Hematopoietic Growth Factors and Their Receptors in Acute Leukemia

By Bob Löwenberg and Ivo P. Touw

Blood cells are derived from small numbers of self-renewing pluripotent hematopoietic stem cells that reside in the bone marrow and generate progenitor cells committed to proceed along one of the maturation pathways. Because the life span of blood cells is limited, the production rate of blood cells in the marrow is high, even during steady-state conditions. The marrow system has the ability to adapt to sudden changes in the needs of different cell compartments by elevating the production rate of blood cells of specific cell lineages. To satisfy these variable needs, a tight control of the processes of cell renewal, commitment, maturation, and survival for each of the differentiation stages within each blood cell lineage is required. The hematopoietic growth factors (HGFs) play a critical role in regulating these processes.

Acute leukemia is characterized by an arrest of maturation and the accumulation of undifferentiated cells in marrow, blood, and other tissues. Similar to normal hematopoiesis, the majority of leukemic cells descend from a relatively small pool of progenitor cells with high proliferative activity. In vitro culture methods used as tools to analyze human leukemia progenitor cells have in recent years been considerably improved as the result of the availability of the recombinant HGFs. We review here current knowledge about the growth properties of acute leukemia progenitor cells derived from the application of in vitro assays. We discuss the significance of these findings in view of the pathogenesis, diagnosis, and treatment of acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL).

Dependence of AML Progenitor Cells on HGFs

Models of hematopoiesis of around 1930 postulated the existence of pluripotent stem cells capable of taking alternative pathways of self-reproduction and differentiation towards fully developed elements. Not until 1961 was a cell population identified that formed visible colonies on the surface of the spleen 7 to 10 days after transplantation of small numbers of marrow cells into lethally irradiated mice. The spleen colony-forming cells fulfilled the requirement of pluripotency, i.e., self-renewal capacity and extensive repopulation ability. Since then, culture techniques have been advanced first for murine cells and, subsequently, for human marrow cells that allowed the clonal proliferation from hematopoietic progenitor cells in vitro. These developments have created the basis for assays of hematopoietic progenitors of various differentiation lineages that allowed the characterization of stem cell and progenitor cell populations of different primitivity. Before the cloning and isolation of the growth factors known as colony-stimulating factors (CSFs) through recombinant DNA technology, it had already become evident that AML progenitor cells from most patients depend on CSFs for proliferation in vitro. The stimulating activity at that time was ill defined. In the "classical" colony cultures that included conditioned media from cell lines or feeder cells as crude sources of colony-stimulating activity, AML colonies were formed during incubation for 7 to 14 days. Typically, only 0.1% to 1% of AML blasts were capable of forming a colony, lending support to the concept of leukemic "stem cells." Studies based on immunophenotypic analysis and cell sorting confirmed that the largely nonproliferative population of AML cells tends to be somewhat more mature than the in vitro clonogenic cells. Generally, AML progenitor cells require HGFs for survival and for proliferation. The recombinant HGFs interleukin-3 (IL-3), granulocyte-macrophage-CSF (GM-CSF), and granulocyte-CSF (G-CSF) induce leukemic colonies or activate DNA synthesis in more than 80% of AMLs. Macrophage-CSF (M-CSF) stimulates AML cell proliferation in vitro in approximately 50% of cases. When IL-3, GM-CSF, and G-CSF are supplemented to culture in combination, their proliferative action on AML progenitors is often enhanced. Recently, it has become evident that klf ligand (KL), also referred to as stem cell factor (SCF), Steel factor, or mast cell growth factor, is also capable of inducing the proliferation of AML cells. Most notably, KL may greatly enhance the individual stimulating activities of IL-3, GM-CSF, or G-CSF and amplify the response by 10- to 20-fold. IL-6 may infrequently promote AML colony growth. Thus, several regulatory molecules that normally stimulate hemopoiesis exert profound positive effects on AML progenitor cells and accelerate growth. These features would indicate that variations of the tissue concentrations of HGFs in vivo will determine or affect the growth rate of human leukemia.

No clear relationship has become apparent between IL-3, GM-CSF, G-CSF, and M-CSF responses and the French-American-British (FAB) classification. This lack of correlation includes the infrequent AML blast responsiveness to erythropoietin (EPO), which is not restricted to erythroleukemias (FAB M6). EPO, in combination with GM-CSF or phytohemagglutinin-leukocyte conditioned media (PHA-LCM) as a crude source of growth factors, may also induce the proliferation of leukemic cells of other cytologic subclasses of AML (FAB-M1, FAB-M2, and FAB-M5) or AML (FAB-M1, FAB-M2, and FAB-M5).

