MINIREVIEW

Leukemia Cells and the Cytokine Network

(44311)

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Abstract. **The cytokine network plays an important role in the growth and differentiation of normal and leukemic cells. Stimulation of this network, which has positive and negative regulators, results in the induction or inhibition of certain hematopoietic events. A cytokine can have multiple effects on various cell types, and combinations of cytokines with each other or with other exogenous substances produce more pronounced effects than any cytokine or agent individually. The mechanisms by which cytokines affect normal and leukemic cell growth and viability may vary depending on the target cell or the cytokine(s) in question. Diseases such as leukemia may result from abnormalities in the cytokine network or their receptors.**

Cytokines play a major role in leukemogenesis. Normally, hematopoietic cells require certain cytokines for their viability and growth. When the viability factors are withdrawn, apoptotic cell death naturally occurs. Prevention of programmed cell death by the abnormal production of a cytokine may release the cell from normal growth control leading to malignant transformation. Disregulation of genes for hematopoietic growth factors and their receptors may be one of the events that leads to leukemogenesis through an aberrant autocrine growth mechanism. However, cytokines have been used as therapeutic agents in various ways. Differentiation therapy has been widely investigated and proven effective in certain types of cancer. Gene therapy, where the cytokine cDNA is used to reduce tumorigenicity and/or increase immunogenicity is promising. Another kind of therapy using alkylated growth factors has been under focus. This review summarizes the actions and interactions of cytokines that are related to leukemic cell viability and growth. The use of cytokines as therapeutic agents is also discussed. [P.S.E.B.M. 1998, Vol 219]

Cytokines, which include colony-stimulating factors (CSFs), other growth factors, and interleukins (ILs) are major regulatory molecules of hematopoiesis. These cytokines interact, forming a network that has positive and negative regulators of hematopoiesis (1–4). In general, CSFs and many ILs are positive regulators, whereas other cytokines such as tumor necrosis factor (TNF) and transforming growth factor β (TGF-β) act as negative regulators of hematopoiesis (1–7). The action of one cytokine can be amplified by the induction of other cytokines that are required to produce the final cell type. The network of cytokines couples the events of growth and differentiation. CSFs induce colony formation and stimulate expression of other cytokines in order to induce differentiation of various cell lineages. Interleukins can also induce the release of CSFs and/or their synthesis (2, 3, 5, 7). For example, IL-1 functions as a positive regulator by inducing the release of CSFs and synergizing with IL-6, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage

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0037-9727/98/2191-0008$10.50/0

colony-stimulating factor (GM-CSF). It also acts as a negative regulator by inducing the release of tumor necrosis factor (TNF) and PGE2 (7). In general, interleukins and CSFs synergize or mediate each other's actions to stimulate the production of certain hematopoietic lineage (2, 8–14). Among all hematopoietic growth factors, GM-CSF, G-CSF, and macrophage colony-stimulating factor (M-CSF) regulate primary myelopoietic lineages. However, other cytokines such as stem cell factor (SCF), TNF-α, and Flt-3 (6, 14–17) also participate in the regulation of myelopoiesis.

Knowledge of cytokine receptors is also extremely important since disorders of cytokines and/or their receptors are associated with many human diseases (12, 13, 18, 19). Many cytokines share the same β subunit of their receptor although they have their own specific ligand-binding α subunit as a high-affinity converter and signal transducer (18). Cytoplasmic events that follow engagement of the receptors is the activation of signal transducer pathways (TAK2, STAT5) which lead to the activation of genes related to growth, differentiation, and other cellular events (6, 19). Many hematopoietic growth factors have been identified, and their role in the regulation of proliferation, differentiation, and functional activation of hematopoietic cells has been studied intensively. Understanding the role of cytokines in normal hematopoiesis has led to the study of their role in leukemic cells. The mechanism by which cytokines affect normal and leukemic cells may vary from one cell line to another or from one progenitor cell to another in a given lineage. Some cytokines seem to have similar effects on the neoplastic counterpart of their normal stem cell (12, 18). For example, the majority of acute myeloid leukemia (AML) progenitor cells retain dependence on growth factors for cell viability and multiplication *in vitro*. It is generally recognized that cytokines interact in a cascade to regulate normal and perhaps leukemic cell growth, multiplication, and differentiation (13, 20–23). On the other hand, the induction of one cytokine may require several cytokines. INF-α initiates the immune response against infection; however, optimal production of INF-γ by the natural killer cells is obtained by exposing the cells to IL-2, IL-12, and IL-15 (24).

Leukemia is characterized by uncontrolled clonal expansion of leukemic progenitor cells arrested at a certain stage of differentiation. Cytokine abnormalities have been implicated in the onset and progression of various types of leukemia (5, 13, 25–29). Defective proliferation of hematopoietic precursor cells could be at least in part due to an abnormal production of hematopoietic growth factors or a change in the precursor cell responsiveness to it. Inhibition of proliferation and induction of differentiation of leukemic cells have been a main area of investigation. Consequently, significant interest has been generated in the use of hematopoietic growth factors for this purpose. It is believed that by manipulating the right cytokine(s), it may be possible to modulate the growth characteristics and oncogenic properties of leukemic cells.

Hematopoietic Actions of Cytokines

Humoral factors and the hematopoietic microenvironment regulate hematopoiesis. Figure 1 shows the possible interactions between normal hematopoietic cells, leukemic cells, stromal cells, and cytokine network. Since Dexter *et al.* (14) developed the long-term bone marrow cultures in an attempt to reproduce the bone marrow microenvironment, more interest was generated in the role stromal adherent cells play in hematopoiesis. Stromal adherent cells include fibroblasts, macrophages, reticular and endothelial cells, and adipocytes (2, 14, 16, 17). The multilineage cytokine network interacts with hematopoietic progenitor cells, including cells expressing CD34 and CD38 antigens, and stromal cells to induce and regulate normal hematopoiesis (Fig. 1). The $CD34^+$ cells are primitive hematopoietic progenitors found in normal peripheral blood and can differentiate into many cell types including myeloid, lymphoid, and erythroid lineages (30). When stromal abnormalities in cytokine production and/or function arise, blood diseases such as leukemia may originate (31–39). The interactions between the cytokine network and all the cells in the bone marrow microenvironment are extremely complex. In general, G-CSF, M-CSF, and GM-CSF induce proliferation of their respective hematopoietic cell lineages whereas cytokines such as IL-1, IL-6, IL-11, TNF, leukemia inhibitory factor (LIF), ciliary neurotrophic factor, and oncostatin M can induce differentiation of many types of cell lineages (2, 35–41). For instance, IL-2 and GM-CSF have been shown to stimulate multilineage hematopoiesis (42); TNF may act as an inhibitor of hematopoietic cell multiplication or differentiation induced by another cytokine.

Other cytokines that may act as negative regulators of hematopoietic events include transforming growth factor (TGF)-β, INF-α, INF-β, INFγ, IL-4, IL-10, and IL-13 (2, 7, 43). Leukemic cells accumulate progressively due to either an increase in the rate of mitosis or a decrease in the rate of apoptosis. The developmentally regulated death process called apoptosis is an efficient means of eliminating unwanted cells. It serves to limit the effect of noxious substances released by dying cells on the surrounding healthy tissue. The *bcl*-2 family can either stimulate or inhibit apoptosis. If *bcl*-2 expression is altered in developing cells, they may become leukemic. In many B-cell lymphomas, for example, *bcl*-2 expression is altered by a chromosomal transduction, t[14;18] (45). Under normal circumstances, immature B cells die by apoptosis if deprived of IL-3. However, due to the translocation of the *bcl*-2 gene, apoptosis is prevented in the IL-3-deprived B cells. *Bcl*-2 blocks apoptosis, and cells become leukemic. Therefore, normal induction of apoptosis plays an important role in tumor suppression. Deregulation of this process is a step in carcinogenesis. Consequently, selective induction of apoptosis in leukemic cells by chemotherapeutic agents may be one of the ways to the therapy of leukemia (44, 45). The Philadelphia chromosome (Ph1), which is one of the well-character-

