Chromosomal and Growth Factor Abnormalities in Leukemia

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Understanding of the complex intracellular and extracellular growth regulatory pathways within the hematopoietic system has increased greatly within recent years, and we have also learned something of the fundamental alterations within these pathways associated with hematopoietic neoplasia. The latter effort has been greatly aided by cytogenetic studies of leukemias and lymphomas, which have provided clues to the location of genes significantly involved in the pathogenesis of these tumors. The techniques of molecular genetics have made it possible to begin isolating and characterizing a number of these "oncogenes," both known and previously unknown. Similarly, better understanding of the nature and action of the multiple local growth factors that regulate normal hematopoiesis have come from the cloning of the genes that code for these factors and their receptors.

As both the neoplastic and normal processes of blood cell formation have been dissected, it is increasingly clear that the regulatory pathways are enormously complex and that many pieces of the puzzle remain to be defined. This meeting was organized to consider our current knowledge regarding lymphoid and myeloid growth factors and the information that cytogenetics has provided in helping to define the alterations in these factors and in other aspects of growth regulation that lead to hemic neoplasia.

Growth Regulation in Lymphoid Cells

T-lymphocytes and macrophages are major sources of a great variety of polypeptide cytokines (interleukins, colony-stimulating factors) that serve autocrine and paracrine roles as growth and differentiation factors in all of the hematopoietic lineages. A number of these have been recently grouped as the "IL-4 family of lymphokines" (Paul, NIH) because of many common characteristics. This group includes IL-2, IL-3, IL-4, IL-10, γ interferon, and GMCSF (CSF2), all produced by activated T-cells. Many of these genes are linked on the long arm of chromosome 5, and their receptors, where characterized, are structurally members of the "hematopoetin receptor supergene family." IL-2 and IL-4 are particularly important as stimulants of T-cell proliferation, and all members of the group act as differentiation or inhibit growth of some myeloid leukemic lines. To begin to understand how these multiple effects are mediated, a nuclear factor (NF-IL-6) involved in IL-6 gene expression has been cloned, and detailed analysis of the IL-6 receptor is also under way (Kishimoto, Osaka). The IL-6 receptor associates with another membrane glycoprotein (gp130) in the formation of high-affinity IL-6 binding sites and signal transduction. The intracytoplasmic region of this receptor complex has GTP binding motifs, suggesting the possibility of a RAS-related transmembrane signaling process. Still another lymphokine, IL-7, has been shown, among its functions, to stimulate the growth of pre-B-cells. Studies in pre-B-cell lymphomas induced by Abelson virus suggest that autocrine IL-7 production may be a factor in the pathogenesis of such neoplasms (Weissman, Stanford; Witte, UCLA). The stimulatory effect of IL-7 is in these cells is associated with expression of a metalloproteinase, 6C3/BP1, on the cell surface.

Generally, it has been difficult to demonstrate abnormal structure or function of specific lymphokines or interleukins in association with human lymphoid neoplasia. One exception is the overexpression of IL-2 receptors on circulating T-cells in the preleukemic and leukemic phases of adult T-cell leukemia induced by the retrovirus human T-cell lymphotrophic virus type 1. This appears to be mediated by a trans-activating gene product (tax-1) encoded by the virus. This protein has other effects as well, including augmentation of viral transcription, increased IL-2 gene expression, and perhaps reduction of β-polymerase activity with resultant genomic instability in the infected cells (Waldmann, NIH). These various effects lead to a polyclonal T-cell expansion and increased probability for a specific genetic alteration that allows a frankly neoplastic clone of T-cells to emerge. A monoclonal antibody, anti-Tac, directed against the IL-2 receptor is now being utilized in clinical trials of patients with adult T-cell leukemia. This role for a virus in the pathogenesis of a human leukemia is somewhat similar to that of the Epstein-Barr virus in the development of endemic Burkitt's lymphoma (Klein, Karolinska Institute, Stockholm). Chronic infection with the Epstein-Barr virus appears also to produce a polyclonal expansion of lymphoid cells (in this case, B-cells) perhaps by preventing the normal programmed movement of these cells from the cycling compartment to a resting stage. Again, this expanded, proliferating population provides the opportunity for a specific genetic alteration to occur in one cell (the 8;14 chromosome translocation that results in deregulation of the MYC protooncogene), and the clonal progeny of that cell then emerge as an aggressive neoplasm.