Induction of Maturation of AML

Although in general AML progenitor cells have retained the responsiveness to HGF stimulation, the cells usually show little maturation under the influence of these regulators. In various in vitro systems supplemented with growth factors, terminal maturation of AML blasts does not appear according to cytologic, immunologic, or functional criteria. This lack of maturation generally remains even when IL-3, GM-CSF,
Programmed cell death (apoptosis) is a physiologic phenomenon associated with the extinction of terminally differentiated myeloid cells. The inability of AML cells to mature towards the terminally differentiated, nondividing, myeloid end products with a short life span may also prevent the progression of the cells to cell death. As a matter of fact, in certain murine myeloid leukemic cell lines, maturation can be effectively induced after exposure of the leukemic blasts to differentiation-inducing agents. With progressive maturation, the latter cells lose self-renewal abilities, finally resulting in the extinction of clonogenicity. The maturation blockade in these leukemic cells appeared coupled to tumor expansion, and induction of maturation was associated with loss of proliferative potential. The observations that the leukemic cells had maintained the capacity to differentiate into nondividing cells have potential therapeutic impact. Mice bearing the differentiation competent subclones of a myeloid leukemia cell line responded favorably to treatment with the differentiation-inducing compound. Conversely, recipients of differentiation-incompetent subclones of the cell line failed to benefit from the attempts at induction of maturation. In human leukemia cell lines, particularly the myeloid cell line HL-60, differentiation can be induced with GM-CSF or G-CSF in vitro. In clinical AML, the cytologically distinct subgroup of the acute promyelocytic leukemias (FAB-M3) represents a notable example in which the maturation block can be abrogated. Acute promyelocytic leukemia (FAB-M3) cells with the typical translocation to retinoic acid. This observation has resulted in the effective use of retinoids in the treatment of acute promyelocytic leukemia. Relatively potent pro- liferation-inducing effects on BCP-ALL cells have been observed with BCGF, a partially purified supernatant of mitogen-activated leukocytes that contains BCGF(s). The structural identity of BCGF remains obscure. The impure preparation of BCGF contains mixed contaminant proteins with profound growth-promoting effects on nonlymphoid cells. This has hampered a critical evaluation of the role of BCGF in BCP-ALL cell proliferation.

The observation that mouse B-cell precursors can be maintained in long-term culture, when seeded onto an in vitro analog of bone marrow stroma, has suggested that stroma-derived components rather than leukocyte-derived factors are particularly capable of stimulating in vitro proliferation of normal and neoplastic B-cell precursors. Three factors produced by bone marrow stroma cells have meanwhile been identified and molecularly cloned: IL-7, KL, and IL-11. IL-7 exerts stimulatory effects on mouse B-cell precursors, mature T cells, and T-cell precursors. Given this spectrum of activities on mouse cells, a role of IL-7 in stimulating the proliferation of ALL cells has been anticipated and subsequently human IL-7 was demonstrated to stimulate DNA synthesis in BCP-ALL and T-ALL. However, the effects are generally quite moderate. Further, in a significant proportion of cases, the ALL cells do not respond to IL-7. How the observed heterogeneity in IL-7 responsiveness relates to the maturation stage of the BCP-ALL and T-ALL cells is not yet clear. In vitro experiments with mouse bone marrow cells established that, in addition to IL-7, other stroma-derived components are involved in normal B-cell development. Further, the most primitive B-cell precursors (pro-B cells) do not respond to IL-7 alone. KL has been reported to synergize with IL-7 in inducing the proliferation of murine primitive pro-B cells. In contrast, no consistent stimulative effects of KL, alone or synergistic with IL-7, on BCP-ALL cell proliferation have been demonstrated (unpublished observations). This appears to be in agreement with the observation that ALL cells usually do not express c-kit. Finally, IL-11, a factor with "IL-6-like" activities on murine plasmacytoma cells and multilineage blast colony-forming cells, also appears to lack growth-promoting activity on ALL cells (unpublished observations).

GROWTH FACTOR REQUIREMENTS OF ALL

Despite efforts by different research groups, progress in understanding the mechanisms involved in the growth control of ALL cells of both the B- and T-cell lineages has been modest. The abilities of ALL clonogenic cells to form colonies in semisolid media containing various sources of growth factors are restricted, and suggest that ideal culture conditions for ALL cells have not yet been disclosed. Although successful colony formation of B-cell precursor ALL (BCP-ALL) progenitors upon stimulation with PHA-LCM and a nonrecombinant preparation of B-cell growth factors (BCGFs) has been reported, it has remained difficult to routinely obtain ALL colonies under these conditions. For this reason, recent studies dealing with HGF responses of ALL cells have been performed with DNA synthesis (3H-thymidine uptake) rather than colony assays.