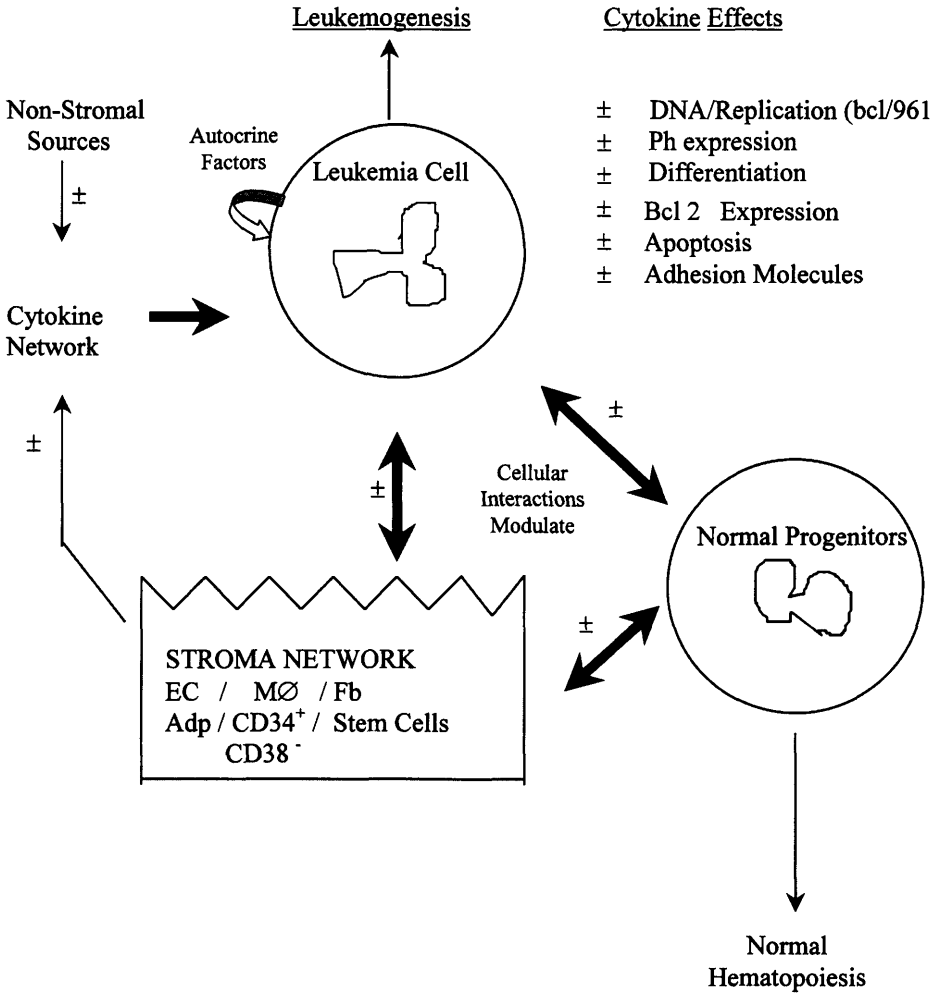

Figure 1. Interactions of stroma, leukemia cell and the cytokine network.

Key:

Endothelial Cells	---- EC
Macrophages	---- MØ
Fibroblasts	---- Fb
Adipocytes	---- Adp

ized cytogenetic abnormalities in leukemia, has been implicated as the cause of some leukemias (44–47). It is detected in many types of leukemias such as chronic mylogenous leukemia (CML), acute lymphoblastic leukemia (ALL), and monocytic leukemia (44–46). In CML, the Ph1 chromosome is formed by a reciprocal translocation, between chromosomes 9 and 22, that fuses BCR-encoded sequences upstream of exon 2 of c-ABL. The BCR-ABL fusion creates a gene whose protein product, p210BCR-ABL, has been implicated as the cause of the disease (46). The Ph1 has been shown to be an independent risk factor in some leukemias such as ALL (47).

Effects of Cytokines on Normal Cell Growth

It is not within the scope of this manuscript to describe the action of every known cytokine; however, it may be an advantage to the reader to summarize some of the main actions of the well-known cytokines.

Erythropoietin (Epo) is the main cytokine that regulates erythropoiesis. It binds to a distinct transmembrane receptor expressed by erythroid cells. Epo stimulates the proliferation of erythroid-burst-forming units and induces differentiation of erythroid-colony-forming units (31). Thrombopoietin (TPO) has recently gained a great deal of attention. It has been shown to have stimulatory effects on various hematopoietic compartments. In addition to stimulating growth and differentiation of megakaryocytes, TPO dramatically increases the number of circulating platelets (48). It has been shown to improve hematopoietic recovery in myelosuppressed mice affecting multiple cell lineages. Administration of TPO in myelosuppressed mice accelerates recovery of RBC and neutrophils by enhancing proliferation of erythroid and myeloid progenitor cells (48–50).

M-CSF or CSF-1 is a dimeric glycoprotein (51) that has multiple functions such as monocytic colony-forming activity where it induces the proliferation and differentiation of

monocyte progenitors (27, 29, 52–55). In addition to its nonhematopoietic actions (54–56), M-CSF is reported to enhance chemotaxis, tumoricidal activity, and cytokine production by monocytes (55). Recently, CSF-1 was demonstrated to have a dual effect on bone marrow stromal cells *in vitro* (52). As the concentration of CSF-1 or the cell density increased, CSF-1 demonstrated an inhibitory effect on cell growth. In contrast, lower concentrations of CSF-1 were stimulatory to bone marrow stromal cell growth. GM-CSF can be produced by a wide spectrum of nonhematopoietic cells including fibroblasts, epithelial, endothelial, and smooth muscle cells in addition to normal and leukemic hematopoietic cells, and was found to play a role in the pathological processes of nonhematopoietic illnesses (51, 52, 57). GM-CSF and G-CSF regulate myelopoiesis (58). GM-CSF with IL-3 stimulates *in vitro* megakaryocyte colony formation (59). G-CSF stimulates proliferation and maturation of precursor cells in the bone marrow into fully differentiated leukocytes. It enhances the functional activity of mature leukocytes (60). Stem cell factor (SCM) selectively controls stem cell self-renewal, proliferation, and differentiation. It synergizes with G-CSF, GM-CSF, IL-3, and IL-11 (10, 11) to stimulate various hematopoietic compartments (61).

At least 18 interleukins have been described to date, and many of them have been studied and used in clinical trials. The biological activity of IL-1 is mediated by two different gene products: IL-1-α and IL-1-β (62). IL-1 induces release of G-CSF and IL-6, and synergizes with G-CSF, GM-CSF, and IL-3 in stimulation of hematopoietic progenitor cells (7). IL-1 shares functional similarity with IL6, LIF, and oncostatin M (OSM). It has been suggested that a common signal transduction mechanism may be used by all four cytokines. Several reports (63–66) demonstrated that these four cytokines share the same signal transducer, gp130. These reports have partially uncovered some of the control mechanisms that may be responsible for the overlap in the biological action of these cytokines. Recently, the actions of IL-1 receptor antagonist has been reported (67). It is naturally occurring and produced by monocytes, fibroblasts, and macrophages in addition to other cells. Using an *in vivo* model of human glioblastoma, IL-1 was found to inhibit tumor growth and tumor angiogenesis. The IL-1 receptor antagonist seems to play a role in the inflammatory process through its mediators IL-1 and TNF (68). IL-2 is a T-cell growth factor. Its activity on T cells leads to the expression of other cytokines, one of which is TNF-α. It is thought that IL-2 deficiency is involved in the induction of cell suicide or programmed cell death of some lymphoid cells (69). Human IL-3 is produced by T lymphocytes and is a mediator of cell growth and differentiation of various cells and plays an important role in regulation of hematogenesis and immunoresistance. In humans, IL-3 increases the cellularity and cycling of bone marrow progenitor cell populations. In sequence with GM-CSF, it has been shown to stimulate multilineage hematopoiesis (42).

IL-4 has pleiotropic actions on B cell, T cells, thymocytes, mast cells, macrophages, basophils, and eosinophils (70–72). It enhances colony formation by granulocyte, granulocyte-macrophage, megakaryocyte, erythroid, and multipotential progenitor cells. IL-4 inhibits IL-3-dependent colony formation by G-M progenitor cells and by multipotential progenitors (1, 73, 74). IL-4 suppresses the proliferation of M-CSF or GM-CSF-dependent macrophage progenitors (75). Interleukin-5 is produced by T cells, and it stimulates production and activation of eosinophils (76, 77) IL-6 is a multifunctional cytokine produced by a variety of cell types including T lymphocytes, macrophages, fibroblasts, endothelial cells, and bone marrow stromal cells (53, 54, 78). IL-6 stimulates growth and normal functions of many cell types such as B lymphocytes, cytotoxic T lymphocytes, mesangial cells, neural cells, and bone marrow multipotential hematopoietic progenitors (26, 54, 79). Other activities of IL6 include stimulation of B-lymphocyte differentiation and antibody production, promotion of megakaryocyte maturation and platelet production, and induction of cytotoxic T-lymphocyte differentiation. (26, 53, 54). The activity of IL-6 is exerted through binding to a high affinity receptor complex of which gp130 protein is a subunit (53). IL-7 is a pre-B-cell and mature T-cell growth factor (80, 81), which enhances IL-2 dependent T-cell immune activity (82).

