The Roles of Stem Cell Self-Renewal and Autocrine Growth Factor Production in the Biology of Myeloid Leukemia

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One approach to achieving an understanding of the general nature of cancer is to reduce the biological complexity of cancer to the minimum number of abnormalities exhibited in common by all cancer populations. If this reductionist approach is coupled with an exclusion of host-cancer interrelationships, what emerges is the recognition that the characteristic feature of all cancer populations is their progressive expansion in size.

This type of approach has directed attention in recent years to two particular aspects of neoplastic populations: (a) the abnormal level of self-generation exhibited by the clonogenic stem cells in the population; and (b) the possibility that cancer cells have acquired the capacity to produce their own specific growth factors (autocrine stimulation). As shall become evident, neither abnormality alone is sufficient to result in a neoplastic cell, although abnormally high self-generation (>50% of progeny remaining parental in type) is a mandatory property for all neoplastic stem cells.

The Nature of Myeloid Leukemia

Myeloid leukemic populations certainly exhibit the progressive size expansion characteristic of neoplastic populations. Supporting evidence for the neoplastic nature of the diseases includes (a) the presence of mitotically active myeloid cells in tissues not normally the location of significant numbers of such cells, (b) the monoclonal nature of most leukemic populations, (c) the presence of characteristic chromosomal translocations involving the abl oncogene in CML and other translocations in various types of AML, (d) the occurrence of maturation arrest and/or abnormalities in cellular maturation and function, and (e) the transplantability of superficially similar animal myeloid leukemias.

It is necessary to emphasize these neoplastic features of the myeloid leukemias because a number of unusual features of these diseases have raised some doubts regarding their neoplastic nature. These include (a) the common termination of CML in blast transformation, suggesting that the initial chronic phase of CML might be merely a myelodysplastic or preleukemic state, (b) the striking dependency in vitro of most myeloid leukemic cells on normal growth regulators, (c) the occurrence of apparently complete, long-term remissions during which the leukemic population is suppressed but not eliminated, and (d) the ability of normal regulators to suppress some myeloid leukemic populations.

The myeloid leukemias therefore exhibit curious features which, if capable of explanation, might throw light on the nature of other cancers where such features may exist but be less apparent.

Regulation of Normal Myeloid Populations

In vitro studies have established that the proliferation and functional activity of granulocyte-macrophage populations are regulated by a group of specific glycoproteins—the four CSFs and IL-6 (Table 1; Refs. 1 and 2). Each of these glycoproteins has been purified, cDNA and genomic clones for each have been isolated, and recombinant molecules have been produced using a variety of expression systems.

The CSFs are the products of many cell types including endothelial, fibroblast, stromal, and some epithelial cells as well as monocytes and T- and B-lymphocytes (3). Some cells have the ability to synthesize simultaneously more than one type of CSF. Under basal conditions, CSF production is low, and in some instances, the molecules may not be released into the circulation but be retained in or near the cell membrane. CSF production rises rapidly after inductive signaling, the effective signal varying with the cell type involved, e.g., endotoxin or IL-1 for endothelial or stromal cells and antigens for T-lymphocytes. In natural inductive activations such as occur during acute infections, levels of more than one type of CSF are elevated.

The four CSFs are unrelated at the amino acid sequence level, but there are some situations in which coordinated transcription may occur (4), and there are suggestions from the structure and physical proximity of the genes that the CSFs might be related ancestrally. It is intriguing that, in humans, the genes for GM-CSF, Multi-CSF (IL-3), M-CSF, and the M-CSF receptor (c-fms) are closely grouped on chromosome 5 together with the genes for two allied hemopoietic regulators, IL-4 and IL-5. This region of chromosome 5 spans the deletion site in the 5q-abnormality associated with clonal proliferative disorders of granulocytes and monocytes and common in secondary AML (5–8).

The CSFs also exhibit coordinated interactions on responding GM populations that may in part be based on the ability of receptors for other types of CSF to exhibit down-modulation when one CSF binds to its unique membrane receptors (9).