HGFs with activities on the more mature stages of B- and T-cell development only rarely induce significant proliferative responses in ALL cells, despite the fact that high-affinity membrane receptors for certain HGFs have been detected on ALL cells. Incidentally, IL-2 may activate the proliferation of T-ALL cells, sometimes only in combination with phorbol ester or PHA as coactivating agents. IL-3, a growth factor of primitive hematopoietic cells, has some stimulatory effects in a minority of cases of BCP-ALL. Relatively potent proliferation-inducing effects on BCP-ALL cells have been observed with BCGF, a partially purified supernatant of mitogen-activated leukocytes that contains BCGF(s). The structural identity of BCGF remains obscure. The impure preparation of BCGF contains mixed contaminant proteins with profound growth-promoting effects on nonlymphoid cells. This has hampered a critical evaluation of the role of BCGF in BCP-ALL cell proliferation.

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GROWTH MODULATORS OF AML AND ALL CELLS

In addition to the HGFs, a variety of regulatory factors exist that can synergize or antagonize the proliferative response of leukemic progenitors to HGFs, but lack direct stimulatory effects. In this respect, the role of tumor necrosis factor (TNF) as a regulator of both normal and leukemic hematopoiesis has been firmly established. TNF can modify HGF-dependent proliferation of hematopoietic progenitors, apparently via mechanisms other than growth factor release. TNF enhances IL-3- or GM-CSF-induced eosin-
ophilic colony formation. Conversely, TNF strongly inhibits G-CSF-induced neutrophilic colony growth as well as EPO-stimulated erythroid burst formation. These in vitro observations would suggest that TNF, when available at sufficient tissue concentrations in the marrow, may drive hematopoietic development within the progenitor cell compartment into different directions.

Multiple effects of TNF on AML blasts have been described as well. It was found that, similar to results in normal hematopoiesis, TNF can synergize with IL-3 or GM-CSF in the induction of AML colony growth.69 TNF antagonizes G-CSF responsiveness of AML–colony-forming units (AML-CFU) in a fashion comparable to normal CFU-granulocyte (CFU-G).64,65 The positive and negative effects of TNF on AML-CFU are most probably mediated through transmodulation of HGF receptors. In vitro incubation of AML blasts with TNF significantly increases the numbers of GM-CSF and IL-3 receptors on their membranes.70 This phenomenon can be blocked by cycloheximide, indicating that active protein synthesis is involved. Conversely, TNF rapidly down-regulates the expression of G-CSF receptors on AML blasts as well as normal peripheral blood granulocytes.71 The latter phenomenon is mediated through the activation of protein kinase C, followed by the release of a proteolytic activity capable of cleaving off the ligand-binding domain of the G-CSF receptor.72 TNF and IL-1 induce the release of HGFs from different sources, eg, G-CSF from fibroblasts73 and GM-CSF from fibroblasts and endothelial cells.65,66 Through these mechanisms of HGF release, TNF and IL-1 can indirectly stimulate hematopoietic cell proliferation. TNF may thus combine its modulating activities with the ability to induce the production of growth factors by the leukemic cells, thereby initiating an autocrine cycle of AML growth stimulation. In addition, TNF can induce CSF release by endothelial cells and activate paracrine mechanisms of AML growth control.55

Recently, two different receptors for TNF, TNF-R(p55) or type I and TNF-R(p75) or type II, have been identified and molecularly cloned.64,65 By using monoclonal antibodies that specifically recognize TNF-R(p55) and TNF-R(p75),66 the role of these receptors in mediating the various TNF responses of AML cells has been investigated. The synergistic (with IL-3/GM-CSF), antagonistic (with G-CSF), and maturation-inducing effects of TNF are all exclusively mediated through TNF-R(p55). Activation of TNF-R(p75) appears to be involved in the induction of GM-CSF release by TNF.66 Zhou et al suggested that TNF frequently inhibits the in vitro proliferation of BCP-ALL cells. The inhibitory effect of TNF on BCP-ALL cells is mediated exclusively through TNF-R(p55), although TNF-R(p75) is more abundantly expressed on these cells.