Our understanding of the sequence of events in transformation has been enhanced by the use of the transgenic mouse system. The type of tumor produced (B-cell, T-cell, or macrophage) depends on the oncogene and the promoter used, as well as the mouse strain in which the construct is studied (Cory, Melbourne). Thus, E-mu-v- abl mice develop plasmacytomas or...
Cytogenetics and Molecular Genetics of Lymphoid Neoplasia

The translocation in Burkitt's lymphoma represents just one of some two dozen nonrandom chromosome translocations in both B-cell and T-cell tumors that have been actively investigated with molecular genetic techniques during the last decade. In many cases, it has been shown that these rearrangements result in a growth regulatory gene (protooncogene) being brought into association with a transcriptionally active immunoglobulin gene in a B-lymphocyte or with a T-cell receptor gene in a T-lymphocyte. This association results in "deregulation" of the protooncogene, contributing significantly to expansion of a neoplastic clone from the affected cell. The only previously known oncogene involved in these rearrangements is MYC, and recent evidence indicates that it can be deregulated in B-cell tumors not only by translocation adjacent to an immunoglobulin gene but also by being brought into juxtaposition with a previously unknown gene (designated BCL3) on the long arm of chromosome 17 at band 17q22 (Crocé, Temple University). Other genes associated with the immunoglobulin heavy chain locus in B-cell tumors have been designated BCL1, BCL2, and a second "BCL3" in the translocations t(11;14)(q13;q32), t(14;18)(q32;q21), and t(14;19)(q32;q13), respectively. The best characterized of these is the BCL2 gene, which is involved, through the t(14;18) translocation, in a large percentage of low-grade follicular lymphomas and, through submicroscopic rearrangements, in a significant number of cases of B-cell chronic lymphocytic leukemia (Crocé). The gene codes for a protein associated with the inner mitochondrial membrane and its deregulation may prevent programmed B-cell death, contributing to the slow expansion of a neoplastic B-cell clone (Korsmeyer, Hughes Institute, Washington University). Thus, BCL2 may represent a third class of transforming genes, not oncogenes or tumor suppressor genes, but rather "programmed cell death" genes. In transgenic models, a bcl2/igh fusion gene has resulted in increased numbers of nonneoplastic B-cells and eventually some B-cell lymphomas, as well as T-cell neoplasms (Korsmeyer, Cory). When crossed with transgenic mice carrying a myc/igh fusion gene, aggressive lymphoid neoplasms of a very primitive phenotype developed (Cory). The latter may mimic a phenomenon observed in patients, where a low-grade B-cell neoplasm with a t(14;18) involving BCL2 may progress to a high-grade malignancy with the acquisition of a second chromosome translocation, t(8;14), involving MYC (Nowell, University of Pennsylvania).

The BCL1 protooncogene has not yet been isolated and characterized, but the BCL3 gene involved in the 14:19 translocation in some cases of B-chronic lymphocytic leukemia has been cloned and sequenced (McKeithan, University of Chicago). Like BCL2, this gene is apparently inducible by mitogenic stimulation of resting lymphocytes, suggesting a role in normal growth regulation, and some characteristics of the protein suggest that it may function as a nuclear transcription factor. The latter is the case with another oncogenic translocation in B-cell tumors, the t(1:19) rearrangement that characterizes a group of pre-B-cell leukemias. As a result of this translocation, a fusion gene is formed that retains portions of the e2a gene from chromosome 19, which codes for immunoglobulin enhancer binding proteins but has its DNA binding motifs replaced by those of another gene (designated pBL1) from chromosome 1. Presumably, the nuclear binding product of this e2a/pBL1 fusion gene inappropriately activates one or more growth regulatory genes and contributes to development of the leukemia (Cleary, Stanford University).

Interestingly, although MYC is involved in translocations in B- and T-cell tumors, none of the new protooncogenes tentatively identified in B-cell neoplasms appears to participate in the development of T-cell tumors. Conversely, the five or six such genes identified in T-cell tumors seem restricted to the T-cell lineage. The latter are "deregulated" by translocations that bring them into association with either the complex α/β T-cell receptor locus at chromosome band 14q11 or with the TCR-β locus at 7q35. The putative protooncogenes involved are located at chromosome bands 1p32, 9q34 (proximal to the ABL gene), 10q24, 11p13, 11p15, and 14q32 (proximal to the immunoglobulin gene) (Crocé). A few have been cloned and partially characterized, and again the preliminary evidence suggests that many of them code for nuclear binding proteins. One such gene, rhombotin, is involved in the t(11;14)(p15;q11) translocation observed in a subset of T-cell leukemias (Rabbitts, Cambridge). This gene encodes a cysteine-rich protein that is expressed in mouse brain, suggesting a role in normal development, but how this relates to its putative function in T-cell growth regulation and leukemia remains to be determined.

