-CSF, and G-CSF has shown that these proteins are coded for by different genes (reviewed in ref. 34). Another cytokine identified more recently, stem cell factor (SCF) (35), has weak CSF activity and requires higher concentrations to induce colony formation. SCF induces colonies that contain myeloid cells in early stages of differentiation and can synergize with IL-3 and IL-6. The cytokines IL-3 and SCF appear to be capable of acting on more primitive myeloid precursor cells and to recruit them to respond to other CSFs. The ability of different CSFs to carry out similar and sometimes overlapping functions in the myeloid lineages demonstrates the flexibility of this multigene system. The receptors for M-CSF (c-*fms*) and SCF (c-*kit*) have a related intracellular tyrosine kinase domain (reviewed in ref. 36), whereas the receptors for GM-CSF, G-CSF, and IL-3, which do not have a tyrosine kinase domain, recruit *src*-related protein tyrosine kinases and the JAK2 protein tyrosine kinase to transmit their signals intracellularly (reviewed in ref. 37).

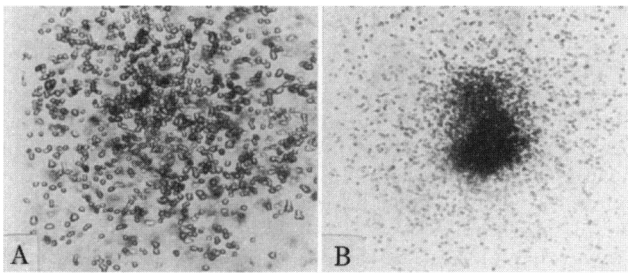

FIG. 1. Clones of normal macrophages (*A*) and granulocytes (*B*) in cultures of hematopoietic precursors incubated with the appropriate cytokine (3).

Differentiation Inducing Cytokines

The development of clones with terminally differentiated, nondividing, mature cells such as granulocytes and macrophages from precursor cells requires induction of cell viability, multiplication, and differentiation associated with the arrest of cell multiplication. It therefore seemed unlikely that a CSF that induces cell multiplication is also a direct differentiation inducer whose action includes stopping cell multiplication in mature cells. I therefore looked for a protein that acts as a myeloid cell differentiation inducer but does not have colony-stimulating activity, and found such a protein that was called macrophage/granulocyte inducer type 2 (reviewed in refs. 25–27). Studies on amino acid sequence of purified protein and myeloid cell differentiation inducing activity of recombinant protein showed that macrophage/granulocyte inducer type 2 is IL-6 (27, 38), and it was postulated that there are presumably other normal hematopoietic cell differentiation inducers. Studies on myeloid leukemic cells have identified other differentiation inducing proteins called D-factor and differentiation-inducing factor (reviewed in ref. 28). D-factor was identified as a protein that has also been called HILDA and leukemia-inhibitory factor, and differentiation-inducing factor was found to be a form of TNF. Another cytokine, IL-1, can also induce differentiation in some clones of myeloid leukemic cells, and this is mediated by induction of IL-6 (27). IL-6 and IL-1 can induce viability and differentiation of normal myeloid precursors, and leukemia-inhibitory factor like IL-1 and IL-6 can synergize with IL-3 in normal myeloid colony formation.

Three other cytokines, IL-11, oncostatin M, and ciliary neurotrophic factor, show some structural and functional similarities and use the same cell surface signal transducing protein, gp130, that is used by IL-6 and leukemia-inhibitory factor (reviewed in ref. 37). These cytokines do not have CSF activity on normal hematopoietic cells, but IL-11 synergizes with IL-3 in normal hematopoiesis. There is another cytokine, IL-5, that induces differentiation to eosinophils (39). The IL-5 receptor shares with IL-3 and GM-CSF receptors the β chain that is involved in signal transduction (reviewed in ref. 37).