Other cytokines with modulating effects on AML cell proliferation are interleukin-2 (IFN-γ), transforming growth factor-β (TGF-β), and IL-4. As compared with TNF, IL-4 has been reported to express opposing effects; IL-4 can counteract IL-3, GM-CSF, and enhance G-CSF–mediated proliferation of AML-CFU.67 However, in a comparable study, IL-4 exerted more diverse effects on AML-CFU in combination with these growth factors.70 IFN-γ can antagonize the stimulatory effects of various crude sources of HGFs on the growth of AML progenitors. Using purified HGFs in their culture assay, Murohashi and Hoang71 reported that IFN-γ alone had little effect on the proliferation of the AML progenitors, but synergized with GM-CSF and IL-3 in the stimulation of AML colony formation. Ernst et al72 have recently shown that IFN-γ upregulates the expression of the IL-3R/GM-CSFR common β chain structure in monocytes. Hence, it is possible that the synergistic effect of IFN-γ on AML is caused by the upregulation of IL-3 and GM-CSF receptors (IL-3R/GM-CSFR). Further, Murohashi and Hoang71 showed that enhancement of AML cell proliferation by IFN-γ is to a large degree mediated through the induction of TNF-α production. These data are in agreement with the role of TNF-α as an upregulator of IL-3 and GM-CSFR expression of AML cells in vitro.73 Multiple forms of TGF-β have been identified that result from the homodimeric and heterodimeric association of subunits.74 TGF-β1 strongly inhibits the proliferation of murine multipotential hematopoietic progenitor cells as well as IL-3-dependent myeloid leukemia cell lines.75 Generally, human AML cell proliferation in response to IL-3, GM-CSF, and G-CSF is also inhibited by TGF-β1, although, in incidental cases, growth may be enhanced.76-78 The contrasting effects of TGF-β on hematopoietic cells may be explained, at least in part, by its bidirectional effects on HGF receptor expression.79

AUTOCRINE MECHANISMS ACTIVATING THE PROLIFERATION OF AML AND ALL PROGENITORS

Autocrine mechanisms of growth may render neoplasms independent on exogenous growth factor stimulation, thereby removing one normal mechanism of growth control. In experimental models, autocrine growth factor stimulation can be a critical step in myeloid leukemogenesis. For instance, in avian leukemia, activation of monocytic growth factor production results in autocrine growth and development of myeloid leukemia by cooperation with transforming genes.80-82 Notably, evidence has been obtained that autocrine growth may also occur exclusively through intracellular action, ie, not necessarily requiring excretion of the growth factor and subsequent activation of receptors expressed on the cell membrane. Transgenic mice constitutively expressing the GM-CSF gene and mice reconstituted with bone marrow, GM-CSF, or IL-3 cDNA cells, which produce GM-CSF or IL-3 as a result of retroviral gene transfer, develop myeloproliferative syndromes,84-86 but not acute leukemia. Clearly, autocrine growth stimulation by itself is not sufficient to induce leukemia, but must be accompanied by other genetic events that block differentiation.

Although, as has been discussed above, maximal proliferation of human AML-CFU generally depends on exogenous growth factors, in the majority of cases the leukemic progenitors show a certain level of spontaneous proliferative activity in vitro. Frequently this “spontaneous” growth is mediated by autocrine growth stimulation. In the original studies by Young and Griffin,87 the presence of GM-CSF messenger RNA (mRNA) was demonstrated in primary AML blasts and the leukemic blasts proliferated in response to the released GM-CSF. However, in many cases, the leukemic blasts can produce multiple HGFs (GM-CSF, G-CSF, and M-CSF) and
other cytokines (IL-6, IL-1, and TNF)⁹⁷ (Fig 1). Generally, the presence of the transcripts of GM-CSF, G-CSF, and M-CSF by AML cells and the release of these cytokines do not occur constitutively. They are induced or augmented after in vitro treatment of AML cells with either IL-1 or TNF. IL-1 induces the release of GM-CSF, G-CSF, and M-CSF by AML cells.⁸⁸,⁹⁹ IL-1 may also induce the release of IL-1 itself by AML cells and thus not only initiate, but also sustain the circuit of autocrine stimulation (Fig 1). Similarly, TNF may elicit the release of HGFs in AML blasts, although less frequently.⁹⁸ Obviously, the autocrine potential will be amplified in association with the presence of the transcripts of GM-CSF, G-CSF, and M-CSF by AML cells and thus not only initiate, but also sustain the circuit of autocrine stimulation (Fig 1). The ultimate magnitude of autocrine stimulation may thus vary depending on the effective concentrations of the various factors.

The question of whether the features of autocrine growth of clinical leukemia really have a role in the pathophysiology of the disease is difficult to resolve. In an analysis of clinical parameters of 114 cases of AML, a prognostic disadvantage was apparent for those cases of AML that exhibited high spontaneous growth.⁹² In this study, cases of AML showing spontaneous proliferation had a low probability of achieving complete remission and, in addition, complete responders after chemotherapy tended to be at high risk for relapse. These findings could suggest that autocrine growth influences disease outcome of human AML and characterizes a more aggressive form of leukemia.