Despite the structural dissimilarity of the CSFs, they exhibit common functional activities. Each stimulates by direct action the proliferation of GM precursors, the CSF concentration determining the length of the cell cycle and the total number of progeny produced. In addition, the CSFs exhibit three other functions: (a) maintenance of membrane integrity and, thus, promotion of GM cell survival; (b) stimulation of the functional activity of mature granulocytes and macrophages; and (c) the ability to irreversibly commit bipotential precursors to enter a restricted pathway of exclusive granulocyte or macrophage formation (3).

Less is known of the biology of IL-6 or its action on GM cells, but it can exhibit a direct capacity to stimulate granulocyte...
Granulocyte colony-stimulating factor (G-CSF) is a dimer of two identical subunits. Due to a variable carbohydrate content, M-CSF is a dimer of two identical subunits.

Table 1 The hemopoietic regulators of human granulocyte-monocyte cells

<table>
<thead>
<tr>
<th>Name</th>
<th>Acronyms</th>
<th>Molecular weight</th>
<th>Chromosomal location of gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte-macrophage colony-stimulating factor</td>
<td>GM-CSF</td>
<td>18,000–30,000</td>
<td>5q23–31</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor</td>
<td>G-CSF</td>
<td>20,000</td>
<td>17q11.2–21</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor</td>
<td>M-CSF</td>
<td>70,000–90,000</td>
<td>5q33.1</td>
</tr>
<tr>
<td>Multipotent colony-stimulating factor (interleukin 3)</td>
<td>Multi-CSF (IL-3)</td>
<td>15,000–30,000</td>
<td>5q23–31</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>IL-6</td>
<td>19,000–21,000</td>
<td>7p15</td>
</tr>
</tbody>
</table>

Formation in addition to its actions on B-lymphocytes and other cells (10).

Stem Cell Self-Renewal in Myeloid Leukemic Populations

In clonal cultures of established murine or human myeloid leukemic cell lines such as WEHI-3B or HL60, it is easy to document the existence of clonogenic (colony-forming) cells in the population. It can also be shown by transplantation of such murine colonies to syngeneic mice that the clonogenic cells are stem cells able to generate transplanted leukemias (11). By reculturing individual colonies, it can be demonstrated that the initiating clonogenic cell is capable of self-renewal and that the level of self-renewal divisions exceeds 50% (and often exceeds 90%) (12). These observations formally document the proposition that abnormally high stem cell self-generation is a consistent property of neoplastic stem cells. As shall be discussed below, if self-generation is markedly reduced, a leukemic population can eventually be completely suppressed.

However, there is now equally clear evidence that extremely high levels of self-generation are alone not sufficient to make cells leukemogenic. Many cloned hemopoietic cell lines have been developed, such as FDC-P1 and 32D, that are immortalized and remain dependent on one or other CSF for survival and proliferation. Such cell lines often exhibit abnormal morphology, an inability to generate differentiating progeny, and chromosomal abnormalities. The clonogenic cells in the lines exhibit the same abnormally high self-renewal capacity (often exceeding 90%) as the clonogenic cells of leukemic cell lines (13). However, such cell lines are nonleukemic when injected into syngeneic mice unless secondary changes subsequently occur in the cells (see below). It is therefore not possible to distinguish a leukemic from a nonleukemic clone in vitro on the basis of either abnormal self-regenerative capacity or failure to differentiate.

When primary human myeloid leukemic cells are grown in clonal cultures, a high proportion of colony-forming cells can usually be detected in CML populations (14, 15). With AML populations, the proliferative pattern is highly variable, with many instances the formation of only small transient clusters (16, 17). While chromosomal analysis has indicated that the colonies and clusters are derived from leukemic cells, reculture usually fails to detect clonogenic cells within them. Even with culture conditions modified to permit the formation of so-called blast colonies, the reconstituting potential of such colonies is strictly limited (18).

The basis for this radical difference between the in vitro behavior of established cell lines and primary leukemic populations has not been established. Possibilities not eliminated are that the true stem cells in primary myeloid leukemias have yet to be detected or that the culture systems used have suppressed the capacity of stem cells for the self-renewal exhibited by such cells in vivo. This unsatisfactory situation has forced more emphasis to be placed on leukemic cell lines for work on self-renewal capacity, with the attendant risk that the data may be misleading because of the possible presence of secondary changes in established cell lines.