Network of Hematopoietic Cytokines

The production of different types of hematopoietic cells with a limited lifespan, both under normal conditions and in

different emergency situations such as infections, wound healing, and various diseases, requires a system with considerable flexibility. A multigene family of interacting cytokines is more useful for the functions required today and for adaptation to functions that may be required in the future, than the existence of only single cytokines with high specificity where a lack of function and lack of flexibility would be lethal. A family of cytokines, some of which have overlapping functions, is also a useful safeguard, so that if one cytokine does not function effectively under certain conditions another can take over. A good way to obtain flexibility would be for different factors to function within a network of interactions and there is such a network of hematopoietic cytokines.

It was found that all four CSFs, GM-CSF, G-CSF, M-CSF, and IL-3, can induce the production of IL-6 that does not induce the formation of colonies but can induce myeloid precursor cells to differentiate (reviewed in refs. 26–29 and 40). In a colony with differentiated cells, induction of a colony by the CSFs is thus followed by production of another cytokine, IL-6, which can induce differentiation of different cell lineages. This induction of a differentiation factor by a growth factor serves as an effective mechanism to couple growth and differentiation. The CSFs and IL-6 may also switch on other, so far unidentified, cytokines that are required to determine the specificity of the final cell type. In addition, IL-6 and GM-CSF can positively autoregulate their own induction (41) and can induce expression of other cytokines including M-CSF, GM-CSF, IL-6, IL-1α, IL-1β, and TNF (42). There is thus a cytokine network which allows amplification of signals and indirect activity of cytokines by inducing production of other cytokines.

IL-6 and GM-CSF also induce expression of genes for transcription factors, including c-*jun*, *jun*-B, and c-*fos* (43), which can participate in the activation of other genes including genes for other cytokines. Another transcription factor, Egr-1/Zif 268, was expressed in GM-CSF-inducible leukemic cells but not in IL-6-inducible leukemic cells (43). Different cytokines may thus differentially induce expression of different transcription factors, which can result in differences in expression of specific cell functions in different cell types. The network allows amplification of the signals leading to increased hematopoiesis under stress conditions such as infections, resulting in production of cytokines such as IL-1, IL-3, IL-6, G-CSF, M-CSF, GM-CSF, and possibly also other cytokines, and these induced cytokines can amplify the system by switching on other cytokines.

Production of specific cell types has to be induced when new cells are required and has to stop when sufficient cells have been produced. This requires an appropriate balance between inducers and inhibitors of development. The network of interactions between hematopoietic cytokines (Fig. 2) thus includes cytokines that can function as inhibitors of hematopoietic cell multiplication such as TNF. Another inhibitory cytokine, type β1 transforming growth factor (TGF-β1), which is part of this network (42) can selectively inhibit the activity and the production of some CSFs and ILs (44). Other cytokines which may also participate in the network include interferons α/β and γ and IL-4, IL-10, and IL-13. These cytokines can exert negative effects on the responsiveness of hematopoietic myeloid cells to certain CSFs and on the production of these cytokines (45–47).

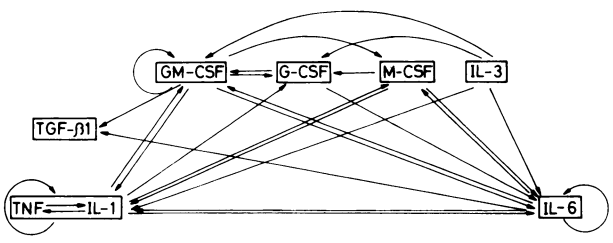

FIG. 2. Network of hematopoietic cytokine interactions.

The network of interactions between molecules that regulate the hematopoietic system has yet another level of control involving the cytokine receptors. For example, induction of differentiation with IL-6 can induce expression of surface receptors for M-CSF and IL-3 which were not expressed before differentiation (48). TGF-β1 can inhibit cytokine production and decrease the expression of receptors for cytokines (49), thus ensuring the feedback inhibition of hematopoiesis at the level of both cytokines and receptors. There can also be a separate regulation of cytokine genes and cytokine receptor genes. This is shown in IL-6-treated leukemic cells that develop IL-3 receptors during differentiation but do not express IL-3, and before differentiation have G-CSF receptors and do not express G-CSF (42, 45). Other leukemic cells that are induced to differentiate with GM-CSF are induced to express IL-6 and M-CSF (42) but do not express receptors for IL-6 (50) or M-CSF (c-*fms*) (42). A separate regulation of cytokine genes and cytokine receptor genes provides normal myeloid cells with an additional control system to prevent autocrine growth.