Similar to the observations in AML, BCP-ALL cells in vitro frequently incorporate ³H-TdR in the absence of exogenous growth factors.⁵⁶,⁶¹,⁷⁰ Several lines of evidence, all indirect or incomplete, suggest that autocrine loops of stimulation may be involved in the proliferation of leukemic B-cell precursors. Overell et al⁹³ showed that murine IL-7–dependent pre-B cells, upon introduction and stable expression of the IL-7 cDNA, became tumorigenic when injected into BALB/cAnN mice. These findings indicated that an autocrine loop of IL-7 stimulation can participate in the malignant transformation of IL-7–dependent pre-B cells. However, after a similar experimental approach, Young et al⁹⁴ showed that overexpression of IL-7 per se is neither sufficient nor necessary for inducing tumorigenicity, and concluded that other mechanisms determine malignant transformation of pre-B cells. Nevertheless, based on the detection of IL-7 transcripts in murine v-abl–transformed pre-B-cell clones,⁹⁴ the possibility that upregulation of endogenous IL-7 production may be one of the multiple events that accompany or condition for malignant transformation of B-cell precursors remains open. Whether these findings are relevant to clinical ALL has not been settled. Thus far, experiments with neutralizing anti–IL-3 antibodies affect the growth of t(5;14) ALL cells.

Cohen et al⁹⁷ demonstrated the constitutive expression of IL-1 in a cell line derived from case of t(4;11) ALL and showed that IL-1 controlled the growth of this cell line. Obviously, the growth properties of a cell line cannot be taken as representative for primary ALL cell samples and the relevance of this mechanism for the growth of t(4;11) ALL needs further study.

**HGF RESPONSE IN RELATION TO CYTOGENETIC FEATURES OF ACUTE LEUKEMIA**

The occurrence of nonrandom chromosome translocations in hematologic malignancies may give clues to the role of specific genes that affect HGF responses (Table 1). Although IL-3, GM-CSF, G-CSF, and, less frequently, M-CSF are common growth factors for AML, other cytokines have infrequently been found to stimulate the proliferation of AML cells in vitro. Responsiveness to IL-5 has predominantly been observed in the cytogenetic subtype of AML with the translocation t(8;21)(q22;q22). Ema et al⁹⁸ reported in two t(8;21) cases of AML that the leukemic cells formed colonies in response to IL-5 that expressed features of eosinophilic maturation. In a recent survey among 52 AML patients, IL-5 induced a proliferative response in 9 cases.³¹ In addition to IL-5, the leukemic cells of these patients responded also to GM-CSF, IL-3, and G-CSF. In one of the IL-5–reactive cases, the AML cells had an apparently normal karyotype. In seven patients, the leukemic cells carried the translocation t(8;21). Interestingly, the ninth case expressed an isodicentric translocation in chromosome 21, i.e, dic (21)(q22;q22). Thus, the cytogenetic abnormalities in eight of the nine patients included rearrangements involving 21q22, suggesting that a gene on chromosome 21 plays a role in the leukemic transformation in IL-5–responsive AML. This is a clinical example showing a unique relationship between a cytogenetic abnormality and a growth factor response pattern. Chromosomal breakpoints involved in the t(8;21) have been cloned.⁹⁹ The gene activated as a result of this translocation has now also been identified and termed AML1.¹⁰⁰ The AML1 gene en-
The function of HGF receptors expressed on the surface of leukemic cells has become an important focus of research. Radioactive HGF preparations have been used extensively to determine the binding characteristics (receptor density and affinity) and the kinetics (eg, receptor internalization after ligand binding) of the HGF membrane receptors on AML and ALL cells. These studies have established that primary AML cells express high-affinity receptors for IL-3, GM-CSF, and G-CSF and less frequently for other growth factors (eg, IL-5 and IL-6). ALL cells have been reported to express high-affinity receptors for IL-3 and IL-7. These cellular properties open the possibility that ALL and AML are sensitive to treatment with growth factor-toxin fusion proteins, a strategy that is, eg, currently being optimized for IL-6 toxin conjugates for clinical use in myeloma patients.

AML and ALL cells are heterogeneous in their responses to growth factors and the presence of a high-affinity receptor for a certain growth factor per se does not predict that the cells proliferate in response to the corresponding growth factor. This is particularly evident in ALL, in which expression of high-affinity GM-CSF and IL-3 receptors for IL-3 and IL-7 does not strictly correlate with mitogenic responses to these factors. Although it has been suggested that defects in receptor function in nonresponsive cases may explain such discrepancies, it appears more likely that the cells require additional activating agents for growth. Alternatively, certain high-affinity receptors may be primarily involved in activation of cellular function rather than proliferation.