Dependency or Autonomy of Myeloid Leukemic Cells

Most long-established myeloid leukemic cell lines exhibit autonomous proliferation in vitro and, for murine cell lines where tests are possible, in every situation where a myeloid cell line exhibits autonomous growth, the cells can be shown to be leukemogenic when transplanted to syngeneic recipients. Autonomy can therefore be equated with some confidence with fully developed leukemic transformation. However, the converse is not always true; as shall be discussed below, leukemic cells do not necessarily exhibit autonomy in culture.

For primary myeloid leukemic populations, it is characteristic that they exhibit apparent complete dependency on extrinsic CSF for survival and proliferation in vitro (19). This is true for most primary murine myeloid leukemias, all human CML populations, and most human AML populations. Extensive surveys over the past 15 yr have documented the remarkable situation that leukemic cells from patients with CML and AML not only exhibit dependency on extrinsic CSF but usually exhibit a near-normal quantitative responsiveness to CSF stimulation. However, cells from an important subset of AML patients differ in exhibiting autonomous proliferation in vitro based on an acquired capacity of the cells to synthesize one or more of the above regulators, and these seem to represent examples of genuine autocrine growth stimulation (20, 21).

The remarkable CSF dependency of myeloid leukemic populations indicates that CSFs are essential cofactors in the development of myeloid leukemia. Without the necessary proliferative stimulation by these CSFs, it would not be possible for a transformed myeloid leukemic cell to generate the expanding leukemic cell clone that is myeloid leukemia.

The true interpretation of the extensive clonal culture data accumulated in many laboratories on the apparent dependency of primary myeloid leukemic cells on extrinsic CSF is now in need of careful reappraisal because of recent work on the leukemic transformation of immortalized CSF-dependent hemopoietic cell lines.

Leukemic Transformation of Immortalized Hemopoietic Cell Lines

As discussed earlier, many immortalized murine hemopoietic cell lines have been developed that exhibit continuing CSF dependency, high stem cell self-renewal and abnormal differentiation but are not leukemogenic. Such immortalized cell lines can be transformed to highly leukemogenic cells by a number of procedures.

When FDC-P1 cells (dependent on Multi-CSF or GM-CSF) are infected with the Abelson virus, they are transformed to autonomous, leukemic cells. Initial studies suggested that this transformation did not involve the formation of abnormal, or abnormal numbers of, CSF receptors or the acquired autoproduction of CSF (22, 23). More recent studies have indicated, however, that in some cases, such cells may have been induced to produce IL-4 (24) and also GM-CSF and Multi-CSF (25). Where transformation was achieved using a bcr-abl construct,
transformed cells were shown to produce IL-3 (Multi-CSF) (26).

Nonleukemic FDC-P1 cells, when injected into syngeneic mice, can slowly evolve to fully leukemogenic cells. This process is accelerated by prior whole-body irradiation of the recipient mouse (27). Analysis of such leukemic populations indicated that many had acquired the autocrine capacity to produce either GM-CSF or Multi-CSF (28), a process that, in at least some cases, involved structural rearrangement of the CSF genes.4

More direct evidence that the acquired autocrine capacity to produce CSF can result in leukemic transformation has been obtained by insertion of CSF cDNAs into immortalized cell lines. Retroviral insertion of either the GM-CSF or Multi-CSF (IL-3) gene with a viral long terminal repeat promoter resulted in leukemic transformation (29, 30). In these cases, the only obvious explanation is that the acquired capacity for autocrine production of CSF was the final critical event resulting in leukemic transformation. While such cells secrete detectable levels of CSF, there has been some evidence to suggest that secreted CSF may not be as important for self-stimulation as CSF remaining inside the producing cells (29).