Parts of this cytokine network were found to function not only within the hematopoietic cell system but also in some nonhematopoietic cell types. Endothelial cells that make blood vessels produce IL-6 at the time of new blood vessel formation (angiogenesis), and the production of IL-6 is switched off when angiogenesis has been completed (51). The transient expression of IL-6 in the endothelial cells indicates a role for IL-6 in angiogenesis in addition to its role in regulating the development of myeloid and lymphoid hematopoietic cells. IL-6 can also induce the production of acute phase proteins in liver cells (22). The pleiotropic effects of a cytokine such as IL-6 raise the question whether these effects on different cell types are direct or are indirectly mediated by IL-6 switching on production of other regulators that vary in the different cell types. Interpretation of experimental data on the effect of each cytokine therefore has to take into account that the cytokine functions in a network of interactions, so as to avoid an incorrect assignment of a specific effect to a direct action of a particular cytokine. This network has also to be taken into account in the clinical use of these cytokines. What can be therapeutically useful may be due to the direct action of an injected cytokine or to an indirect effect due to other cytokines that are switched on *in vivo*.

A network of interactions allows considerable flexibility depending on which part of the network is activated. It also allows ready amplification of response to a particular stimulus such as bacterial lipopolysaccharide (reviewed in ref. 27). In addition to the flexibility of this network both for the response to present-day infections and to different types of infections that may develop in the future, a network may also be necessary to stabilize the whole system.

Control of Leukemia by Normal Hematopoietic Cytokines and Other Compounds

Identification of the cytokines that control normal hematopoiesis raised the question whether cytokines that induce differentiation of normal hematopoietic cells can also induce leukemic cells to differentiate to mature nondividing cells. It was shown with human (8) and mouse leukemias (52) that there are some myeloid leukemic cells that can be induced to terminally differentiate to mature macrophages or granulocytes by some normal differentiation inducing cytokines. It was suggested already at that time that induction of terminal differentiation of leukemic cells may be of potential value in the therapy of leukemia, thus putting forward the idea of differentiation therapy (8, 15, 53). The results have shown that differentiation-competent clones (called D^+ clones) of mouse

leukemic cells induced to differentiate with a cytokine such as IL-6 to mature nondividing cells by the normal sequence of gene expression (15, 26, 53, 54) were no longer malignant when injected into mice (55). In addition to D^+ clones that can be induced to differentiate by IL-6 and can also be induced to partially differentiate with G-CSF, there are D^+ clones from another myeloid leukemia that can be induced to differentiate with GM-CSF or IL-3 but not with IL-6 or G-CSF (26–29). In clones that respond to these CSFs, the growth inducers presumably induce production of an appropriate differentiation inducer. D^+ leukemic cells that respond to IL-6 can also be induced to differentiate by IL-1α and IL-1β, and this is mediated by the endogenous production of IL-6 (27). The D^+ myeloid leukemic cells have an abnormal chromosome composition (56, 57). Suppression of malignancy in these cells was not associated with chromosome changes and the differentiating leukemic cells did not regain the normal diploid chromosome complement. The stopping of cell multiplication by inducing differentiation to mature cells thus bypassed genetic changes that produced the malignant phenotype, such as a loss of requirement for a normal cytokine for cell viability and multiplication, and a block in the ability of a multiplication inducing cytokine to induce the production of a differentiation inducing cytokine (reviewed in refs. 25–27 and 58).