Growth Regulation in Myeloid Cells

The major regulators stimulating the proliferation of immature granulocytes and macrophages are the four glycoprotein colony-stimulating factors: GMCSF (CSF2), GCSF, MCSF (CSF1), and MultiCSF (IL-3). At least two other regulators, IL-6 and IL-4, have some actions, and the necessary initial generation of committed GM progenitor cells is controlled by regulators which include IL-1 and the newly described c-kit ligand, stem cell factor. The CSFs control not only cell proliferation but also irreversible decisions regarding differentiation, commitment, and initiation of maturation. The CSFs are also powerful activators of the functions of mature granulocytes and monocyte-macrophages. A major role of the CSFs in infection is the activation of neutrophils by GCSF and GMCSF, eosinophils by GMCSF, and IL-3 and macrophages by MCSF, GMCSF, and IL-3, so that these cells function more effectively in host defense (Golde, Los Angeles). There is a complex inductive network for the production of these regulators that may differ in different cell types and involves interactions between bacterial products and a variety of cytokines, including the CSFs themselves, as well as IL-1, IL-6, and tumor necrosis factor (Sachs, Rehovot). Murine cell models of myeloid leukemia can be induced to terminally differentiate by a variety of cytokines, and the responsiveness to differentiation induction by IL-6 (MGI2), leukemia inhibitory factor, GCSF, and IL-3 varies with different leukemic cell lines. The inducibility of these murine cell lines for differentiation is determined by genes present on chromosomes 2 and 12 in the mouse (Sachs). A deletion in one chromosome 2, which is associated with a deletion of a homeobox gene, Hox-4.1, is the most common chromosome change in mouse myeloid leukemia.

The CSFs exert their biological actions through interaction with specific cell surface receptors. The first CSF receptor to be structurally defined was that for CSF1 or MCSF, because of its homology with the viral fms oncogene (Sherr, Memphis). The other CSF receptors (GCSF, GMCSF, IL-3) are not
homologous to the MCSF receptor and do not contain a tyrosine kinase domain. However, along with receptors for several other cytokines (IL-2 β chain, IL-4, IL-6, IL-7, and erythropoietin) they form a new class of related receptors that display a partly conserved extracellular domain (Nicola, Melbourne). The cloned human GMCSF (CSF2) receptor is a low-affinity, non-cross-reactive receptor when displayed on COS cells or murine FDC-P1 cells, even though high-affinity, IL-3 cross-reactive receptors have been described on human hematopoietic cells. Nevertheless, this receptor is capable of signaling proliferation in FDC-P1 cells, and it may be that molecular interactions in hematopoietic cells can generate both high-affinity and cross-reactive receptors. As with other cytokine receptors, there is evidence for alternative transcripts of the human GMCSF (CSF2) receptor that generate altered cytoplasmic domains and soluble forms of the receptor (Golde).

Human myeloid leukemic cells, from either CML or AML, usually exhibit the same dependency on the CSFs for proliferative stimulation as do normal granulocyte-macrophage precursors. Thus, the CSFs are mandatory cofactors for the emergence of the leukemic clone. Further studies using immortalized murine CSF-dependent cell lines have shown that the genes for at least GMCSF (CSF2) and MultiCSF (IL-3) are protooncogenes able to transform such cell lines to leukemic cells. Studies on human myeloid leukemic cells have revealed that, in at least some patients, the leukemic cells can produce one or more of the CSFs either constitutively or following induction by IL-1 (Griffin, Boston; Mertelsmann, Freiberg). It seems likely that this autocrine CSF production is of relevance to the leukemic transformation, although the abnormality in transcriptional regulation responsible has yet to be characterized.

Leukemic clones in vivo behave dominantly and, at least in AML, can markedly suppress the preexisting normal granulocyte-macrophage population by mechanisms that remain unclear. Interestingly, the situation is reversed in long-term cultures of marrow from leukemic patients, with often a substantial reduction or even elimination of the leukemic clone (Eaves, Vancouver). This procedure has now been used in clinical trials of autologous marrow transplants in leukemic patients following routine chemotherapy.