These experiments showed that the CSFs can play a more important role in myeloid leukemia development than simply being necessary to stimulate the expansion of the first clonogenetic leukemic cell. CSF genes are in fact protooncogenes able to transform cells to leukemic cells. It needs to be emphasized that the starting populations in such transformation experiments were already highly abnormal and had the necessary property of high stem cell self-generation. Acquisition by otherwise normal cells of an autocrine capacity to produce CSF has so far not been shown to be leukemogenic. In transgenic GM-CSF mice, the macrophages transcribe and produce high levels of GM-CSF, yet neoplastic transformation does not result in such cells (31). Similarly, when the GM-CSF gene with a strong retroviral promoter is inserted into normal hemopoietic cells and then these cells are used to repopulate irradiated recipients, extremely high levels of CSF result that are associated with extreme elevation of peripheral blood white cells and massive invasion of the liver and lung by proliferating granulocytes and macrophages. Histological examination revealed a leukemia-like pathology, but when the cells were tested by transplantation to normal animals, they were not in fact leukemogenic (32).

These latter experiments suggest that excessive stimulation of normal cells to proliferate either by autocrine or exogenous CSF is not in itself able to induce leukemic transformation if the conventional test of transplantability is used to define the transformed state.

A remarkable feature of some of the CSF-induced transformation experiments was the fact that certain of the resulting leukemogenic cell lines continued to exhibit in clonal culture a dependency on exogenous CSF similar to that exhibited by primary human myeloid leukemic cells (3, 33, 34). Since the leukemic cells were transformed because of the acquired capacity to produce CSF, it is evident that the dependency of leukemic cells on exogenous CSF seen in clonal in vitro cultures can be misleading in apparently excluding a capacity of the cells for autocrine stimulation.

These observations suggest that autocrine production of CSF, possibly based in some cases on physical disruption of sequences controlling CSF transcription, may be more commonly involved in the genesis of myeloid leukemia in humans than previously suggested by the apparent absolute dependency of most human myeloid leukemic cells on exogenous CSF.

Involvement of Other Oncogenes in Myeloid Leukemia Development

While there is growing evidence for the involvement of growth factor genes in the genesis of acute myeloid leukemia, there is difficulty at present in relating this biology to the translocaional chromosomal changes characteristic of the various subtypes of myeloid leukemia, reported abnormal levels of transcription of other protooncogenes, and the literature on the viral induction of myeloid leukemia in mice and chickens. Some of the reported abnormal transcription levels of cellular protooncogenes may merely represent secondary effects of the abnormal differentiation and proliferative activity of transformed cells. However, it would be a major advance in our understanding of myeloid leukemia to be able to link chromosomal changes and leukemogenic viruses with the emerging knowledge of the involvement of growth factors in leukogenesis.

The hallmark of chronic myeloid leukemic cells is the 9;22 translocation or its variants, leading to the production of an abnormal bcr-abl transcript and the synthesis of a presumably functionally abnormal fusion protein (35). The exact function of the abl protein or its abnormal variant is unknown, but the bcr-abl product may be a signal for abnormal cell replication. As noted earlier, however, retroviral insertion of bcr-abl cDNA into an immortalized CSF-dependent murine cell line led to autonomy and leukemic transformation in which the cells were shown to have acquired the capacity to synthesize Multi-CSF. Thus the bcr-abl product may act indirectly by inducing autocrine CSF production. It remains uncertain whether an event of this type is of relevance in the genesis of CML in humans.

None of the common translocations in AML appears to involve loci of CSF genes, but in secondary AMLs, it is common to find deletion of a portion of one chromosome 5 that involves the location of a number of growth factor genes (5–8).

Mutations of N-ras have been reported to be frequent in AML (36, 37). While the mechanism by which the ras gene product might be involved in leukemic transformation is unknown, it is of interest that transgenic mice bearing a mutated human N-ras develop a high frequency of reticulum cell sarcomas (38).

An interesting new approach to determining the role of some retroviruses in the induction of myeloid leukemia has been based on the observations that transformation by retroviruses often involves integration of proviruses near or within cellular genes, altering their expression (39, 40). A number of immortalized CSF-dependent cell lines have been shown to have retroviral insertions adjacent to a gene Evi-1 (ecotropic virus integration site 1). This gene codes for a member of the Zinc-finger family of transcriptional regulatory proteins (41). Since these cells remain CSF dependent, autocrine growth stimulation does not appear to be the consequence of this transcriptional activation. However, the abnormal production of this protein may influence the gene(s) determining the inability of such cells to terminally differentiate and thus to exhibit the abnormally high capacity for self-generation necessary for cells undergoing eventual leukemic transformation.