Studies *in vivo* have shown that normal differentiation of D^+ myeloid leukemic cells can be induced not only in culture but also *in vivo* (Fig. 3) (59, 60). After injection of mouse myeloid leukemic cells into fetuses, D^+ leukemic cells were shown to participate in normal hematopoietic cell differentiation to mature granulocytes and macrophages in apparently healthy adult animals (61, 62). The development of leukemia was inhibited in mice inoculated with these D^+ leukemic cells by increasing the amount of differentiation-inducing cytokine, either by injecting it or by injecting a compound that increased its production by cells in the body (59, 60, 63, 64). Induction of differentiation *in vivo* like *in vitro* can occur directly or by an indirect mechanism that involves induction of the appropriate differentiation-inducing cytokine in cells in the body (60, 63–65).

The study of different clones of myeloid leukemic cells has shown that in addition to D^+ clones there are differentiation defective clones called D^- clones (15, 56, 66–71). Some D^- clones are induced by a normal myeloid differentiation-inducing cytokine to an intermediate stage of differentiation that then slows down the growth of the cells, and others could not be induced to differentiate even to this intermediate stage. It was suggested that D^+ clones are the early stages of leukemia and that the emergence of D^- clones may be later stages in the progression of malignancy (53). Even these D^- cells can be induced to differentiate by other compounds, either singly or in combination, that can induce the differentiation program by alternative pathways. The stopping of cell multiplication by inducing differentiation by these alternative pathways bypasses the genetic changes that inhibit response to the normal differentiation-inducing cytokine (reviewed in refs. 25–27 and 58). Studies on the genetic changes in D^- clones of myeloid leukemias have shown that differentiation defectiveness may be due to changes in homeobox genes. These include rearrangement of the *Hox-2.4* homeobox gene which results in abnormal expression of this gene in the leukemic cells (72). This abnormal expression of *Hox-2.4* inhibits specific pathways of myeloid cell differentiation (73). In other leukemias with a deletion in one chromosome 2 (57) there is a deletion of one copy of *Hox-4.1* (74).

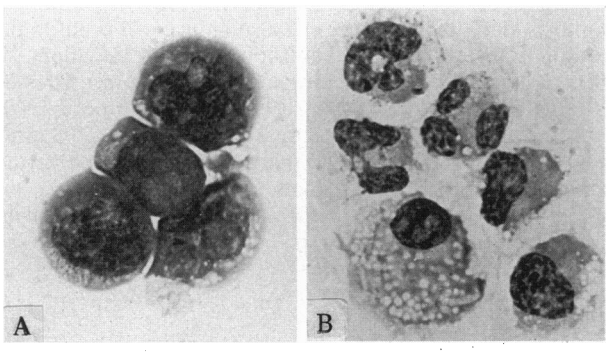

FIG. 3. Differentiation of D^+ myeloid leukemic cells *in vivo*. Shown are leukemic blast cells (*A*) and cells in various stages of differentiation (*B*) (59).

Studies with a variety of compounds, other than normal hematopoietic cytokines, have shown that many of them can induce differentiation in D^+ clones of myeloid leukemic cells. These include glucocorticoid hormones, compounds that are used today in cancer chemotherapy, such as cytosine arabinoside, methotrexate and others, and irradiation. At high doses, irradiation and these compounds used in cancer chemotherapy kill cells by inducing apoptosis, whereas at low doses they can induce differentiation. Not all these compounds are equally active on the same leukemic clone (reviewed in refs. 53 and 54). A variety of compounds can also induce differentiation in clones that are not induced to differentiate by a normal hematopoietic cytokine, and in some D^- clones induction of differentiation requires combined treatment with different compounds (54). In addition to certain steroids, chemotherapeutic compounds, and radiation, other compounds that can induce differentiation in myeloid leukemic cells include insulin, bacterial lipopolysaccharide, certain plant lectins, tumor promoting phorbol esters (reviewed in refs. 53 and 54), and retinoic acid (reviewed in ref. 75). This effect of retinoic acid on differentiation of promyelocytic leukemia cells is now used clinically in the therapy of these leukemias (75), showing the successful application of the concept of differentiation therapy in the clinic. It is possible that all myeloid leukemic cells that are no longer susceptible to the normal hematopoietic cytokines by themselves can be induced to differentiate by the appropriate combination of compounds. The experiments with myeloid leukemic cells have shown that there are different pathways of gene expression for inducing differentiation, and that genetic changes which suppress induction of differentiation by one compound need not affect differentiation by another compound by alternative pathways (refs. 76 and 77; reviewed in refs. 25 and 54). These results show that there is considerable flexibility in the myeloid differentiation program, and this presumably also applies to other cell types.