Interleukin-8 was originally purified as neutrophil chemotactic factor (83). It is involved in the regulation and mobilization of bone marrow progenitor cells into peripheral blood. IL-8 plays an essential role in the neutrophil-mediated acute inflammation processes, and it is a negative regulator of B lymphocytes. In this respect, it was found to inhibit IgE and IgG_1 production by human B lymphocytes, which are stimulated by IL-4. This negative effect may also be produced by activating natural killer cells that are negative regulators of hematopoiesis (83). IL-8 has been reported to inhibit colony growth by $CD34^+$ bone marrow progenitor cells, although receptors on these cells were not detected (83, 84). IL-8 is produced by a wide variety of cells including monocytes, T lymphocytes, keratinocytes, and hepatocytes (85, 86), and its production by human gastric cancer cells is induced by TNF-α and INF-γ (85). Other cytokines that have been identified as inflammatory mediators include IL-1-α, TNF-α, IL-6, TGF-β, and INF-γ (86). IL-9 stimulates erythroid colony formation along with erythropoietin (87) by increasing the number of erythropoietin-dependent erythroid colonies (88). Interleukin-10 is an anti-inflammatory and regulatory cytokine whose involvement extends to many areas of the immune system (89, 90). It has regulatory effects on epithelial cells as well as proliferation and differentiation of glial cells (89). As an inhibitory cytokine, it inhibits many immune parameters such as T-helper cell type 1 (Th1) cytokine production, antigen presentation and antigen-specific T-cell proliferation (90).

Interleukin-11, which is a multifunctional cytokine, has been discovered as a stromal cell product with multiple

activities in hematopoiesis (8, 61). It has some similar effects as IL-6 on B-cell differentiation, and synergizes with IL-3, IL-4, and Epo in stimulating megakaryopoiesis, and erythropoiesis (9–11). IL-11 expression and synthesis in hematopoietic tissue are induced by IL-1 and TGF-β; IL-11 is also expressed in nonhematopoietic tissues, including brain, spinal cord neurons, gut, and testis (91). IL-11 influences the microenvironment by inhibiting adipocyte differentiation and the breakdown of extracellular matrix (92). Recently INF-α has been identified as a negative regulator of IL-11 (8). In this regard, INF-α is considered a modulator of the cytokine network; it has been defined as an inhibitor of hematopoiesis by regulating the expression of hematopoietic growth factors. For example, it was reported to inhibit the production of GM-CSF, G-CSF, and IL-8 by stromal cells (65).

Interleukin-12 is produced by macrophages, monocytes, dendritic cells and B cells. It acts on T cells, natural killer cells, and cytotoxic cells by increasing their proliferation and cytotoxicity, and by increasing other cytokine production such as INF-γ, IL-2, IL-10, TNF-γ, and GM-CSF (7, 43, 93, 94). The multilineage effects of IL-12 are mediated by INF-α (43). It also enhances colony formation by early progenitor cells and committed hematopoietic progenitor cells. However, its hematopoietic-enhancing effect may be inhibited or masked by INF-γ and TNF-γ, which may be considered a regulatory process (94). Interleukin-13 is an immunoregulatory protein produced by activated T cells. It shares many of its biological activities with interleukin-4, such as the enhancement of the expression of CD23 on monocytes and B cells, and also the induction of IgE production (95). It has an anti-inflammatory function since it inhibits the production of many LPS-induced monokines, such as IL-1α, IL-1β, IL-6, IL-8, IL-10, TNF-α, MIP-1α, GM-CSF, and G-CSF (96).

Interleukin-14 is a B-cell growth factor (97). Interleukin-15 plays a significant role in stimulating the immune system. Its actions include stimulation and proliferation of activated $CD4^+$, $CD8^+$ cells, and other subsets (98). It acts as a stimulator with IL-2 to facilitate the synthesis of INF-γ and TNF-α (99). IL-15 induces proliferation and immunoglobulin synthesis by B cells (100), and it may play a role in differentiation within the immune system. In this respect, its message has been demonstrated in thymic epithelial cells and in bone marrow stromal cells (100–102). IL-16 is secreted by $CD8^+$ T lymphocytes and acts on $CD4^+$ T-lymphocytes (103). All of IL-16's biological activities depend upon the cells surface expression of CD4 through which it transmits its functional signals independent of the expression of CD3. In addition to its chemotactic activity, IL-16 induces HLA-DR expression in a CD4-dependent, interferon-independent pathway in CD4 monocytes, eosinophils, and CD4 T cells (103, 104). Human IL-17 is produced by stimulated $CD4^+$ T cells. It induces the production of IL-6 and IL-8 and enhances the expression of adhesion molecules in fibroblasts (24, 105). Finally, IL-18, originally called INF-γ-inducing factor, is the most recent cytokine at the time of this report. It is produced by Kupffer cells, activated macrophages, and epidermal keratinocytes (106). IL-18 induces INF-γ production in the presence of IL-12, activates T-helper type-1 cells, and induces the production of IL-2 (106–108).

Other growth factors include fibroblast growth factors (FGF), which are a family of heparin-binding growth factors. To date, seven FGFs have been identified (109). The basic bFGF and the acidic FGF (aFGF) are the best characterized. The bFGF and aFGF have been shown to enhance colony formation by multipotent colony-forming units CFU-GEMM, CFU-GM, CFU-megakaryocytes (CFU-Mk), and BFU-E in the presence of IL-3, GM-CSF, and Epo (110). Furthermore, bFGF seems to act on the hematopoietic microenvironment since it was reported to stimulate myelopoiesis in long-term human bone marrow culture (LT-BMC) (111) and stimulate the production of M-CSF by stromal cells (112). Filt3 ligand is a potent stimulator of erythroid progenitors and co-stimulator of normal bone marrow myeloid progenitors. Bone marrow stromal cells produce it, and its receptor is expressed on leukemic cells (15). Transforming growth factor (TGF)-β1 exerts a down regulatory effect on the synthesis and release of proinflammatory cytokines by phagocytic cells. Therefore, it is an inhibitor of endotoxin-induced inflammatory reaction (113).

Effects of Cytokines on Leukemic Cells

The effects of cytokines on leukemic cell growth can be direct, indirect, or synergistic. In addition to the tremendous variation in the actions of cytokines, the action(s) of a given cytokine can vary from one cell line to another, and from one cell to the other in the same cell line. G-CSF, GM-CSF, and IL-3 have been reported to stimulate the proliferation of myeloid leukemia cells *in vitro* (114). IL-6 (115), TNF-α (116), LIF (117), INF-γ, and TGF (118) have been shown to suppress leukemic cell growth and/or induce cell differentiation. IL-6 has been shown to modulate clonogenic blast cell growth in complex ways in acute myeloblastic leukemia (AML) when used either as a single factor or in different hematopoietic growth factor combinations. The inhibitory effect of IL-6 on blast cell colony formation was retained when IL-6 was combined with G-CSF, but was lost if IL-6 was used in combination with mast cell growth factor, IL-3, GM-CSF, or IL-4 (119). IL-6 had a neutral effect on colony growth in seven cases with AML. However, in these cases, IL-6 significantly stimulated clonogenic cell growth if combined with mast cell growth factor or GM-CSF (119). In another study, IL-6 showed little effect on the proliferation of blast precursors present in the peripheral blood of patients with AML, however, it synergized with GM-CSF and IL-3 in the stimulation of AML blast colony formation (26). Other cytokines, such as INF-α induce remission in patients with early stages of B-cell chronic lymphocytic leukemia (B-CLL). B-CLL cells are long-lived cells characterized by accumulation in peripheral blood and bone marrow. B-CLL

cells were found to be protected from apoptosis *in vitro* when co-cultured with cytokines such as IL-1, IL-2, IL-4, IL-6, INF-γ (120), and IL-8 (121). Without these cytokines B-CLL cells die rapidly *in vitro* (120). This protection was demonstrated to correlate with *bcl*-2 expression. *Bcl*-2 is an oncoprotein that protects cells from apoptosis. Others have reported that IL-8 increases the expression of *bcl*-2 mRNA in B-CLL cells (121).

Responses of leukemic cells to cytokines are complicated. For example, IL-4 inhibits proliferation of leukemic cells from one patient, whereas it has stimulatory effects on the same AML cell from another patient (72, 122). This heterogeneity of responses may be due to the *de novo* characteristics of individual leukemic cells. Maekua *et al.* (117), demonstrated that IL-4 and G-CSF synergistically suppressed the self-renewal ability or clonogenicity of U937 leukemia cells. Others reported that U937 cell numbers did not decrease in cultures containing IL-4 (73). These results showed that the effects of a given cytokine or combination of cytokines, on the same type of leukemic cells, may vary from one patient to the other. However, as mentioned previously, combinations of cytokines seem to be more effective in promoting effects on leukemic cells. Whereas G-CSF showed inhibitory effects on colony formation by U937 leukemia cells, combinations of IL-4 and G-CSF were more effective in reducing their clonogenicity than either one alone. There are well-defined effects of many cytokines on certain hematopoietic compartments. This fact makes the use of these cytokines as therapeutic agents or adjunct to therapeutic agents justifiable. A well-defined feature of INF-α is the suppression of myelopoiesis (67). Therefore, INF-α has been used for the treatment of myeloproliferative disorders such as chronic myelogenous leukemia (123), polycythemia vera (124), and essential thrombocytopenia (125).