Further information regarding the transformation events following retroviral infection has emerged from an elegant series of studies analyzing leukemogenesis induced by two avian viruses, each containing two oncogenes. The avian erythroblas-
tosis virus contains the v-erbA and v-erbB oncogenes and induces erythroleukemia; the MH2 virus contains the v-mil and v-myc oncogenes and induces monocycol leukemia. Analysis has shown that, in each case, it is the cooperation between a cytoplasmic and nuclear oncogene that is crucial for leukogenesis. For myeloid cells, abnormal self-renewal appeared to be induced by v-myc, while the role of v-mil was to induce the autocrine production of CSF (42).

Despite these examples linking oncogenes to a final common pathway of acquired growth factor production, there is no particular reason why autonomous growth needs to involve growth factors or their receptors. Binding of a growth factor to its receptor presumably achieves eventual cell division through a sequential series of mediators within the cell. In principle, an oncogene product could be, or could influence, one of these mediators and effectively deliver a proliferative signal to a cell, so bypassing completely the normal initial growth factor-receptor step.

Indeed, it remains quite possible that leukemic transformation could be the end consequence of a number of quite different abnormalities. Some caution needs to be exercised, therefore, in attempting to unify all of the existing information into a single pathogenetic sequence involving induced abnormal self-generation and autocrine production of growth factors.

Influence of Leukemic Clones on Other Hemoipoietic Cells

In CML, the vast majority of GM progenitors are members of the Ph1 leukemic clone. Nevertheless, careful examination has revealed that normal progenitors do persist and that these can be shown by calculation to represent unaltered numbers of normal GM progenitor cells. Thus, the dominant CML clone does not suppress normal GM populations; it merely dilutes them almost beyond detection (15).

The situation is strikingly different in AML where it is characteristic that no normal GM progenitors are demonstrable (19). The suppression of such progenitors is paralleled by a comparable suppression of normal erythroid, eosinophilic, and megakaryocytic progenitors and provides the cellular basis for the anemia and thrombocytopenia of acute leukemia.

Suppression of normal hemoipoietic populations by the leukemic clone does not include the normal multipotent stem cells or, if it does, is incomplete. When a remission is induced by chemotherapy, the cells repopulating the marrow can be demonstrated in most cases to be derived from surviving normal stem cells or cells of a preleukemic clone with a more normal capacity for proliferation and differentiation (43).

Interestingly, the dominance exhibited by the leukemic clone in vivo is not necessarily seen when marrow from leukemic patients is cultured in vitro for prolonged periods. In such long-term cultures, it is common to observe a decline in the leukemic population and an associated reappearance of normal hemoipoietic cells (44, 45).

The cellular basis for the suppression exhibited by leukemic cells over normal cells in vivo is poorly understood. One possibility worthy of further exploration is that the leukemic cells damage or suppress the functional activity of hemoipoietic stromal cells necessary for maintaining normal hemoipoiesis. Stromal cells are complex in nature, but some at least can be grown clonally in primary cultures of marrow cells. The frequency of such clonogenic stromal cells (fibroblast colony-forming unit) is abnormally low in the marrow of AML and returns to normal during remissions (46).

The Nature of a Leukemic Remission

A characteristic of AML is that, following successful first courses of chemotherapy, patients can enter a remission phase of relatively long duration where the cellular composition of the marrow returns to apparent normality. Such chemotherapy frequently fails to eliminate all leukemic cells, and the subsequent relapse is usually due to reexpansion of cells of the original leukemic clone. What is surprising about this phenomenon is the duration of many such remissions, given the known persistence of leukemic cells.