Control of Programmed Cell Death (Apoptosis) by Cytokines

Normal hematopoietic cells cannot be maintained in culture unless certain viability factors or viability factor producing cells are provided (reviewed in refs. 15 and 26–28). When deprived of viability-inducing cytokines, which include CSFs and ILs, normal myeloid progenitor cells rapidly lose viability (32, 78) by a process called programmed cell death which has also been called apoptosis (79, 80). This process is associated with cell shrinkage, nuclear fragmentation, and DNA fragmentation into oligonucleosome size fragments (79). Although viability-inducing cytokines such as the CSFs also induce cell multiplication, viability and multiplication are separately regulated (reviewed in ref. 33). The program for apoptotic cell death activated in the absence of hematopoietic factors is present not only in normal hematopoietic progenitor cells but throughout the differentiation program to mature neutrophils, eosinophils and macrophages (reviewed in refs. 33 and 81). Viability factor withdrawal also induces apoptosis in hematopoietic factor-dependent cell lines that are no longer normal but not yet malignant.

Cancer cells are not immortal and maintain the program for apoptotic cell death which can be activated. Myeloid leukemic cells from many patients behave *in vitro* like normal cells in still requiring hematopoietic viability factors (reviewed in ref. 82) and in the absence of these factors die by apoptosis. There are also myeloid leukemic cells from some patients that do not require an exogenous source of cytokines for viability (82) and myeloid leukemic cell lines established in culture that are independent of exogenous viability-inducing cytokines. Induction of differentiation in such lines of D^+ leukemic cells with IL-6 induces in the differentiating cells a viability factor-dependent state so that the cells lose viability by apoptosis following withdrawal of IL-6 (32, 48, 83). This induction of the program for cell death occurs before terminal differentiation, and the differentiating cells can be rescued from apoptosis by re-adding IL-6, or by adding IL-3, M-CSF, G-CSF, SCF, or IL-1 (48, 81, 83). The differentiating leukemic cells can also be rescued from apoptosis by the tumor promoting phorbol ester 12-*0*-tetradecanoylphorbol 13-acetate (TPA) but not by the nonpromoting isomer 4-α-TPA (32). TPA rescues the differentiating cells from apoptosis by a different pathway than rescue with these cytokines. TPA can thus act as a tumor promoter by inhibiting apoptosis (32). It is therefore possible to activate the normal physiological process of apoptosis in myeloid leukemic cells, and this can be used to suppress leukemia even without induction of terminal differentiation. This has been shown by the decreased *in vivo* leukemogenicity of IL-6-pretreated leukemic cells with a viability factor-dependent state (83) and by the ability of antibody to IL-6 or to its receptor to suppress the development of multiple myeloma by IL-6-dependent myeloma cells (84, 85).

TGF-β1 and different cytotoxic agents (including irradiation) that are currently used in cancer therapy can induce apoptosis in myeloid leukemic cells (33, 81, 86). Hematopoietic cytokines including IL-3, IL-6, G-CSF, and GM-CSF inhibited induction of apoptosis in viability factor-independent myeloid leukemic cell lines treated with TGF-β, irradiation or with different cytotoxic cancer chemotherapy compounds (33, 86). Interferon-γ but not interferon-α/β, also inhibited induction of apoptosis in normal and leukemic myeloid cells by different apoptosis-inducing agents (87). Studies with primary human myeloid leukemic cells have shown that GM-CSF and IL-3 can protect myeloid leukemic cells from patients with acute myeloid leukemia against induction of apoptosis by a chemotherapeutic compound such as the anthracyclin doxorubicin (88). It was also shown that IL-6 and other cytokines such as IL-1, IL-2, and IL-4 can suppress induction of apoptosis in normal and leukemic lymphoid cells by various cytotoxic agents (reviewed in refs. 33 and 81). Different hematopoietic cytokines can thus decrease the effectiveness of cancer therapeutic compounds against leukemic cells, and this has implications for cancer development and cancer therapy (33, 81).