The CSFs have also been used in clinical trials in myeloid leukemia, either to force leukemic cells into cell cycle to heighten their susceptibility to cycle specific cytotoxic agents or to enhance normal hematopoietic regeneration following chemotherapy (Mertelsmann).

Because the CSFs are also capable of initiating differentiation commitment in myeloid cells, they can induce a comparable differentiation in certain myeloid leukemic cell lines. This results in suppression of self-renewal by the clonogenic cells and suppression of the leukemic population with or without associated morphological maturation. IL-6 and leukemia inhibitory factor have comparable suppressive actions (Lotem, Rehovot; Metcalf, Melbourne), and combinations of multiple myeloid regulators achieve more substantial suppression than single agents. It has yet to be established whether this action of myeloid regulators will play a useful role in the clinical management of myeloid leukemia, but such studies are in progress. Oddly, leukemia inhibitory factor also has the opposite effect of inhibiting differentiation in normal embryonic stem cells, indicating that the exact response to a particular regulator is determined by the gene program operating in the responding cell (Metcalf).

The leukemic transformation of myeloid cells can involve not only an acquired autocrine capacity to produce relevant growth factors but also an altered ability to differentiate in response to these factors. A murine model system in which tumorigenic variants of the murine FDC-P1 cell line were isolated by selection in vivo for tumor formation in irradiated syngeneic mice was particularly intriguing. Although many of the leukemic clones derived were able to grow in vitro in a growth factor independent manner, a significant number remained fully growth factor dependent. When autocrine CSF production was demonstrated, this was in many cases associated with insertion of an IAP particle upstream of the relevant gene (Gough, Melbourne).

In order to identify genes that are able to alter the ability of normal CSF dependent hematopoietic cells to differentiate, Ihle’s group (Memphis) examined viral integration sites in a series of CSF dependent cell lines isolated from retrovirus-induced myeloid leukemias in mice. Five of 37 cell lines contained provirus in a common viral integration site termed the ectopic virus integration site (EviI). These integrations had occurred in the vicinity of and had activated a novel gene, encoding a M, 145,000 DNA-binding protein with 10 zinc finger motifs. The normal expression of EviI is not in fetal myeloid cells but in kidney, liver, and ovary. However, ectopic expression in myeloid cells is transforming. The human EviI gene, which is highly homologous to the mouse gene, maps to 3q26. It is not normally expressed in AML, but it is expressed in 8 of 19 AMLs with 3q26 alterations (Ihle).

Ectopic expression of homeobox genes can also apparently contribute to the generation of myeloid leukemia. In the mouse myeloid leukemia WEHI-3B, endogenous retroviral-like (IAP) genomes have integrated just 5' to both the I3 gene and the Hox-2.4 homeobox gene, inducing their expression (Blatt, Rehovot; Adams, Melbourne). This finding suggested that deregulated expression of Hox-2.4 might be oncogenic. A test of the IAP-driven Hox-2.4 gene in the NIH3T3 fibroblast cell line showed that transplantation of fibroblasts expressing high levels of Hox-2.4 mRNA into nude mice produced tumors (Blatt). Other evidence for leukemogenic action was provided by the demonstration that retroviral delivery of the I3 and Hox-2.4 genes to normal bone marrow cells generated autonomous immortal myeloid cell lines that were highly leukemogenic in vivo (Adams). The leukemogenic effects of enforced Hox-2.4 expression appeared to reflect its enhancement of self-renewal capacity. This evidence that deregulated expression of a normal homeobox gene product can be oncogenic complements the finding discussed above that the t(1;19) frequently associated with childhood pre-B ALL creates a structurally altered homeobox gene (Cleary). Thus, the numerous homeobox genes may constitute a previously unsuspected reservoir of potential oncogenes.