The receptor molecules for a wide range of HGFs, including those of GM-CSF, G-CSF, EPO, IL-3, IL-4, IL-5, IL-6, and IL-7, share certain structural features of the extracellular domain and are therefore grouped in the hematopoietin receptor family. The binding features of IL-3R, GM-CSFR, and G-CSFR on AML cells from individual patients show some heterogeneity, but not to an extent that is markedly different from normal bone marrow or peripheral blood cells. In most cases of AML, IL-3, GM-CSF, and G-CSF receptor numbers are quite low (50 to 250 receptors per cell). While receptor overexpression has been implicated as one of the mechanisms through which ligand-independent activation of M-CSF receptors can occur, this phenomenon has not been demonstrated in AML. However, these values are based on average cell estimates and not on single cell or subpopulation analysis, so that the level of receptor expression for the proliferating progenitors in AML has not been specifically determined.

A striking feature is that GM-CSF and IL-3 cross-compete for cellular binding to AML cells. Thus, the high-affinity binding of GM-CSF can completely be competed for by IL-3. Vice versa, high-affinity IL-3 binding is totally or partially inhibited in the presence of excess GM-CSF. The IL-3/GM-CSF cross-competition is explained by the fact that high-affinity GM-CSFR and IL-3R are heteromeric structures consisting of a and b chains. The human b chain (KH79) was discovered by virtue of its homology to the murine IL-

### Table 1. Chromosomal Translocations Resulting in Specific Growth Characteristics of Acute Leukemia Cells

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Leukemia type</th>
<th>Gene involved</th>
<th>Leukemic cell response</th>
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<tbody>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>AML (FAB-M2)</td>
<td>AML 1 (chr 21); AML 1 protein function unknown, possibly a transcription factor</td>
<td>Proliferation in response to multiple HGFs, including IL-5; IL-5 induces eosinophilic maturation; G-CSF induces neutrophilic maturation</td>
</tr>
<tr>
<td>t(15;17)(q22;q11.2-12)</td>
<td>APL (FAB-M3)</td>
<td>PML (chr 15); zinc finger protein, putative transcription factor; RARalpha (chr 17); retinoic acid alpha receptor</td>
<td>Proliferation predominantly stimulated by G-CSF; Terminal myeloid differentiation inducible by retinoic acids</td>
</tr>
<tr>
<td>t(5;14)(q31;q32)</td>
<td>BCP-ALL</td>
<td>IL-3 (chr 5); IgH (chr 14)</td>
<td>Putative autocrine role of IL-3</td>
</tr>
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Abbreviation: chr, chromosome.
3R homologues AIC2A and AIC2B. In humans, the β chains are shared by the unique α chains of IL-3R, GM-CSFR, and IL-5R in the formation of the respective high-affinity receptor complexes. Competition occurs because the β chains are expressed at limiting density as compared with the α chains. IL-3/GM-CSF cross-competition was apparent in all AML cases so far examined. The IL-3/GM-CSF cross-competitive binding is also found in normal peripheral blood monocytes. A similar competition applies to the IL-6 signal transducer GP130, which is shared in the formation of high-affinity IL-6R, leukemia-inhibitory factor receptor (LIF-R), and Oncostatin M receptors. The involvement of common receptor structures in the formation of different high-affinity receptor complexes essentially narrows down a multitude of growth factor responses of AML cells into a more limited spectrum of signalling pathways. This information may be relevant in view of the design of growth factor antagonists that could be applied to inhibit, for instance, the IL-3/GM-CSF/IL-5–activated replication of AML cells. This makes the study of antagonists that prevent association of the common β chain to these growth factors particularly useful.

Certain viral oncogenes encode for truncated (transforming) forms of growth factor receptors. Hence, the possibility that mutations in cellular HGF receptor genes can be involved in the development of human leukemia now receives considerable attention. The cellular counterparts of the viral oncogenes v-fms and v-kit, encoding for the receptors of M-CSF (CSF-1) and KL, respectively, are candidate genes in which activating mutations could occur. Unlike most HGF receptors, the receptors for CSF-1 and KL belong to the family of intrinsic tyrosine kinase receptors, which act through phosphorylation on tyrosine residues of primary downstream target substrates involved in intracellular signal transduction. The complex mechanism of action of this type of receptors and the oncogenic potential of various mutated forms of these structures have been the subject of recent reviews. Despite the fact that experimental evidence indicates that certain mutations in c-fms cause leukemia in mice, there is currently no proof for such a relationship in clinical AML or myelodysplasia (MDS). Although a substitution of serine at codon 301 of the human c-fms gene may render its product transforming when introduced into NIH-3T3 cells, in a series of 110 patients (67 with MDS of which 5 developed AML, and 43 primary AML cases), only 1 case was reported to express a mutation in c-fms at codon 301 (Leu → Phe). Even the significance of this single finding is doubtful, because a 301 (Leu → Phe) substitution mutant was reported to lack transforming activity in NIH-3T3 cells. In another group of 41 patients, only 1 case with a mutation at codon 301 was identified (Leu → Val). In the latter two studies, the incidence of mutations at position 969 was significantly greater. At the 969 position, a Tyr is normally phosphorylated after activation, thereby negatively regulating the tyrosine kinase activity of the receptor. In 20 of the 151 patients (100 MDS and 51 AML) mutations within codon 969 were detected. However, one of 51 normal subjects, investigated by Ridge et al., also expressed a 969 substitution, indicating that alterations at this position per se do not cause MDS or AML, or that they are insufficient by themselves, or that there is a low degree of polymorphism. The demonstration of certain new sites for activating mutations in the extracellular domain of CSF-1R may serve as a basis for additional investigations to establish a potential role of c-fms mutations in AML and MDS.