M-CSF has antitumor activity and is thought to involve antibody-dependent monocyte-mediated cytotoxicity that is augmented by increasing the number and affinity of F_c receptors on monocytes (78). GM-CSF, which normally stimulates growth and differentiation of granulocyte-macrophage colonies, was reported to be necessary for the growth and proliferation of many myelocytic and erythrocytic-transformed cell lines. Many malignant cells seem to produce GM-CSF by an autocrine mechanism (reviewed in Ref 12). Hematological malignancies, such as myelogenous leukemia, lymphoblastic leukemia, diffuse large cell lymphoma, multiple myeloma (16), and L1210 lymphocytic leukemia (27), have been shown to express M-CSF. These leukemias also promote bone marrow depression. It has been shown that higher concentrations of M-CSF show inhibitory effects on bone marrow stromal cells (16). It is possible that localized high levels of M-CSF produced by malignant cells in these hematological malignancies, exert inhibitory effects on bone marrow stromal cells. This is an area that needs to be examined further since there are cytokines such as TNF that stimulate M-CSF levels when administered to patients (126). An immediate leukopenia followed by leukocytosis has been associated with exposure to TNF. Cancer patients treated with TNF had elevated plasma levels of M-CSF and G-CSF but not GM-CSF (126). This suggested that the effects of TNF are not mediated by induction of the cytokine GM-CSF, although others have reported that TNF induces production of GM-CSF in many normal and certain malignant cells (127). The increase in baseline levels of G-CSF and M-CSF after the administration of TNF indicates that there is an *in vivo* cumulative enhancement of the TNF effect. Furthermore, the increase of M-CSF by TNF causes an increase in the numbers of monocytes and their progenitors, which may respond to further treatment with TNF (126). Intravenous administrations of G-CSF, GM-CSF, or M-CSF have also been shown to cause leukopenia followed by leukocytosis in humans (128, 60).

FGFs are also involved in leukemic hematopoiesis. In fact FGF receptors are expressed on some leukemic cell lines such as K-562, HL60, KG-1, and ML-2 (129). Recent reports (109) indicate that aFGF and bFGF do not directly affect the growth and proliferation of leukemic cells; however, they may modulate the cytokine network by synergizing or antagonizing the effects of other growth factors on leukemic cell growth. Nara *et al.* (109) examined the actions of bFGF and aFGF using acute myelocytic and chronic myelocytic leukemia cells from 14 patients. Results indicated that aFGF and bFGF stimulated blast-colony formation by leukemic blast progenitors from two patients. However, in the rest of the patients, effects varied between stimulation and inhibition depending on the cytokine(s) used in combination with either aFGF, bFGF, or both. The cytokines tested were IL-3, G-CSF, GM-CSF, and SCF.

Synergistic Effects of Cytokines on Leukemic Cells. An overlap in biological activities of various cytokines and growth factors is very common because they can share either the same receptors or key components of intracellular signaling pathways. IL-6 induces transient expression of the α-chain of the IL-2 receptor (IL-2R)-α in murine myeloid leukemia M1 cells (130). IL-2R-α is absent on M1 cells, but it appears after induction by IL-6 (130). It is thought that IL-1 and IL-6 synergise in the induction of IL-2R-α (131). IL-6 can induce WEHL-3B and M1 cells to differentiate from blast cells to mature macrophages (132, 133). Finally, a synergistic interaction by interleukin-1, interferon-β, and tumor necrosis factor was reported to stimulate terminal differentiation of the mouse myeloid leukemic cell line (M1) into macrophages (134). IL-4 and G-CSF have synergistic effects on the clonogenicity of U937 cells. The reason for this synergistic effect is thought to be because of a close association in the transduction system of IL-4 and G-CSF since homology of their intracellular domains has been reported (117). This combination was not effective against other cell lines such as KG-1 and HL60 although both cell lines carry a significant number of receptors for IL-4 (117). Another example is that by IL-1 and

TNF (74). Tumor necrosis factor (TNF) induces differentiation of myeloid leukemia cell lines to monocytes/macrophages (135–137). The differentiating effect of TNF on myeloid leukemia cell lines has been controversial, and this may be due to the use of different cell lines or various concentrations of TNF. However, combination of TNF with other cytokines, such as interferon gamma (IFN-γ) (138, 139), PGE_2 (140), and IL-1 (134), are synergistic in the induction of myeloid leukemia cell differentiation. IL-6 also overlaps in its function with LIF, ciliary neurotrophic factor (CNTF), and IL-11. The molecular basis for this overlap seems to involve the common signal transducer molecule gp130. By manipulating this molecule, it seems possible to modulate the actions of many cytokines. IL-12 effects on leukemic cells have not been fully elucidated yet, however, recombinant IL-12 induces regression of some types of solid tumors in association with INF-γ production (141, 142). It has immunoregulatory effects (43), and enhances the production of other cytokines that effect proliferation and differentiation of leukemic cells. IL-12 was reported to have an antitumor activity since it inhibits angiogenesis by tumor cells (43, 94).

Autocrine Production of Cytokines by Leukemic Cells

Some tumor cells have been shown to grow and form colonies in the absence of exogenous growth factors (145–147). They can produce the cytokine they need for their endogenous growth (28). Growth factors such as GM-CSF, G-CSF, and M-CSF are the most common and intensively studied cytokines produced by tumor cells. Juvenile chronic myelogenous leukemia (JCML) occurs early in life. It is a rare pediatric malignancy characterized by leukocytosis with prominent monocytosis. The CFU-GM progenitor cells in this disease proliferate spontaneously at very low densities in the absence of exogenous growth factors (29). Monocytes have been shown to produce IL-1 and GM-CSF endogenously, and that may account for spontaneous GM colony production (28, 145). Spontaneous CFU-GM proliferation was abolished after depletion of monocytes. This suggested a paracrine mode of cellular proliferation. Furthermore, the addition of anti-GM-CSF inhibited colony formation by mononuclear cells from JCML peripheral blood (29). Sawai *et al.* (145) demonstrated that GM progenitor cells grew in the presence of SCF, GM-CSF, IL-3, or G-CSF in serum-deprived media. However, combination of SCF and IL-3 stimulated generation of more GM colonies in the bone marrow of JCML patients than any other two-factor combination and was comparable to all factor combination (146). It may be of significance that normal GM progenitor cells responded most prominently to G-CSF plus SCF, however, more colonies were generated in bone marrow of JCML patients than in the bone marrow of normal controls.

L1210 lymphocytic leukemia is a rapidly growing murine lymphocytic leukemia associated with leukocytosis and anemia (148). In a study of Moqattash *et al.* (27), L1210 cells were reported to generate growth factors that stimulated their proliferation, in addition to stimulating the proliferation of CFU-GM progenitor cells. Media conditioned by L1210 cells contained colony-stimulating activity which stimulated normal murine bone marrow myeloid colony growth. L1210-conditioned media also stimulated L1210 cell growth in suspension and semisolid cultures. Preliminary analysis of the conditioned media showed that it contained GM-CSF and G-CSF. However, neither GM-CSF nor G-CSF were responsible for the autocrine activity by L1210 cells *in vitro* (27, 148), Other cytokine(s) may be directly or indirectly involved in L1210 cell autocrine activity. Other examples of autocrine dependence are the B-cell chronic lymphocytic leukemia (B-CLL), which was found to be growth-dependent on IL-8 (121) among other cytokines such as IL-1, IL-2, and IL-4 (120). B-CLL cells are long-lived cells that are characterized by their severe accumulation in peripheral blood and bone marrow.

A human leukemia cell line AML193, which was established from the bone marrow of an M5-type acute monocytic leukemia patient, is cytokine-dependent. GM-CSF and IL-3 stimulate AML-193 proliferation. On the other hand, AML-193 cells have been shown to produce autocrine GM-CSF when stimulated by IL-1 or tumor necrosis factor-α (TNF-α). Pretreatment of AML-193 cells with IL-6 enhanced their proliferative response to GM-CSF and IL-3 through upregulation of their receptors by a mechanism that seems to involve the direct activation of gp130 and *lyn* kinase (53). Additionally, filt3 ligand has been shown to play a role in the autocrine and paracrine loops sustaining acute myeloid leukemia cell growth. In a study by Sonoda *et al.* (15), the Filt3 ligand was found to stimulate proliferation of AML blast cells, and it also enhanced the proliferative response of AML cells to G-CSF or GM-CSF. The Filt3 ligand prevented apoptosis in AML cells by decreasing the expression of *bax* rather than increasing the expression of *bcl*-2. Its antiapoptotic effects were doubled when combined with G-CSF and GM-CSF.