Cytogenetics and Molecular Genetics of Myeloid Neoplasia

The “oncogenic” genes associated with nonrandom chromosome alterations in myeloid tumors have proved much more difficult to identify than in lymphoid neoplasms. The one most extensively characterized remains the BCR/ABL fusion gene resulting from the 9;22 translocation that produces the Philadelphia chromosome and characterizes chronic myelogenous leukemia as well as Ph1 positive acute leukemias (Witte, UCLA). Detailed studies of the fusion gene suggest that a cis-acting function of the BCR sequences from chromosome 22 helps to deregulate or alter the tyrosine kinase domain of the
ABL gene from chromosome 9. Depending on the location of the breakpoint within BCR, the fusion gene product is either a p210 or p185 protein resulting, respectively, in either a chronic or an acute leukemia. Transfection studies are under way to try to explain why these two aberrant tyrosine kinases have such differing degrees of "mitogenicity." It is also unclear why the cells of CML, which carry the Philadelphia chromosome, typically acquire additional genetic alterations that lead to the aggressive terminal phase of the disease. Compared to chronic B-cell leukemia, for example, the cells of CML appear to be much more genetically unstable and prone to the acquisition of additional changes, but the mechanisms underlying this and other instances of such instability in many neoplasms remain largely speculative (Cairns, Harvard; Nowell).

With respect to other myeloid disorders, three laboratories have recently succeeded in cloning the breakpoint region of the 15;17 translocation that characterizes acute promyelocytic leukemia and have demonstrated structural alteration in the retinoic acid receptor \( \alpha \)-gene (\( rARA \)), located adjacent to the breakpoint on chromosome 17 (De Jean, Institut Pasteur, Paris; Alcalay, Instituto Clinica Medica, Rome; Goddard, Imperial Cancer Research Fund, London). The translocation results in a fusion gene composed of the 5' portion of the MYL gene (presently uncharacterized) with the \( rARA \) gene minus its first exon. The break points on both chromosomes 15 and 17 are tightly clustered. The fact that acute promyelocytic leukemia has been shown to respond clinically to large doses of retinoic acid suggests strongly that this rearrangement is involved in the pathogenesis of the disease. Further exploration of this and other soluble factors, such as the transforming growth factor \( \beta \) family, that have both stimulatory and suppressive actions in different cell lineages and under different circumstances (Sporn, NIH) may provide additional approaches to the control of certain hemic neoplasms.

Significant progress has also recently been made in characterizing two previously unknown genes involved in the t(6;9)(p23;q34) rearrangement associated with another subgroup of acute myeloid leukemias (Grosfeld, Erasmus University, Rotterdam). This translocation produces a fusion gene containing sequences from a gene on chromosome 9, designated CAN and pronounced "cain" (because it is adjacent to ABL), and sequences from a gene on chromosome 6, designated DEK. The fusion gene product, with a molecular weight of approximately 165,000, appears to retain the nuclear binding characteristics of one of the parent genes (DEK) and is currently being further characterized.

Less progress has been made in identifying the gene (or genes) at band 11q23 that is involved in a variety of chromosome translocations in both myeloid and lymphoid tumors. With the use of cosmid probes and yeast artificial chromosomes, it has been possible to show that in four of these translocations, t(4;11), t(6;11), t(9;11), and t(11;19), the break point is within the same 320-kilobase region, and efforts to clone the involved gene(s) are under way (Rowley, University of Chicago). Similarly, detailed dissection has continued of the region in the long arm of chromosome 5 that is frequently deleted in myeloid leukemias and preleukemias. Many CSF and interleukin genes have been mapped to this region, as well as the genes of other growth factors and their receptors, but the function of none of these has been shown to be consistently altered in the resultant neoplasms (LeBeau, University of Chicago). It is possible that the early growth response gene (EGRI), which is also within this region and codes for a nuclear binding protein, may function as a tumor suppressor gene the loss of function of which could be important in myeloid tumorigenesis; this suggestion is currently being explored. The recent mapping of the GMCSF (CSF2) receptor gene to the pseudoautosomal region of the sex chromosomes may also provide a useful lead, since another group of acute myeloid leukemias, associated with the 8;21 translocation, show the loss of an X or Y chromosome in almost 70% of patients (Gough). One can speculate that a decrease in these receptors could significantly impair normal myeloid differentiation and thus contribute to tumor development.

Scientific discovery involves "oscillations of understanding between clarity and confusion" (Cairns). It is clear that much progress has been made but that much remains to be learned, not only about the interactions among various hematopoietic growth factors but also about the many intracellular proteins the altered structure or function of which contributes importantly to the development of hemic tumors. Thus far, the only known growth factor gene with a function altered as a consequence of a chromosome rearrangement is \( IL3 \), in the t(5;14) of some acute lymphoblastic leukemias. It seems quite likely that, in the future, the increasing interplay between cytogenticists and growth factors will contribute substantially more to our understanding of the role of both areas in leukemogenesis.

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**References**

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