The hypothesis that the c-kit proto-oncogene product, a putative intrinsic tyrosine kinase receptor, could be involved in leukemogenesis received additional interest when its ligand was identified. As indicated above, KL is a potent (co)stimulator of proliferation in certain cases of AML. Analysis at the protein and RNA level indicated that c-kit is expressed in a subgroup of AML patients. In one study, fresh primary AML cells were found to have detectable levels of c-kit tyrosine phosphorylation without added KL. The possibility has been raised that high expression levels of c-kit in AML may be associated with poor prognosis of the disease. Evidence that transforming mutations in the c-kit gene are involved in AML is currently lacking.

The observation that the myeloproliferative leukemia virus (MPLV) encodes a fusion protein, partially consisting of the envelope protein and partially of a truncated structure of a hematopoietin receptor, supports the possible involvement of mutated forms of the hematopoietin receptor structures in the leukemic transformation of hematopoietic cells. MPL, the human cellular homolog of this receptor structure, has recently been cloned and characterized. MPL is highly expressed in the human erythroleukemia (HEL) cell line and at a low level in a variety of other hematopoietic cell lines. The ligand for MPL is still unknown. Additional direct evidence that mutated forms of hematopoietin receptor structures can be oncogenic comes from random mutagenesis of the murine EPO-R. The latter investigations showed that a point mutation in the extracellular domain of the EPO-R (Arg129 → Cys129) results in the ligand-independent activation of the receptor and contributes to the development of erythroleukemia in mice.

MECHANISMS OF LEUKEMIC CELL SURVIVAL

The possibility that leukemic cells may accumulate because of delayed cell death has been recognized for a long time. The interest in this concept has revived when it was discovered that hematopoietic cells are prone to actively programmed cell death (or apoptosis) after withdrawal of growth factors. Certain leukemic events may interfere with programmed cell death and extend cell survival. The finding that the product encoded by the proto-oncogene bcl-2, which is overexpressed in human follicular lymphomas expressing the translocation t(14;18), blocks apoptosis strongly favors this notion. Notably, high expression of bcl-2 is not restricted to follicular lymphoma, but can also be observed in long-lived B lymphocytes (memory cells) and in hematopoietic neoplasms, including chronic myelogenous leukemia. Normal myeloid precursors (myeloblasts, promyelocytes, and myelocytes) also express significant levels of the bcl-2 gene product, but in more mature stages of myeloid development the protein gradually disappears. Whether the expression of bcl-2 in normal and leukemic hematopoietic precursors is influenced by growth factors is presently unclear. In contrast to the bcl-
2 gene product, the protein encoded by the tumor-suppressor gene p53 exerts a negative effect on the survival of hematopoietic cells. Introduction and expression of the wild-type p53 gene in the spontaneously growing myeloid leukemia line M1, which originally lacks p53, induces apoptosis in these cells. The addition of IL-6 counteracts p53-induced apoptosis in the M1 cells, thus providing evidence that, even in cells that are not absolutely dependent on growth factors, growth factors can prevent programmed cell death. In agreement with these findings, apoptosis of murine IL-3–dependent cells ectopically expressing functional human IL-6 receptors could be counteracted by IL-6. Although these observations have not yet been extended to clinical leukemia, they could imply that leukemic cells capable of IL-6 production have prolonged survival times, possibly as a result of suppression of p53 activity.