Some diseases result not only from disorders in the cytokine mode of secretion, but also from receptor disorders. Disorders of IL-2 and IL-2 receptors (IL-2R) expression occur in association with infection by human T-cell lymphotropic virus I (HTLV-I) (143). The HTLV-I-related adult T-cell leukemia (ATL) is associated with this virus. Initially, ATL shows an autocrine system that involves the secretion of IL-2 and the expression of IL-2R. However, late-phase ATL cells and a cell line (HuT-102) derived from ATL patients, do not produce IL-2 and do not express its receptor. These cells on the other hand were found to produce IL-15 (143). It is of significance that normal resting or activated T cells do not express IL-15 mRNA, whereas the HuT-102 cell line does. It has been suggested that transcription is not the predominant site of regulation by IL-15, and that it is perhaps regulated at the translational level, in contrast to other cytokines. Therefore, one of the important

tools in studying functions of cytokines, and the treatment of diseases that result from disorders in cytokines, is the use of antibodies and antagonists to the cytokine receptors. For example, IL-1 receptor blockade has been shown to reduce the metastatic capacity of melanoma cells (144).

Cytokines and Leukemogenesis

Cytokines have been postulated to contribute to neoplastic cell growth, and many *in vitro* studies have confirmed this prediction, but little is known about the *in vivo* role of these growth factors. Cell lines developed from patients with lymphomas of the B-cell type non-Hodgkin's lymphoma (NHL-B) produced IL-14 and expressed the receptor for it (97). The autocrine or paracrine production of IL-14 may play a significant role in the transformation and rapid proliferation of aggressive NHL-B. NHL-B cells were unable to survive *in vitro* in the presence of IL-14 antibodies (97). Therefore, interruption of the autocrine or paracrine pathways may be a useful goal of therapy in this and other types of leukemia.

Autocrine production of growth factors by transformed cells has been shown to be an important step in leukemogenesis (21–23, 149). However, autocrine growth stimulation is not sufficient by itself to induce leukemia. Progenitor cell lines, such as GM-CSF, IL-3, and Epo (64, 150), that express growth factors as a result of retroviral gene transfer have been shown to develop myoproliferative syndromes but not leukemia (149, 151). Therefore, another mutation may be required to be associated with autocrine stimulation to transform hematopoietic cells. In this respect, cells surviving after chemo or radiation therapy with damaged DNA could lead to misrepair of DNA and propagation of cells with genetic alterations (152). It has been reported that patients who went into complete remission after chemotherapy developed a different type of leukemia after they received IL-2 as maintenance therapy. The role of the cytokine network in apoptosis of leukemia cells is not clear, however, there is some evidence that apoptosis may require a previous cytokine signal such as Epo for terminal erythropoiesis and cell death (153).

Other studies have shown that oncogenesis may be the result of interference with apoptosis (152, 154), this seems to be the initiating event in some malignancies (152, 155). In this regard, samples from patients with various types of leukemias were analyzed for the expression of IL-1β and INF-α (156). These cytokine expressions were found in leukemia cells from lymphoblastic, monocytic, and myeloid leukemias. The findings suggested that IL-1β and INF-α may be involved in the leukemic process. It appears that abnormal expression of growth factors is a common event with CML Phl progression. It is strongly thought that autocrine production of IL-1 stimulates production of various growth factors by stromal cells, and this process may play a role in the disease progression (157). Leukemic cells from acute type of ATL synthesize IL-4 receptors (IL-4R), without stimulation, and at much higher levels than normal resting lymphocytes. There is also correlation between the proliferative response of acute ATL and the expression of IL-4R. Therefore, IL-4 seems to be involved in the leukemogenesis of this disease (158).

Interactions Between Stroma and Leukemic Cells: The Role of Cytokines

The bone marrow hematopoietic microenvironment consists of cells, a vascular network, extracellular matrices (ECM), and a complex of growth factors. Bone marrow stromal (BMS) cells are composed of fibroblasts, macrophages, adipocytes, endothelial cells, and reticular cells. Blood cell formation involves the interaction of hematopoietic progenitor cells and stromal cells of the bone marrow microenvironment (159–162). Stromal cells have been found to deposit extracellular matrix molecules such as fibronectin, laminin, collagen, and hyaluronic acid (159, 163). Candidate hematopoietic stem cells as well as developing precursor cells express matrix adhesion molecules in addition to the receptors for cytokines. A number of studies have shown that cytokines regulate not only hematopoietic cell survival, growth, and differentiation, but also adhesion of progenitor cells to bone marrow ECM molecules such as Fn, Lam, and collagen. Specific adhesion receptors on progenitor cells have been described to mediate interaction with ECM (159, 164, 165). Stromal cells and matrices provide attachment sites for hematopoietic progenitor cells and regulate their proliferation and maturation by secreting stimulatory and inhibitory cytokines (159). An understanding of normal and abnormal hematopoiesis requires a detailed study of the interaction of not only the stimulatory and inhibitory factors but also other types of factors such as heme, arachidonic acid, and prostaglandins, which are beyond the scope of this paper. However, the interaction of heme and cytokines and heme's role in hematopoiesis will be discussed later.

Shigetaka (166) examined the interaction of normal and leukemic cells with stromal cells by video. $CD34^+$ normal and leukemic cells were implanted on human stromal cell layers from normal donors. Both normal hematopoietic and leukemic cells divided in a similar manner, and macrophages moved actively around dividing cells. However, G-CSF promoted normal cell division more than leukemic cells. This model was also used to examine the effect of INF-α on the interaction of hematopoietic cells with stromal cells during remission.

Microenvironmental abnormalities were reported in many leukemias. These abnormalities were not only due to abnormal cytokine production, but also to cell-to-cell interaction and cell composition (34, 167, 168). A reduced number of macrophages, fibroblasts, and adipocytes were reported in myeloid leukemias (168). Other investigators suggested that abnormalities in the bone marrow microenvironment are due, at least partially, to malignant macrophages (32). Manipulating the interactions of leukemic cells with bone marrow stroma using various cytokines may be

therapeutically useful in patients with some types of leukemia. Cellular adhesion, which is involved in physiological events such as differentiation, migration, and inflammation, has recently gained attention due to the special relationship found between leukemic cell growth and adhesion (169). Like their normal counterparts, acute leukemia cells (AML) adhere to bone marrow stroma and extracellular matrix components such as fibronectin. It has been demonstrated that direct contact between leukemic cells and BMS is essential for the leukemic cell viability. Interruption of their contact increases apoptosis and results in cell death (40, 170). Normal hematopoietic cells as well as leukemic progenitors rely on the bone marrow microenvironment for survival and growth (40, 41). Leukemic cells come in direct contact with reticular cells, extracellular matrix, cytotoxic cells, soluble and membrane bound growth factors, and macrophages. This contact inhibits apoptosis of malignant cells and prolongs their survival (35, 41, 170). Adhesion of malignant cells to BMS is influenced by soluble and membrane-bound cytokines. Bendall *et al.* (169) reported that AML cell adhesion to bone marrow fibroblasts (BMF) was enhanced when BMFs were treated with TNF alone or in combination with IL-4 or IFN. Other cytokines such as G-CSF, GM-CSF, SCF, and IL-3 were also found to increase AML cell adhesion to BMF in some cases. ALL cells also adhere to BMFs (36). Media conditioned by cytokine-treated BMFs had no effect on adhesion of AML cells suggesting that increased binding of AML cells results from induction or activation of a ligand on the cell surface rather than a soluble factor secreted by the cytokine-stimulated BMFs. However, this area requires further investigation since many cells are involved in the *in vivo* situation, and increased or decreased adhesion may be the result of interaction of many soluble or bound cytokines with cell surface or soluble ligands. Different mechanisms may be involved in the adhesion of leukemic cells to BMS. Therefore, alteration or manipulation of the adhesion characteristics of leukemic cells to BMS cells, the leukemic cell surface proteins, and the cytokines involved in leukemic cell adhesion to BMS may increase the likelihood of cytokine or cell-mediated killing.

Tachykinins are potential regulators of hematopoiesis and have important effects on the nervous system. They are members of a family of structurally related peptides that share a common -COOH terminal sequence Phe-X-Gly-Leu-Met-NH_2. Neurokinin A (NKA) and substance P (SP) are members of the tachykinin family (171). Therefore substances/factors produced by neural tissues also have effects on hematopoiesis. Bone marrow stromal cells carry tachykinin receptors that induce the production of several important cytokines. In this respect, SP and NKA can regulate proliferation of myeloid progenitors, CFU-GM. SP is a positive regulator, and NKA is a negative regulator. IL-1 induces SP and in turn, SP induces the production of hematopoietic growth factors by stromal cells. Examples of these growth factors include SCF, IL-1, IL-6, GM-CSF, and c-*kit* ligand. On the other hand, NKA induces the production of negative regulators such as MIP-1α and TGF-β (171). Therefore, it is apparent that tachykinins and cytokines may interact to modulate hematopoiesis. The potential sources of SP are BMS cells, macrophages, endothelial cells, and possibly other resident cells in bone marrow (172). SP binding sites are present on hematopoietic cells, T cells, macrophages, and endothelial cells, whereas the NKA gene is expressed in bone marrow, stromal cells and T cells (171). When SP is released by nerve endings, bone marrow cells are induced to release IL-1. Induction of IL-1 and its specific receptor IL-1R1 produce a chain reaction that results in the induction of various hematopoietic lineages in addition to an increase in SP receptors on cells within the BMS. The interaction between SP and IL-1 is probably one of many ways in which cytokines and other growth factors interact to regulate hematopoiesis. However, this interaction may also represent a model in which BMS cells play an intermediate role in the communication between BMS cells, cytokines, and the regulation of hematopoietic progenitor cell growth and differentiation. Therefore, abnormalities in the bidirectional, neurohematopoietic communications may be related to the development of some malignancies. Such malignancies may involve the receptors on BMS cells, cytokines induced by tachykinins, or the production of tachykinins themselves. Knowledge of such possible abnormalities may represent a new approach to the understanding of some types of leukemia and perhaps to new therapeutic strategies.