The preferential development and metastasis of leukemia and other types of cancer cells in specific tissues in the body can be due to better protection against apoptosis at these sites due to the presence of appropriate viability factors. In view of the ability of hematopoietic cytokines to inhibit apoptosis in leukemic cells induced by irradiation and a variety of chemotherapeutic agents (33, 81, 86, 88), endogenous viability factors may decrease the effectiveness of cytotoxic therapy. This protective effect of hematopoietic viability factors can explain the poorer complete remission rates after chemotherapy in human acute myeloid leukemia patients whose cells show a higher responsiveness to hematopoietic viability-inducing cytokines *in vitro* (82). Leukemic cells from acute myeloid leukemia patients can express high levels of the *bcl*-2 gene (89) which suppresses apoptosis (90), and in these leukemic cells GM-CSF upregulated expression of *bcl*-2 (89). It has been suggested that reducing the level of viability-inducing cytokines or the responsiveness of cancer cells to these cytokines may be clinically useful in cancer therapy (33, 81, 86).

Control of Apoptosis by Wild-Type and Mutant p53

The regulation of apoptosis by cytokines involves interactions with other cellular genes that control apoptosis. A variety of such apoptosis-inducing and apoptosis-inhibiting genes have been identified (90–93), and my studies on such interactions started with wild-type p53 and mutant p53 (94–96). Wild-type p53 protein is a product of a tumor suppressor gene that is no longer expressed in many types of tumors including myeloid leukemias (97). There is a clone of M1 myeloid leukemic cells that completely lacks expression of p53 protein and mRNA (94). This p53 negative clone of myeloid leukemic cells was transfected with DNA encoding a temperature-sensitive p53 mutant (Ala → Val change at position 135). The Val-135 mutant behaves like other p53 mutants at 37.5°C but like wild-type p53 at 32.5°C. There was no change in the behavior of the transfected cells at 37.5°C but activation of the wild-type p53 protein at 32.5°C resulted in apoptotic cell death (94). This induction of apoptosis was not associated with differentiation (94). Apoptosis can, therefore, be induced by expression of wild-type p53 in undifferentiated leukemic cells. This apoptosis inducing effect of wild-type p53 was confirmed in other cell types (reviewed in refs. 33 and 81). Induction of apoptosis by wild-type p53 in M1 myeloid leukemic cells was inhibited by cytokines such as IL-6 (94) and interferon-γ (87). Experiments with p53 knock-out mice have shown that wild-type p53 is also involved in mediating apoptosis in normal myeloid precursors deprived of the cytokines required for cell viability, and that this induction of apoptosis by wild-type p53 in normal myeloid precursors is inhibited by adding the appropriate cytokine concentration (96). Experiments with p53 knock-out mice have also shown wild-type p53 mediated apoptosis after irradiation in myeloid precursors (96), thymocytes (96, 98, 99), and intestinal epithelium cells (100) and that there are wild-type p53 dependent and p53 independent pathways of inducing apoptosis (96, 98, 99).