ADMINISTRATION OF HGFs TO PATIENTS WITH AML

Recombinant human GM-CSF and G-CSF have now been used widely in cancer chemotherapy to ameliorate neutropenia. Because myelosuppression is a particularly severe side-effect of antileukemic therapy, there has been considerable interest in the efficacy and safety of administering CSFs to patients with AML undergoing induction chemotherapy. As noted above, IL-3, GM-CSF, and G-CSF stimulate in vitro proliferation of blast cells from most patients with AML without supporting leukemic cell differentiation. These results suggested that CSFs have the potential of stimulating rapid leukemic cell proliferation in vivo, at least in those patients in whom leukemic cell growth is limited by a supply of growth factors. Early studies in patients with MDS suggested that leukemic proliferation could be induced in patients who have a higher percentage of blasts in marrow or blood. In some, but not all of the patients, the increase in blast proliferation was reversible upon discontinuation of the CSF. However, the majority of patients, even those with high blast counts, did not show any significant hyperproliferation of myeloblasts in response to either GM-CSF or G-CSF. Many of these MDS patients responded to GM-CSF or G-CSF with a beneficial increase in circulating neutrophils. These results suggest that some AML patients may also benefit from administration of CSF, provided that any potential for excessive blast proliferation is controlled by the coadministration of chemotherapy. This concept has now been tested in vivo in a number of studies, showing that coadministration of GM-CSF and G-CSF is generally safe and appears to improve the rate of recovery from myelosuppression without affecting the rate of complete response. For example, Ohno et al. randomized 108 patients with various types of relapsed acute leukemia (67 with AML, 30 with ALL, 9 with CML blast crisis, and 2 with secondary AML) to receive G-CSF or nothing, starting 2 days after completion of induction chemotherapy. G-CSF accelerated the recovery of neutrophils by approximately 7 days and may have significantly decreased the number of documented infections. The rate of complete remission and relapse was almost the same in the two groups. Similarly, Büchner et al. administered recombinant human GM-CSF to 30 elderly AML patients and compared the outcome to results from a historical control group of 56 patients. The complete remission rate was 50% in patients receiving GM-CSF versus 32% in controls, and the early death rate was decreased in the GM-CSF–treated patients. Two patients showed marked leukemic regrowth that was completely reversible in one patient and GM-CSF–independent in the other patient. GM-CSF appeared to accelerate neutrophil recovery by approximately 7 days. These studies, and others, support the notion that GM-CSF and G-CSF can be administered to patients with AML relatively safely, at least in the setting of coadministration with chemotherapy. Fortunately, in many of the cases in which GM-CSF appears to induce accelerated leukemic regrowth, this effect is reversed after abrogation of the CSF treatment, and long-term remissions have still been observed.

Other investigators have asked whether coadministration of HGF and cytarabine could result in enhanced leukemic cell kill. In vitro studies of the effects of G-CSF, GM-CSF, and IL-3 on the kinetics of AML clonogenic cell proliferation indicated that these HGFs could modestly increase the fraction of leukemic cells in S phase. Because cytarabine ( Ara-C), the most effective agent for the therapy of AML, is S-phase specific, the question has been raised as to whether administration of GM-CSF or IL-3 before Ara-C therapy would actually enhance killing of leukemic cells. This was shown to occur in a number of in vitro studies, but requires prolonged exposure of the leukemic cells to the CSF. Recombinant HGF stimulation of leukemic cells in vitro may alter the intrinsic sensitivity to chemotherapeutic drugs. For instance, (pre)stimulation of human AML cells with IL-3 or GM-CSF in vitro may significantly raise the cytotoxicity of cytarabine to clonogenic cells. The combined in vitro exposure of AML blasts to Ara-C and IL-3 or GM-CSF may also enhance the intracellular cytarabine triphosphate (Ara-CTP) levels and result in a dose-dependent increase of incorporation of Ara-C into the cellular DNA and accordingly increase antileukemic Ara-C toxicity significantly. Thus, HGF stimulation may influence not only the cell cycle status, but also stimulate metabolism affecting intracellular drug pharmacology favorably. IL-3 and GM-CSF are among the most attractive factors to be used for this purpose. Clinical information along this line is still scarce. Preliminary clinical trials indicate that the fraction of leukemic cells in S phase in vivo can be transiently increased by the administration of GM-CSF. A phase II study in newly diagnosed patients has yielded a satisfactory complete remission rate, but the follow-up and number of patients are too limited to permit any further conclusion. One other study using GM-CSF in combination with daunomycin and high-dose Ara-C has reported inferior complete remission and survival rates as compared with historical controls. The latter study differs from most of the ongoing studies by the prolonged GM-CSF prephase before chemotherapy. This urged for frequent early discontinuation of GM-CSF. Carefully conducted, randomized trials will be absolutely necessary to determine if factor-induced alteration of cell cycle kinetics and intracellular drug metabolism will alter the outcome of chemotherapeutic treatment in AML significantly as regards frequency and quality of complete remission. The results of these random-
ized studies using different schedules of GM-CSF administration may clarify the potential utility of this new approach of biologic therapy of AML within a few years.

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