Simple kinetic considerations and the overt capacity of leukemic cells to suppress normal cells would lead to the expectation that, during regeneration of hemoipoiesis after the chemotherapy-induced aplasia, leukemic cells would again dominate the regenerating population and that no phase of apparent normality would ever be achieved. It seems evident that some major readjustment of regulatory control occurs by either stromal cells or regulatory molecules akin to the situation in long-term cultures with conditions, for a time, allowing normal cells to be dominant over leukemic cells. The phenomenon is in fact far more complex than it might appear, since recent evidence indicates that the apparently normal hemoipoietic cells reappearing during remission can be clonal in nature (47). This implies either a capacity of leukemic cells to revert to the production of maturing normal cells, or more likely the temporary reemergence of a preleukemic clone with such properties.

Suppression of Myeloid Leukemic Cells by Normal Regulators

The continuing dependency of myeloid leukemic cells on growth stimulation by normal regulators and the phenomenon of remission induction both predict that myeloid leukemic populations might be suppressible by the action of normal regulators, either humoral or membrane displayed.

The first indication that this is indeed possible was the demonstration by Ichikawa (48, 49) that addition of media conditioned by various tissues was able to induce differentiation in colonies of the murine myeloid leukemic cell line, M1. A similar phenomenon was demonstrable with another murine myelomonocytic cell line, the WEHI-3B, and the human line HL60. Considerable confusion resulted from the assumption that a single active molecule was likely to be involved, in view of the similar types of conditioned media able to elicit such effects.

Studies using the WEHI-3B model established that one active molecule in such conditioned media was G-CSF (M, 23,000) (50), but studies using the M1 system resulted in the purification of an active molecule, D-factor, with a molecular weight 58,000 (51). Furthermore, it was shown that tumor necrosis factor α was the active molecule in at least some conditioned media able to induce differentiation in HL60 cells (52). More recent studies have somewhat clarified the situation with the demonstration that M1 cells can be induced to differentiate by two other distinct glycoproteins that were present in the starting materials—the LIF (M, 58,000) (53) and IL-6 (M, 22,000) (54). LIF is identical to the previously identified D-factor. WEHI-3B cells respond to both G-CSF and IL-6, while M1 cells respond to G-CSF, LIF, and IL-6. Human HL60 cells respond not only to tumor necrosis factor α and probably the related molecule lymphotoxin but also to GM-CSF, particularly in the presence of LIF (Ref. 55; Footnote 5). Marked suppression of

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5 T. Maekawa and D. Metcalf, unpublished data.
the human line U937 has been observed with GM-CSF.

These studies need to be interpreted with some caution, since differentiation induction in some myeloid leukemic lines can be achieved in vitro by biological molecules such as endotoxin and steroid hormones that seem unlikely to play an important role in vivo (56). Furthermore, a wide variety of chemicals can induce similar differentiation (57, 58). A phenomenon that could be of particular relevance is the ability of a number of drugs used in the therapy of AML to induce responsiveness of leukemic cells to differentiation-induction by biological regulators (56, 57).

There are certain aspects of the three regulators G-CSF, LIF, and IL-6 that warrant comment. These three molecules are coded by genes in quite separate locations (Table 1), and the molecules themselves are dissimilar although there is a minor degree of sequence homology shared by G-CSF and IL-6. G-CSF is a proliferative stimulus for normal and leukemic granulocytic cells (50, 59), IL-6 has some proliferative effects on normal and leukemic granulocytic populations (10, 60), but LIF has no proliferative action yet detected on normal hematopoietic cells (61).

Both G-CSF and LIF are able to induce differentiation at the same molar concentration (10^{-10} to 10^{-12}) and by direct action on the leukemic cells. However, analysis of the action of G-CSF on WEHI-3B or M1 leukemic cells indicates that its differentiation-inducing action is slow, no overt differentiation in colony cells being seen before 5 days (12, 61). Differentiation induction by G-CSF appears to require cell division and is notably asymmetrical, only some daughter or granddaughter cells exhibiting differentiation (12). In contrast, LIF and IL-6 are able to induce differentiation within 48 h, and differentiation induction is associated with marked or complete suppression of colonies (61, 62). In part, this appears to be due to the induction of CSF-dependent macrophage differentiation, since addition of M-CSF partially prevents death of colony cells (61). IL-6 appears to be very similar to LIF in its actions on M1 cells although higher concentrations (25-fold) are required.