Apoptosis under growth-restrictive conditions in certain nonmalignant factor-dependent myeloid cell lines and in Rat-1 fibroblasts can be enhanced by deregulated expression of c-*myc* (101, 102). In myeloid leukemic cells that do not express wild-type p53, deregulated expression of c-*myc* enhanced cell susceptibility to various apoptosis inducing treatments (95). Deregulated expression of c-*myc* in myeloid leukemic cells coexpressing deregulated mutant p53 did not result in enhancement of susceptibility to apoptosis, showing that mutant p53 suppressed the apoptosis enhancing effect of deregulated c-*myc* (95). An oncogene such as mutant p53 can thus promote the viability of these leukemic cells after treatment with apoptosis-inducing agents. The suppression of apoptosis by mutant p53 can explain (29) the high frequency of mutant p53 in many types of tumors (97). In addition to enhancing apoptosis, deregulated c-*myc* can also induce cell proliferation and inhibit differentiation. The suppression of the apoptosis-enhancing effect of c-*myc* by mutant p53 thus allows expression of these other functions of c-*myc* (95). *bcl*-2 also suppresses the apoptosis-enhancing effect of deregulated c-*myc* (103, 104). Therefore it was suggested (96) that c-*myc* and mutant p53 may cooperate in tumor development as occurs with c-*myc* and *bcl*-2 (105, 106). This was confirmed in experiments showing that coexpression of mutant p53 in myeloid leukemic cells expressing deregulated c-*myc* increased leukemogenicity *in vivo* compared to cells only expressing deregulated c-*myc* (Fig. 4) (107).

In view of the role of wild-type p53 in certain pathways leading to apoptosis, mutations that inactivate this tumor suppressor can be expected to prevent apoptosis, leading to illegitimate survival of DNA-damaged cells. Thus bone mar-

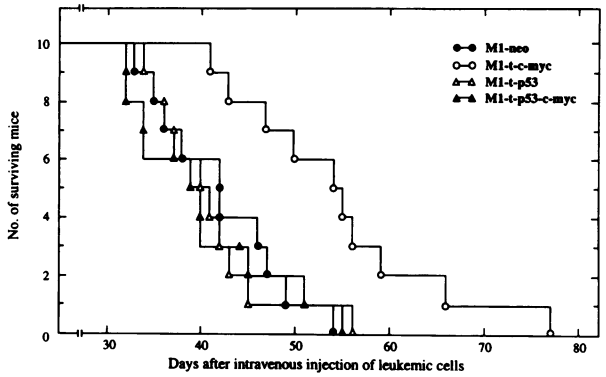

FIG. 4. Survival time of mice inoculated with M1 myeloid leukemic clones transfected with a neomycin resistance gene (M1-*neo*), deregulated c-*myc* (M1-t-c-*myc*), mutant p53 (m1-t-p53), or with both mutant p53 and deregulated c-*myc* (M1-t-p53-c-*myc*) (107).

row hematopoietic progenitors and thymocytes from transgenic mice that overexpress mutant p53 are more resistant to apoptotic cell death compared to normal mice (108). Mutant p53 can inactivate wild-type p53 (97), and a similar effect can be obtained with the adenovirus gene E1B 55k and simian virus 40 large tumor antigen that inhibit wild-type p53-mediated apoptosis (109, 110). Illegitimate survival of DNA-damaged cells is presumably responsible for the high incidence of tumors in wild-type p53-defective mice (111). High-level expression of mutant p53 in human tumors is also associated with a poor prognosis and transition from low to high aggressive states are often associated with an increased level of mutant p53 (reviewed in refs. 33 and 81).

Genetic manipulation resulting in reexpression of wild-type p53 in tumor cells *in vivo* can induce apoptosis (112). It has also been suggested (33, 81) that treatments that reduce mutant p53 expression may increase susceptibility of cancer cells to therapy, a suggestion that can apply to other apoptosis-suppressing genes. Members of the *bcl*-2 family (90, 91) were used to test this. The level of *bcl*-2 expression in certain lines of leukemic cells is associated with the degree of resistance to induction of apoptosis (95), and expression of *bcl*-2 can be downregulated in myeloid leukemic cells by cytokines such as IL-6 and G-CSF and the steroid dexamethasone (113, 114) and in promyelocytic leukemic cells by all-trans-retinoic acid (115). In the myeloid leukemic cells, downregulation of *bcl*-2 by the cytokines increased cell susceptibility to induction of apoptosis by cytotoxic compounds used in cancer therapy. But there was no such increased susceptibility after downregulation of *bcl*-2 by dexamethasone (113, 114) owing to upregulation of another apoptosis suppressing gene *bcl*-X_L (114). Apoptosis is controlled by the balance between apoptosis inducing and apoptosis-suppressing genes. To increase cancer cell susceptibility to induction of apoptosis, it will be necessary to select the appropriate compound for downregulation of those apoptosis-suppressing genes that suppress apoptosis in a particular cancer.