Bone marrow stromal cells are responsible for providing the cytokine and cell-to-cell interactions required for normal hematopoiesis (37, 38). Additionally, some stromal cells can also differentiate into adipocytes responding to adipogenic agonists such as glucocorticoids and adipogenic antagonists such as TNF-α, IL-1-α and TGF-β (39). The bone marrow stromal cell line, called BMS2 supports B-lymphcyte differentiation and myelopoiesis (38). Some cytokines such as TNF-α, IL-1, and TGF-β were found to induce the levels of IL-6 mRNA and IL-6 bioactive materials in BMS2 cells (39, 59). IL-6 mRNA can also be induced by IL-1α and TNF-α in human lung fibroblasts (173). Other human stromal cell lines, which were developed from fetal liver (ST-1) and adult bone marrow (SV-MSC), were found capable of supporting myelopoiesis *in vitro* (174). Again in these two stromal cell lines IL-1-α induces IL-6 mRNA in the ST-1 cell line, and TNF-α induces IL-6 mRNA in the SV-MSC cell line. However, variation in IL-6 mRNA half-life was found that reflected the heterogeneity within the bone marrow stromal cell lines (10, 38). These reports provide some insight into the role of bone marrow stromal cells in hematopoiesis and the regulatory role played by IL-6 in lymphopoiesis, myelopoiesis, and hematopoiesis in general.

It has always been assumed that human primitive progenitor cells do not respond to cytokines produced by stromal cells from other species. Recent reports proved otherwise. A novel unidentified factor has been shown to cross

species barriers (146, 175). Murine stromal cell activity has been shown to promote maintenance and differentiation of many primitive human stem cells. For example, a murine bone marrow–derived stromal cell line, MS-5, was found to stimulate proliferation of a human leukemic cell line UT-7 (175). Early human stem cells have been maintained *in vivo* (176, 177) when grafted in the marrow environment of a xenogenic host, and *in vitro* (146, 178–180) when cocultured with xenogenic stromal cells in the absence of the human cytokines normally required. These studies suggest the existence of novel stromal-derived, species-cross-reactive hematopoietic regulator(s). These studies also suggest that certain interactions between some known proteins and the cell surface may also cross species barriers. Serum proteins serve as carrier proteins for cytokines.

Cytokines and Apoptosis

The programmed destiny of cells (to live or die) is regulated by a process called apoptosis. This process is regulated by intracellular and extracellular factors that signal viability or death through the activation of certain genes within the cell (181, 182). Abnormalities in this program result either in death of vital cells or in abnormal survival of damaged cells that would normally die. There are genes that induce apoptosis and those that suppress it. Genes that induce apoptosis include tumor suppressor wild-type p53, c-*myc* and *bax,* and those that suppress it include the oncogene mutant p53, *bcl*-2, *bcl*-x1 (181).

Growth factors and interleukins may maintain viability of normal and leukemic cells by suppressing apoptosis. Patients with myelodysplastic syndrome (MDS) suffer from ineffective hematopoiesis. In this disease the ratio of apoptosis-related oncogenes c-*myc* to *bcl*-2 is increased (15). c-*Myc* expression enhances apoptosis whereas *bcl*-2 expression decreases it. This has been suggested to explain in part the cause of ineffective hematopoiesis in MDS patients. Experiments with $CD34^+$ cells showed that treatment of those patients with G-CSF plus Epo resulted in an enhancement of hematopoiesis. This enhancement was associated with decreased levels of programmed cell death within $CD34^+$ marrow cells, and the ratio of c-*myc* and *bcl*-2 was altered (15). In general, withdrawal of the necessary cytokine(s) results in the induction of apoptosis and cell death (19, 34). Therefore, manipulating the levels of cytokine(s) required for the survival of leukemic cells may induce apoptotic genes, reduce their leukemogenicity, and consequently render leukemic cells more susceptible to anti-cancer agents. On the other hand, hematopoietic cytokines can decrease the effectiveness of anticancer agents against leukemic cells. This can explain the reason for poor remission in AML patients where leukemic cells are responsive to growth factors such as G-CSF (182, 183). Thus, reducing the level of the leukemic-cell-viability factor or the responsiveness of leukemic cells to that factor, as the anticancer agent is administered, may result in a more successful therapy. For example, the use of antibody to epidermal growth factor receptor together with doxorubicin resulted in complete regression of autonomously growing human carcinoma cells grown as a xenograft in mice. Neither the antibody nor doxorubicin alone caused a similar regression (182, 184). Recent studies by Brodsky *et al.* (152) and others (150) raised the possibility that growth factors may mediate the suppression of the apoptotic death of hematopoietic progenitors damaged by chemotherapy, and that may contribute to their leukemic transformation. In this respect, the National Cancer Institute announced that patients receiving doxorubicin/cyclophosphamide therapy for breast cancer may be at a higher risk of developing a secondary AML. It is thought that hematopoietic growth factors used to decrease the toxicity related to myelosuppression may play an important role in the development of secondary AML (185). Another example of the leukemogenic properties of cytokines has been demonstrated in a patient who had entered complete remission after chemotherapy. Low doses of IL-2 were used for 6 weeks after which the patient went into relapse. The phenotype of the blast cells after relapse was different from that of the originally diagnosed cells (186). The authors concluded that IL-2 may have promoted the growth of leukemic cells.

Apoptosis is a normal physiological process by which cells may be eliminated during normal embryonic development and adult life (155). In some cases, growth factors play a vital role in regulating hematopoietic cell survival by interfering with apoptosis. Hematopoietic cells deprived of growth factors rapidly undergo apoptosis (185–187). In fact, apoptosis is also the common pathway by which the anticancer agents induce cell death (150, 154, 188). Since growth factors have multiple effects on both normal and malignant cells, the pleiotropic effects of the growth factors used in the treatment should be taken into consideration. Growth factors, which stimulate leukemia cell proliferation and survival, appear to cause drug resistance (189) and relapse in some patients (190). In addition, multiple genetic factors that inhibit apoptosis have been shown to induce cellular resistance to most cytotoxic anticancer agents (150, 154, 188). Similarly, cytokines were reported to protect hematopoietic cells from cytotoxic chemotherapy and radiation by inhibiting apoptosis (191, 192). That is, by suppressing the apoptotic death of damaged hematopoietic progenitor cells, growth factors stimulate proliferation of undamaged cells and ameliorate defects in hematopoiesis.

On the other hand, without the presence of certain cytokines, the program of apoptotic cell death may become activated (153). Many myeloid leukemic cells require certain cytokines for their growth. Apoptosis is activated in these leukemic cells if they are deprived of the cytokines they require, the result is less leukemogenicity (182). GM-CSF and IL-1 were found to inhibit apoptosis in isolated human monocytes and the U937 monocytic cell line, whereas TNF-α was found to induce DNA fragmentation of U937 cells (193). Enhancement of apoptosis by TNF-α caused a diminished host defense against an infection by

Candida albicans, whereas GM-CSF, which inhibited programmed cell death, intensified host defense. Apoptosis of the monocytic cell line U937 and freshly isolated monocytes was inhibited during an infection with C. albicans. The C. albicans was found to inhibit apoptosis by inducing and enhancing the production of PGE_2 synthesis by the monocytic cells. That was regarded as a mechanism by which the host immune system intensifies the defense against infection. However, U937 cells were resistant to TNF-mediated apoptosis during retinoic acid and 1,25-dihydroxyvitamin D3–induced differentiation (194). These findings indicate that cellular response to apoptotic induction is related to the stage of the cycle in which the cell is found. Further investigation is required in this area. It is necessary to determine the sensitivity of leukemic and normal cells to apoptotic inducers in various states or stages of the cell cycle. These investigations may lead to the determination of the appropriate apoptotic inducers for certain types of leukemia.