The membrane receptors for G-CSF, LIF, and IL-6 are distinct and are neither cross-reactive nor subject to cross-down-modulation (63). Nevertheless combined use of LIF plus G-CSF or LIF plus IL-6 can result in enhanced differentiation of M1 leukemic cells even if the biology suggests that these agents may act by different mechanisms. One feature that relates these three regulators is the fact that levels of all three are elevated in the serum of a mouse within 3 h of the injection of endotoxin (62). This, plus the fact that several cell types such as macrophages, endothelial cells, and fibroblasts can produce all three molecules, suggests that their normal function is involved in early responses to invading microorganisms.

An intriguing aspect of the actions of these three regulators is their ability to induce quite opposite effects according to the target cell system in use. This is seen most clearly with LIF. The actions of LIF on M1 leukemic cells are to rapidly suppress stem cell self-generation and induce macrophage differentiation (61). However studies have shown LIF to be the molecule previously named differentiation-inhibitory activity because of its ability to inhibit differentiation in cultured normal embryonic stem cells. In culture these cells tend to undergo spontaneous differentiation commitment and lose their totipotentiality. However, in the presence of the same concentration of LIF as used to induce differentiation in M1 cells, embryonic stem cells retain their totipotentiality indefinitely (64).

To a lesser degree this extraordinary duality of action is also seen with G-CSF and IL-6. For example, IL-6 is a proliferative stimulus for myeloma cells and likely to be the autocrine growth factor in this neoplasm (65), a function it could not exhibit in such cells if its action was associated only with rapid differentiation commitment.

The opposing effects of these agents do not correlate simply with whether the target cells are normal or neoplastic, and the association of these opposite properties with a single molecule and receptor suggests strongly that a single chromosomal control element may determine whether or not differentiation commitment is initiated. Previous studies have pointed out the all-or-none aspect of terminal differentiation commitment in model leukemic cell systems (66, 67). The possibility is therefore that an on-off switch mechanism is able to be activated by an agent such as LIF, the direction of the switch being determined by the existing gene program of the cell concerned. If this relatively simple model is valid, then other agents must be capable of initiating signals that ultimately act on the same switch mechanism.

While LIF, G-CSF, and IL-6 have been discussed in some detail, it needs to be emphasized that these are not the only hematopoietic regulators able to induce, or collaborate in inducing, the suppression of myeloid leukemic cells. GM-CSF has some suppressing action on murine WEHI-3B cells and human HL60 cells and dramatic suppressing effects on human U937 cells. Data have been presented suggesting that M-CSF may have suppressing actions on primary human leukemic blast cells that outweigh the usual proliferative actions of the CSFs (68). It may be, therefore, that, according to the particular leukemic population involved, one of a number of possible regulators can exhibit more significant suppressive effects than the others.

G-CSF has been successfully used in mice to suppress transplanted myeloid leukemias (69), and GM-CSF has been reported to suppress a transplanted chloroleukemia in rats (70), but such studies remain some distance from clinical application. Although clinical trials on G-CSF and GM-CSF are now well advanced (71) and these are proving effective nontoxic agents in stimulating normal hematopoietic cells, it remains uncertain whether such agents will have a useful clinical role as selective suppressors of myeloid leukemic populations. Use of CSFs in preleukemic or leukemic patients carries the risk of provoking leukemic cell proliferation and exacerbating the disease. It seems irresponsible to use these agents clinically until better in vitro tests can be developed to identify those leukemias in which a useful suppressive effect might be anticipated.

Concluding Comments

Recent studies on myeloid leukemia are providing strong support for the view that two crucial elements in leukemic transformation are the acquisition by the cells of an abnormal capacity for self-replication and an autocrine capacity to produce specific growth factors. It would be naive, however, to suppose that all types of leukemic transformation necessarily involve the autocrine production of such growth factors. Conversely, there is increasing evidence that normal regulatory factors can suppress leukemic populations by differentiation induction. The availability of these regulatory factors for clinical trial raises the possibility of their future use in the therapy of myeloid leukemia.

References


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