Clinical Use of Hematopoietic Cytokines

In the previous sections I have outlined the identification and function of hematopoietic cytokines in the control of cell viability, multiplication, and differentiation, using the myeloid cell lineages as a model system. Different pathological states are candidates for a new type of therapy based on the use of these cytokines (reviewed in refs. 24–29 and 116–119). The concentration of these proteins can be increased *in vivo* either by injecting cytokines or by injecting a compound that induces their production (refs. 59, 60, 63, 64, and 120; reviewed in ref. 27). Chemotherapy and radiotherapy in cancer patients and the use of immune-suppressing compounds such as cyclosporine A kill normal cells of the myeloid lineage and kill other cells that produce hematopoietic cytokines. Injection of a cytokine such as G-CSF, which stimulates myelopoiesis under normal circumstances, accelerates the recovery of myeloid cells following cytotoxic therapy in cancer patients or following immune-depression treatment for blood cell transplantation. Injection of this cytokine to bone marrow or peripheral blood cell donors before grafting and injection after grafting can increase the success of blood cell transplants. Because of the important functions of mature cells such as granulocytes and other myeloid cells, the increased survival and function of mature cells induced by CSFs can also be clinically helpful to patients with deficiencies in myeloid cell production and functions (reviewed in ref. 27). The finding that apparently normal granulocyte development can be induced in culture with cells from patients with infantile congenital agranulocytosis (8, 121) has led to promising clinical results with G-CSF in children with this genetic disease (122). It has also been shown that injection of erythropoietin, which stimulates the development of erythroid cells, can correct the anemia in patients with chronic renal failure (123).

Another aspect of the clinical use of hematopoietic cytokines is associated with their effect on leukemic cells. The treatments used for killing of leukemic cells by irradiation and various chemotherapeutic compounds induce apoptotic cell death (33, 81, 86, 92). Hematopoietic cytokines including CSFs, ILs, and interferon-γ can inhibit induction of apoptosis induced by cancer chemotherapy compounds and irradiation (33, 81, 86, 87). The clinical use of cytokines to correct therapy-associated suppression of normal hematopoiesis should, therefore, be carefully timed to avoid protection of the malignant cells from the cytotoxic action of therapeutic compounds (33, 81, 86, 88). Many leukemic cells are still cytokine-dependent and undergo apoptotic cell death following cytokine withdrawal (33, 48, 81–83). The combination of such cytokine withdrawal and chemotherapy may thus lead to a higher degree of apoptosis in the malignant cells and to a better clinical outcome of therapy. Another way of improving the outcome of therapy by increasing apoptotic cell death is associated with upregulation of expression of apoptosis inducing genes and downregulation of apoptosis-suppressing genes (81, 113, 114). Hematopoietic cytokines can also affect leukemic cell behavior by controlling cell differentiation. Different CSFs and interleukins such as IL-1 and IL-6 can control the abnormal growth of certain types of leukemic cells and suppress malignancy by inducing cell differentiation (reviewed in refs. 26–29). Like retinoic acid, which induces differentiation in certain types of myeloid leukemic cells and is effectively used in the clinic to treat such patients (75), differentiation-inducing cytokines may also be an effective treatment for certain types of leukemias. The existence of the cytokine network has to be taken into account in the clinical use of cytokines (27).

In conclusion, the development of the cell culture system (1–4) for the clonal development of hematopoietic cells and discovery of the normal myeloid hematopoietic cytokines in cell culture supernatants (3, 6) has led to their successful clinical use for therapy of hematological disorders. The idea of developing these cultures to study the biology of hematopoiesis (1) has thus also turned out to be clinically useful.

I thank for their support the National Foundation for Cancer Research (Bethesda, MD), the Esther Mazor Family (Washington DC), and the Ebner Family Biomedical Research Foundation at the Weizmann Institute of Science in memory of Alfred and Dolfi Ebner.

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