Therapeutic Applications

The variation in the effects of cytokines indicates that the therapeutic regimen for a given type of leukemia may vary from one patient to another. Therefore, for a particular type of leukemia, treatment with cytokines may require the development of a system in which leukemic cells of a patient are screened with the most effective cytokine(s) or type of therapy known to determine the most appropriate cytokine therapy for that patient. INF-α is one of the most useful cytokines in the treatment of hematological malignancies. It has been approved for the treatment of chronic myelogenous leukemia, multiple myeloma, and hairy cell leukemia (160). Long-term survival is expected in some of those patients (68, 120, 195). In addition, INF-α seems to have potential for use in the treatment of several lymphomas (68). Elucidation of the antileukemic mechanism of interferons may be useful as a model, however, the variability of the target cell response and the complexity of the actions of the cytokine network make it a very difficult task. A variety of colony stimulating factors have been used in various ways in the therapy and treatment of some leukemias. They have been used after chemotherapy or after stem cell transplantation to stimulate stem cell proliferation or increase the intensity of the chemotherapeutic dose (68). Cytokines have a great potential for broad clinical application. Four recombinant cytokines, GM-CSF, G-CSF, M-CSF, and IL-3 are being tested in clinical trials. In fact two of these factors, G-CSF and GM-CSF, have been approved by the US FDA (196). GM-CSF was demonstrated to increase the number of neutrophils, eosinophils, and monocytes with corresponding bone marrow changes. Some data suggest that GM-CSF may be applicable to patients with a high risk of infection. G-CSF, studied mainly in the treatment of neutropenia following cytotoxic chemotherapy, was found to decrease the duration of severe neutropenia as well as the risk of infections. G-CSF causes prominent increases in neutrophil levels without affecting eosinophils or monocytes. It may also have a therapeutic role in lymphoid neoplasms complicated by neutropenia. G-CSF is greatest when used as an agent to enhance circulation of stem cells and pre-colony-forming progenitor cells (196, 197). The predominant effect of M-CSF appears to be enhancement of macrophage and monocyte function, which may reduce the severity and duration of fungal infection (196). However, administration of natural M-CSF to patients receiving chemotherapy and those with chronic childhood neutropenia has shown modest neutrophil increases. Preclinical data on IL-3 suggest that this agent increases neutrophils, eosinophils, basophils, reticulocytes, and possibly platelets (197).

Many other interleukins have been used in clinical studies. IL-2 was reported to produce remission in patients with melanoma, solid tumors, and renal cell carcinoma (167, 186). However, in other instances it was reported to have a leukemogenic effect. After remission in a case of acute myelogenous leukemia, IL-2 promoted the growth of leukemic cells when used as maintenance therapy at a low dose (186). In a clinical study with IL-3, Nimer *et al.* (198) evaluated the effect of recombinant human IL-3 (rhIL-3) in 21 patients with aplastic anemia (AA) and myelodysplasia (MDS). Patients received 21-day cycles of IL-3. However, the duration of the treatment and the concentration of rhIL-3 varied from one patient to another. The results of this study showed that hematologic responses were seen in 10 out of 20 patients. Multilineage effects were seen in 25% of the patients, with the majority of those having MDS. Some of the clinically significant results included two patients with AA who became transfusion-independent for 8 months and another who had decreased transfusion requirements. Other patients showed increases in absolute neutrophil and platelet counts. The authors concluded that IL-3 may be used best as part of a combination therapy.

The antagonistic action of some cytokines to other cytokines has been used successfully in some therapeutic regimens. For example, B-CLL cells require cytokines such as IL-1, IL-2, IL-4, IL-6, and INF-γ for their survival. These cytokines seem to protect B-CLL cells from apoptotic death (199), and this protection was found to be correlated with *bcl*-2 expression (120). INF-α was demonstrated to induce remission in patients with early stages of B-CLL (120, 195). It was suggested that INF-α interrupts the growth-factor-dependent survival pathways in B-CLL cells allowing the apoptotic death of these cells. Many cytokines use similar signal transduction pathways. An understanding of these pathways may lead to the development of new therapeutic strategies in which cytokines are used to induce programmed death in leukemic cells.

One of the approaches against leukemia/cancer growth is gene therapy. In this kind of therapy, tumor cells are modified to produce a cytokine that has tumoricidal activity. In recent studies by Feldman *et al.* (200) and Ahmed *et al.* (201), adenovirus-mediated gene transfer techniques were used to transfer INF-α into $CD34^+$ leukemic stem cells to

provide INF-α to bone marrow microenvironment as a means to induce remission of CML. More recent studies are in progress using retrovirus-mediated INF-α gene transfer to give a permanent expression of the INF-α gene. INF-α restores adhesion molecule expression so cells can remain in the stroma and behave normally. This technique has great potential for managing leukemia, hemangiomas, and other types of leukemia. However, the major setback in this type of therapy is that some cytokines such as IL-2 and GM-CSF may stimulate leukemic cell growth (167, 186, 202). Modified tumor cells or target tumor cells may also be autocrine in nature producing their own growth factor(s), and that may interfere in the gene therapy or the tumorigenicity of the growth factor cDNA. Therefore, knowledge of the growth properties of the target tumor cells as well as the modified tumor cells used for immunotherapy is essential. However, immunotherapy using cDNA seems to be promising. Kimura *et al.* (55) examined the antitumor effect of the locally expressed M-CSF when human M-CSF cDNA was transduced into the mouse lymphoid cell line, L1210. Survival of infected mice was prolonged when they were injected with M-CSF-producing subline. M-CSF-expressing cells induced immune protection against the parental cells. Results suggest that M-CSF cDNA is a candidate for use in gene therapy of at least lymphoid leukemia, since other types of leukemia may respond best to other cytokine cDNA. Consequently, other candidate genes need to be examined with other types of leukemia. Expression of M-CSF by genetically modified melanoma cells produced an effective antitumor immune therapy response (205). This approach was attractive since systemic administration of M-CSF has limitations. Local production of M-CSF by modified tumor cells had a greater effect on target tumor cells and induced immune protection (55). Recently gene therapy for advanced cancer using IL-12 was announced by the University of Pittsburg Cancer Institute.

In some cases, chemical modification of human growth factors generates an antagonist to the growth factor itself. Alkylated IL-3 was reported to inhibit the proliferation of MO-7 cells, which require IL-3 for their proliferation (18). IL-3 was chemically modified by incubation with iodoacetate at 37°C in the presence of EDTA and urea. The alkylated form of IL-3 was found to be a potent IL-3 antagonist even though it bound to the receptor. This suggests that some leukemias and other forms of cancer may be suppressed by the alkylated form of the cytokine(s) on which they are dependent.

Specific antibodies for cytokine receptors might serve as useful therapeutic agents by blocking the action of growth factors on the malignancy. However, Shadduck *et al.* (203) reported that malignant cells can show a biphasic response to monoclonal antibodies (McAb). In their report, they found that high concentrations of McAb to M-CSF receptors stimulated the growth of rat normal and myelogenous leukemia cells *in vitro*. Splenic cells from rats bearing this tumor give rise to *in vitro* colonies in response to M-CSF. In contrast, low concentrations of the McAb to M-CSF inhibited colony formation by tumor cells. The costimulatory activity caused by high concentrations of the M-CSF McAb was destroyed by conversion of the antibody into monovalent Fab fragments by papain digestion.

M-CSF was reported to have tumoricidal activity for some tumors *in vivo* (204). It was demonstrated to kill L1210 lymphocytic leukemia cells that were resistant to tumor necrosis factor by inducing the production of O^{-}_{2} and H_2O_2. These reactive oxygen intermediates have an antibody-independent cytotoxic activity against tumor cells (55). It is of interest to mention that retinoic acid (RA) and 1,25-dihydroxyvitamin D3 (VD3), individually and in combination, were found to inhibit colony growth by L1210 cells (27, 148). Similarly the two agents were also found to inhibit generation of growth substances by L1210 cells. The combined action of the two agents was more effective than either one alone (27, 148). It remains possible that a combination of M-CSF with RA and/or VD3 may prove beneficial in the treatment of this type and perhaps other types of leukemia.

The effects of TGF-β1 were evaluated in patients with hematologic malignancies designated as clonal disorders of multipotential stem cells. Murohashi *et al.* (206) have demonstrated that TGF-β1 suppresses the growth of some hematological malignancies that are stimulated with different growth factors. In some of these malignancies, the suppressive effect of TGF-β1 increased with certain growth factors. In other malignancies, TGF-β1 abolished the malignant cell clonogenic growth irrespective of the growth factor used. For example, the growth-suppressive effect of TGF-β1 was increased with G-CSF in patients with essential thrombocytopenia and polycythemia vera; chronic myelogenous leukemia (CML) in the chronic phase; CML in the accelerated phase; CML in myeloid crisis; and acute myeloblastic leukemia (AML). In other causes, the level of suppression increased with GM-CSF, IL-3, and SCF. They also reported that in some patients with CML in the accelerated phase, AML, and CML in myeloid crisis, the TGF-β1 was able to abolish the clonogenic cell growth completely. They concluded that the TGF-β1 suppression of growth seemed to increase with the progression of clonal evolution in these hematological malignancies.

Recombinant thrombopoietin (r-TPO) has recently been shown to be clinically useful. It was found to alleviate thrombocytopenia associated with myelosuppressive and myeloablative therapies for cancer patients (207). When r-TPO was administered to mice treated with sublethal irradiation and carboplatin, the severity of the platelet nadir was reduced, and platelet recovery was accelerated by 10–12 days (49, 207). Similar results were obtained in monkeys (208). However, r-TPO was not effective in other experiments where it had no effect on hematopoiesis in lethally irradiated mice or monkeys (207). These results suggest that r-TPO may require interaction with stromal cells and/or their products to be effective since stromal cells may be

destroyed by lethal irradiation. Further support for this assumption comes from the experiments where lethally irradiated mice transplanted with marrow cells from r-TPO-treated donor mice showed accelerated recovery of platelets and RBCs. However, post-transplant administration of r-TPO had no further effect on this accelerated recovery (209).

Cytokines as Adjunct Therapeutic Agents. One of the main side effects of various types of therapies is the depression of one or more of the hematopoietic lineages (169, 210). To promote normal hematopoiesis, colony-stimulating factors have been widely used to treat these side effects. G-CSF and GM-CSF have been used to reduce the degree and duration of neutropenia. This treatment allows for an increase in the dose intensity of the chemotherapeutic agents used following chemotherapy or stem cell transplantation (68, 211). Administration of hematopoietic growth factors before, after, or during various therapies has been attempted. Results are somewhat controversial. GM-CSF priming before a high-dose chemotherapy (etoposide and cyclophosphamide) did not have any impact on early neutrophil or platelet recovery (210). Other reports showed that GM-CSF expanded progenitor cell mass in patients treated with the CyADIC regimen (212) and rendered progenitors in a quiescent state, decreasing their sensitivity to agents that were cycle sensitive before chemotherapy. In another study when GM-CSF was given to patients with solid tumors, the percentage of their bone marrow burst-forming unit-erythroid (BFU-E) and CFU-GM increased in the S phase; however, 48–96 hr after discontinuation of GM-CSF, the fraction of cycling progenitors dropped below pretreatment levels (213). This indicated that abrupt withdrawal of growth factors might induce a quiescent state in the committed progenitor population. Other studies showed that G-CSF was not effective in stimulating granulopoiesis in dogs treated with radiolabeled immune therapy (RIT) and G-CSF since the granulocytic count in G-CSF-treated dogs was not different from the dogs that received RIT plus G-CSF. This indicated that RIT damages stem cells irrespective of the presence of G-CSF (169). However, in other studies, G-CSF-treated dogs showed an increase in granulocyte levels and were not affected by RIT. In other studies with mice, IL-1 and GM-CSF have been shown to accelerate hematopoietic recovery after RIT (214). Effects of other cytokines such as Epo and thrombopoietin have not yet been fully elucidated in these kinds of studies. It remains possible that combinations of cytokines before or after chemo and immunotherapies may prove to be more effective than administration of a single cytokine. This is because some factors may augment the effects of others, and some progenitor cells may require a combination of cytokines for proper response. Furthermore, combinations of growth factors may provide more protection to progenitor cells. For example, both AZT and INF-α are inhibitors of human immunodeficiency virus type 1 (HIV-1) replication (215, 216). Combination of the two agents results in marked synergistic antiretroviral activity (217). However, both AZT and INF-α inhibit progenitor cell proliferation (218–220). Combinations of AZT and INF-α have additive inhibitory effects on CFU-GM and BFU-E (220). GM-CSF (221) and/or IL-1 (222) were separately reported to partially alleviate the cytotoxic effects of AZT on hematopoiesis. On the other hand, Castello *et al.* (220) reported that neither GM-CSF nor IL-1 alone was able to reduce AZT-induced hematopoietic toxicity. However, they found that combining the two agents at low doses reduced the *in vitro* AZT-cytotoxicity on CFU-GM. Moqattash *et al.* have also demonstrated that recombinant hemoglobin alone or in combination with Epo reverses the hematopoietic cytotoxicity induced by AZT in MAIDS and normal mice *in vitro* and *in vivo* (218, 219). In other studies, Abraham *et al.* (222) have shown that both IL-1 and heme improved the hematopoietic recovery from the cytotoxic effects of AZT. Finally, Lutton *et al.* (223) demonstrated that IL-1 and heme can significantly promote and protect normal stromal hematopoiesis after radiation damage.

Cytokines can potentiate the therapeutic effects of other agents. For example, HL60 cells can be induced to differentiate to mature macrophages with VD3. On the other hand, TNF-α shows antiproliferative effects on HL60 cells, but fails to trigger HL60 cells to differentiation. However, if TNF-α is used in combination with VD3, it increases the number of morphologically mature cells (137). HL60 cells exposed to VD3 acquired the ability to secrete cytokines such as IL-1-β, PGE2, and GM-CSF. These observations led to the conclusion that treatment of leukemic cells with combinations of cytokines and vitamin derivatives, such as RA and VD3, represent an attractive area of research. The combination of IL-1 and GM-CSF may be interesting among differentiating agents since both are natural products and may act under physiological or pathophysiological conditions to stimulate differentiation of transformed cells. Finally, cytokines can make leukemic cells more susceptible to apoptosis. Lotem and Sachs (195) reported that treatment of M1 myeloid leukemia cells with IL-6 causes a down-regulation of the apoptosis-suppressing gene *bcl*-2. As a result, M1 cells become more susceptible to induction of apoptosis by adriamycin or cycloheximide.

Heme and the Cytokine Network

There is increasing evidence indicating that heme may interact with growth factors and other cytokines to form a network that can modulate hematopoiesis. Heme induces heme oxygenase, and this enzyme plays multiple roles in growth and differentiation of hematopoietic cells as well as their response to stress (224). Furthermore, heme oxygenase levels were found to be elevated in leukemic and lymphoma cells (225). The vital role of heme in mammalian physiology is attested to by its function as the prosthetic group in a variety of important hemoproteins that are essential for various cellular processes. Additionally, the role of heme in the regulation of globin and nonglobin protein synthesis is

amply documented (224–231). Cells within the network must have a viable heme metabolism to maintain a functional role within the microenvironment (224, 225, 227, 231). It has recently been demonstrated that heme upregulates the expression of Epo receptors in murine (MEL) and human (HEL) erythroleukemia cells (226). These observations were supported by the experiments that showed that Epo receptor expression was prevented by incubating cells with succinylacetone, an inhibitor of heme synthesis. Therefore, heme levels can modulate Epo receptor expression and hence the cell response to Epo. Other studies have demonstrated that heme and some heme analogs (Sn-protoporphyrin) synergize with IL-2 in stimulating thymidine incorporation by lymphocytes. In addition, heme has been shown to stimulate TNF-α and IFN-γ production by peripheral blood macrophages in the presence of T lymphocytes and the generation of killer cells (226, 232).

Abraham *et al.* (226, 231) have demonstrated that conditioned media from adherent stromal cells exposed to inhibitors of heme synthesis had a significant inhibitory effect on erythroid and myeloid progenitor cell growth. These results suggested that heme may be necessary for the production of growth-promoting substances by stromal cells, which directly promote hematopoietic colony formation and possibly the expression of growth factors and/or their receptors. As a result, a defect in heme synthesis may lead to an increase in negative regulation and suppression of pluripotent stem cells. On the other hand, adequate heme may stimulate the synthesis and/or release of cytokines from stromal or hematopoietic cells, which in turn stimulate hematopoiesis. Therefore, heme and/or heme oxygenase levels may directly or indirectly affect the production or action of various cytokines to form a network that modulates hematopoiesis. These and other results lend support for the therapeutic use of heme directly or as an adjunct to other therapeutic agents. For example, some therapeutic agents such as AZT cause *in vitro* and *in vivo* suppression of bone marrow erythropoiesis, myelopoiesis, and platelet production. Moqattash *et al.* have demonstrated that the addition of recombinant hemoglobin to AZT-treated bone marrow cells *in vitro,* results in hematopoietic recovery (218). Similarly AIDS patients receiving AZT treatment suffered from anemia, leukocytopenia, and thrombocytopenia. Using a murine model of AIDS, Moqattash *et al.* (219) demonstrated that administration of Epo and/or recombinant hemoglobin to MAIDS mice fed with AZT resulted in erythropoietic and myelopoietic recovery. However, the combination of Epo and recombinant hemoglobin was not more effective than recombinant hemoglobin alone. Other cytokines that affect a wide variety of progenitor cells or earlier progenitor cells may prove to be beneficial if used in combination with recombinant hemoglobin. Furthermore, it is not clear yet whether heme oxygenase (which is induced by hemoglobin or heme) plays a beneficial or harmful role in these experiments. Finally, heme has been shown to have modulatory effects on specific adhesion molecules such as ICAM-1 and VCAM-1. Wagener *et al.* (233) recently demonstrated that heme induces expression of ICAM-1 on endothelial cells and that this has effects on hematopoietic cellular adhesion and cellular traffic in and out of the bone marrow microenvironment. Thus, heme and the heme oxygenase system may have important effects on leukemic cell growth/death response to cytokines/therapeutic agents and cellular interactions within the bone marrow microenvironment.

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